THE MECHANISM OF THE CONTRACTILE ACTION OF ANGIOTENSIN II

By

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PhD Thesis

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The mechanism of the contractile action of angiotensin II was investigated on the rat descending colon and the effect of all experimental treatments were compared with their effect upon equivalent responses to PGE_2 and KCl. Exposure to Ca^{2+}-free Tyrode caused a greater reduction of responses to angiotensin than of responses to PGE_2 and KCl. An increase in $[Ca^{2+}]e$ from 1.8 to 3.6mM potentiated the responses to angiotensin and KCl and a further increase to either 7.2mM or 10.8mM potentiated the responses to angiotensin and PGE_2 but reduced responses to KCl. Ca^{2+}-free Tyrode with SKF525A or verapamil abolished the responses to angiotensin, PGE_2 and KCl. Reintroduction of Ca^{2+} caused incomplete recovery of responses to angiotensin and PGE_2 but no recovery of responses to KCl. Reduction of $[Na^+]e$ increased the initial response to angiotensin and PGE_2 but reduced subsequent responses while responses to KCl were consistently increased. Isoprenaline, theophylline and dibutyryl cyclic AMP caused a greater reduction in responses to angiotensin and PGE_2 than KCl. Indomethacin ($50\mu M$) reduced the responses to all three spasmogens.

Contraction and membrane electrical activity were measured by the sucrose-gap method with KCl as a control spasmogen. Contractions of longitudinal muscle of the rat descending colon to angiotensin were associated with small membrane depolarizations ($<5mV$) which were dependent upon extracellular Ca^{2+} and Na^+ and unaffected by verapamil. Contractions of guinea-pig taenia coli to angiotensin were associated with membrane depolarization and an increase in spike size and frequency. The spikes were dependent on extracellular Ca^{2+} and reduced by verapamil but the membrane depolarization was dependent on extracellular Na^+ and unaffected by verapamil.

Angiotensin and KCl increased cellular ^{45}Ca uptake measured by the lanthanum technique. The increase due to KCl but not to Angiotensin was abolished by verapamil.

The findings suggest that the contractile action of angiotensin upon smooth muscle is direct and may involve Ca^{2+}/Na^+ movement.

Key words: Angiotensin, smooth muscle, calcium-antagonists.

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INTRODUCTION

1 General Introduction

Angiotensin is one of the most potent naturally occurring pressor agents and although some of its pressor effect is due to an indirect action, its direct vasoconstrictor action plays a major role in the generation of its pressor activity (see reviews Gross, 1971; Regoli, Park and Rioux, 1974; Peach, 1977). Since the manipulation of physiological conditions such as an increase in sodium intake may increase the pressor response to angiotensin (Reid and Laragh, 1965; Cowley and McCaa, 1976) a clear understanding of how this and other manipulations may affect pressor responsiveness might come at least in part by understanding the smooth muscle contractile mechanisms of angiotensin.

The objective of this study was to investigate the mechanism underlying the contractile action of angiotensin upon smooth muscle. The contraction of the isolated guinea pig ileum to angiotensin is partly mediated by acetylcholine released from parasympathetic nerve endings (Khairallah and Page, 1961). Unlike guinea pig ileum, it has been reported that the contractile action of angiotensin on rat descending colon is wholly direct and furthermore the myotropic action of different analogues of angiotensin in this tissue has been found to correlate with their pressor action in the same animal (Regoli and

Vane, 1964). This has led to the suggestion that receptors for angiotensin in the rat colon are similar to the pressor receptors in the rat. Therefore, the direct smooth muscle action of angiotensin has been studied on the isolated rat descending colon and the investigation has been extended to include the contractile effect of the peptide on guinea pig taenia coli, in which like rat colon the effect is not mediated by parasympathetic nerves (Ohashi, Nonomura and Ohga, 1967).

In order to determine the mechanism by which angiotensin elicits smooth muscle contraction, the effect of all experimental treatments upon responses to angiotensin has been compared with the effect upon responses to potassium chloride, an agonist whose mechanism is better understood.

Contraction of smooth muscle is the mechanical end product of a series of events that can be initiated by a combination of a stimulant drug with its specific receptor on the smooth muscle cell. Irrespective of whether the agent has a direct or an indirect action, the event that immediately precedes and is responsible for contraction is always an increase in the myoplasmic concentration of ionized calcium (Bohr, 1964; Hurwitz and Suria, 1971). However the events leading to such an increase in activator calcium seem less universal and

depend upon the agent used to initiate the sequence of events (Somlyo, 1972). The calcium necessary for contraction of smooth muscle may originate from extracellular or intracellular sources (Hurwitz and Suria, 1971; Devine, Somlyo and Somlyo, 1973) and the contraction may occur with or without membrane depolarization (Evans, Schild and Thesleff, 1958; Edman and Schild, 1962; Somlyo and Somlyo, 1968a; Speden, 1970). In this study an attempt has been made to elucidate the source of calcium and the role of changes in membrane potential for the contractile action of angiotensin upon smooth muscle. Since the distribution of calcium ions is closely related to that of sodium ions (see review Van Breemen, Aaronson and Loutzenhiser, 1979) the role of sodium ions in contractile action of angiotensin has also been studied.

The action of many peptide hormones following the interaction with their specific receptor at the cell membrane of the target cell is mediated by cyclic AMP (Sutherland and Robison, 1966; Sutherland, Robison and Butcher, 1968; Robison, Butcher and Sutherland, 1971). Evidence suggests that agonist induced contraction of smooth muscle may be mediated by changes in intracellular cyclic AMP concentration (see review Bär, 1974; Rasmussen and Goodman, 1977). Since angiotensin is a large polypeptide molecule that exerts its action by interaction with a specific receptor site located on the cell membrane

(see review Regoli et al, 1974) the possible role of cyclic AMP in the contractile action of angiotensin has also been studied.

Reports in the literature have suggested that angiotensin may cause synthesis and release of prostaglandins which may serve to mediate angiotensin smooth muscle contraction (Chong and Downing, 1973, 1974). In view of this suggestion, the possibility that prostaglandins may act as an alternative secondary mediator in the angiotensin contractile action of rat descending colon was investigated using the prostaglandin synthetase inhibitor, indomethacin (Vane, 1971). In addition, during the initial part of this project PGE_2 was used as an additional agonist in order to inter-relate its contractile action to that of angiotensin.

2 Literature Review

Introduction to Literature Review

The present study has involved an investigation of the mechanism of the contractile action of angiotensin upon isolated smooth muscle preparations. It is therefore appropriate to review our knowledge of the biochemistry and physiological importance of this hormone together with our current knowledge of its mode of action. An extensive review on the contractile action of angiotensin and the mechanism of smooth muscle contraction has been presented in order to facilitate an understanding of the philosophy of the experimental procedures.

The literature review has been presented in three main parts:

(A) The renin-angiotensin system and the hormone angiotensin. History of angiotensin, the biochemistry, physiological and pathophysiological roles of angiotensin.

(B) The mechanism of smooth muscle contraction including a survey of the mode of action of calcium antagonists and a consideration of membrane potential measurement in smooth muscle.

(C) The contractile action of angiotensin upon smooth muscle.

(A) Renin-angiotensin System

(i) History of Discovery of Angiotensin

In 1898 Tigerstedt and Bergman demonstrated that crude extracts of the kidney were pressor when injected into anaesthetized rabbits. They named the active principle in the extract 'renin'. Several years later Goldblatt and co-workers (1934) demonstrated that partial construction of the main renal artery produced a chronic sustained hypertension in the dog. Later, in 1938, Kohlstaedt, Helmer and Page established that renin was an enzyme without direct action on smooth muscle. In 1942, Houssay and Braun-Menendez showed that the ischaemic kidney produced the enzyme renin that was responsible for the formation of a pressor substance in the blood. This pressor substance originally called both hypertensin and angiotonin came to be known as angiotensin (Braun-Menendez and Page, 1958). Since then the biochemistry of its formation, its physiology and pharmacology have been widely studied.

(ii) Factors affecting Renin Release

Enzymes with renin-like activity are found in many tissues including uterus and placenta, brain, pineal gland, hypophysis and in large arteries and veins. The most widely studied to which important physiologic and pathophysiologic roles have been ascribed is produced

by the kidney. Renin is synthesized and stored in the granules of the juxtaglomerular (JG) cells which are located in the renal afferent arterioles of the kidney. The control of renin release from the kidney has been a subject of many reviews (Gross, Brunner and Ziegler, 1965; Vander, 1967; Davies and Freeman, 1976; Peach, 1977; Reid, Morris and Ganong, 1978).

Renal ischaemia (Houssay and Menendez, 1942) induced by renal artery constriction has been found to cause renin release. However Skinner, McCubbin and Page (1964 a, b) have suggested that the afferent arterioles act as a stretch receptor or baroreceptor such that increased intravascular pressure in the arterioles inhibits release of renin and conversely low pressure induces the release of the enzyme. At the beginning of the distal tubule are located the macula densa cells which are in contact with fluid. These cells have an osmoreceptor that enables them to transmit information on the composition of tubular fluid to the JG cells which lie at the entrance of the nephron such that a decreased sodium load at the macula densa increases renin release and an increase in sodium load has the opposite effect (Vander and Miller, 1964; Freeman, Davis, Gotshall and Johnson and Spielman, 1974). In addition the JG cells are sympathetically innervated (Barajas and Muller, 1973) and the stimulation of renal nerves

or infusion of noradrenaline (NA) into the renal artery of nonfiltering kidney increases renin release (Taher, McLain, McDonald and Schrier, 1976). Renin release is also increased when catecholamines are added directly to renal cortical slices or isolated renal cell preparations. This effect on renin secretion is mediated through β-adrenergic stimulation possibly by activation of adenyl cyclase with the resultant formation of cyclic AMP (see review Reid et al, 1978). The CNS may influence renin release by affecting the activity of renal sympathetic nerves (Passo, Assaykeen, Goldfien and Ganong, 1971). Other factors influence renin release. Angiotensin has been shown to inhibit the release of renin and this has been attributed to a direct action upon JG cells (Shade, Davis, Johnson, Gotshall and Spellan, 1973) which is dependent upon the presence of calcium (Van Dongen and Peart, 1974). Similarly the administration of vasopressin into the renal artery inhibits renin secretion possibly by exerting a direct inhibitory action on the JG cells (Vander, 1968; Shade et al, 1973).

(iii) Biochemistry of Angiotensin

Renin acts on an α_2-globulin of the blood plasma to release a poorly active precursor, the decapeptide angiotensin I, which in man, horse, rat and pig has the following structure (see review Callingham, 1976):

1 2 3 4 5 6 7 8 9 10

H_2N-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-COOH

The converting enzyme cleaves off amino acids 9 and 10 to produce the active octapeptide angiotensin II, which will be referred to as angiotensin in this thesis. The conversion from angiotensin I to angiotensin II takes place mainly in the lung (Ng and Vane, 1967, 1970) and to a lesser extent in peripheral tissues (Bakhle, Reynard and Vane, 1969; Oparil, Sanders and Haber, 1970; Oparil, Tregear, Koerner, Barnes and Haber, 1971). Angiotensin II is catabolised by a group of hydrolysing enzymes referred to as angiotensinases (see review Sander and Huggins, 1972). One initial product of angiotensinase action is angiotensin III formed by cleavage of amino acid 1 from angiotensin II. This heptapeptide is the only fragment of angiotensin II that has significant biologic activity, it is however rapidly hydrolysed by tissue enzymes to inactive products (see Peach, 1977).

The structure of angiotensin was elucidated in 1956 (Skeggs, Lentz, Kahn, Woods and Shumway, 1956) and it was synthesized the following year (Schwarz, Bumpus and Page, 1957; Schwyzer, Iselin, Kappeler, Riniker, Rittel and Zuber, 1957). Since then several derivatives of angiotensin have been synthesized in which some of the amino acids have been substituted

(see review Regoli, Park and Rioux, 1974). The result has been an availability of compounds of variable potency which include specific receptor blockers.

(iv) Physiological and Pathophysiological roles of Angiotensin

Angiotensin II is the principal active component of the renin angiotensin system that is found in peripheral venous and arterial plasma (see review, Peach, 1977). It exerts several physiological and pharmacological actions including a pressor action, contraction of smooth muscle, stimulation of aldosterone release from the adrenal cortex and of catecholamine release from the adrenal medulla, modification of epithelium ion transport etc., (see review Gross, 1971; Regoli et al, 1974; Peach, 1977). Of particular interest to this thesis is the pressor action of angiotensin especially the smooth muscle contractile action.

Angiotensin has long been recognised as a pressor agent in the intact animal which has been revealed to have a potency several times that of adrenaline (see review Whelan, Scroop and Walsh, 1969; Gross, 1971; Peach, 1977). Evidence (see reviews Davis, 1971, 1974; Peach, 1977) suggests that angiotensin may be of physiological significance in the control of aldosterone secretion from the zona glomerulosa of the adrenal cortex, which in

turn leads to sodium retention and water reabsorption from the renal tubule cells. This renin-angiotensin-aldosterone system, operates in homeostasis and disease and is present throughout the vertebrates (see Davis, 1971). The intense vaso-constrictor and pressor properties of angiotensin have played a key role in its historical association with hypertensive disease (Johnson and Davis, 1973). Johnson and Davis (1973) have suggested that the angiotensin aldosterone system may play a role in the maintenance of arterial blood pressure. In acute hypotensive haemorrhage and during a decrease in blood pressure due to sodium deficiency renin release is increased (see review Davis and Freeman, 1976). The renin angiotensin system has been variously manipulated in the induction of experimental renal hypertension by procedures that lead to increased production of angiotensin II, these include 1 - or 2 - kidney clipping, figure of eight, cellophane wrapping and aortic ligature between renal arteries. Salt and the mineralocorticoid DOCA have been administered simultaneously to induce experimental hypertension. The renin-angiotensin system has been implicated in the genesis of human renovascular hypertension although measurement of renin values in peripheral venous blood has yielded inconsistent results (see Salvetti, Arzilli, Poli, Pedrinelli, Sassano and Motolese, 1978). Increased peripheral resistance is a fundamental factor in the genesis of hypertension. Although the renin angiotensin

system has also been implicated in essential hypertension, elevated plasma concentrations of either angiotensin or noradrenaline (NA) have not been identified in patients with early and mild essential hypertension (Axelrod, 1976; Salvetti et al, 1978). There is overwhelming evidence of increased sensitivity and responsiveness of the arterial smooth muscle to NA and angiotensin II both in human hypertension (see review Mendlowitz, 1967) and in rats with experimental hypertension such as renal, renal and saline, DOCA and saline hypertension or with spontaneous hypertension (Somlyo and Somlyo, 1970; McGregor and Smirk, 1968; Collins and Alps, 1975; Battachrya, Dadkar and Dohadwala, 1977; Genest, Boucher, Nowaczynski and Kuchel, 1978). It has recently been suggested by Genest and co-workers (1978) that the arterial changes are related to excessive sodium intake in genetically predisposed individuals or to a hypermineralocorticoid activity which lead to alteration in membrane permeability and muscle reactivity to an otherwise normal level of angiotensin.

(B) The Mechanism of Smooth Muscle Contraction

(i) Calcium and Smooth Muscle Contraction

An increase in the free myoplasmic concentration of calcium is of fundamental importance for the contractile activity in smooth muscle (Edman and Schild, 1962; Hurwitz and Suria, 1971; Ruegg, 1971) as well as that of skeletal and cardiac muscle (Weber and Herz, 1963; Nayler, 1967; Ebashi and Endo, 1968). It has been suggested that (Ebashi and Endo, 1968; Ebashi, 1976; Endo, 1977) in skeletal muscle calcium ions act by binding to the troponin tropomyosin system, which when saturated with calcium ions fails to exert an inhibitory effect on the interaction of myosin and actin. This system has also been demonstrated in smooth muscle (Sparrow, Maxwell, Ruegg and Bohr, 1970; Ruegg, 1971). Muscle relaxation occurs as a result of active calcium uptake from the cytoplasm mainly by the sarcoplasmic reticulum leading to a reduction of free cytoplasmic calcium ion concentration and subsequent detachment of calcium from the troponin-tropomyosin system which then exerts its inhibitory effect on actin and myosin.

The concentration of calcium in the myoplasm is determined by a balance between those processes that tend to deliver free calcium to this environment and those that tend to remove calcium. Thus an agonist could

produce a contraction of smooth muscle by an influx of extracellular calcium by increasing the permeability of the smooth muscle membrane to calcium or by inhibiting the active removal of calcium from the cytoplasm. It is therefore essential to understand the factors which regulate intracellular calcium concentration in order to understand how an agonist may produce an increase in activator calcium concentration.

(ii) Factors controlling Cytoplasmic Calcium Concentration

Where it has been measured, the intracellular free calcium concentration is less than 1 μM while the concentration in the extracellular fluid is in the range 1-10 mM (Baker, 1972; Reuter, 1973). The large inward electrochemical calcium (Ca) gradient due to both the concentration and electrical gradients, thus favours a continuous Ca influx.

The very large driving force for Ca entry indicates that there must exist specific mechanisms which reduce intracellular calcium. Calcium stabilizes the membrane, possibly by cross linking of phospholipid head groups with a resultant increase in transmembrane potential field (Frankenhauser and Hodgkin, 1957). Na ions have been postulated by Goodford (1967) to compete with calcium ions for membrane sites; extracellular Na ions therefore

lower the cell membrane permeability to calcium and this helps to maintain the large calcium gradient (Hurwitz and Suria, 1971; Van Breemen, Farinas, Casteels, Gerba Wuytack and Deth, 1973). There is evidence for an outwardly directed calcium pump located on the membrane (Tomita and Watanabe, 1973; Brading, 1973; Hurwitz, Fitzpatrick, Debbas and Landon, 1973). In addition, a calcium extrusion mechanism has been proposed by various workers (Godfraind, 1973; Blaustein, 1977) which is coupled to influx of sodium down the electrochemical gradient maintained by $Na^+ - K^+$ pump. The corollary is that this exchange mechanism is reversible and may serve to increase influx of calcium (Brading, 1973; Rink, 1975). An extensive review on Na - Ca interaction in mammalian smooth muscle has been recently presented by Van Breemen et al (1979). Cellular calcium is also removed through complexation with cytoplasmic constituents (including the internal membrane surface) or by sequestration into intracellular structures, sarcoplasmic reticulum and mitochondria (see review Rosenberger and Triggle, 1978). An ATP dependent calcium re-uptake has been observed in the sarcoplasmic reticulum (SR) of uterine (Carsten, 1969; Batra and Daniel, 1971; Batra, 1973) and vascular muscle (Devine, Somlyo and Somlyo, 1972; Baudouin-Legros and Meyer, 1973) and of uterine mitochondria (Batra and Daniel, 1973; Batra, 1975).

(iii) Spasmogens and Contraction of Smooth Muscle

The reversal of any mechanism that functions to reduce cytoplasmic calcium concentration is a potential mechanism for spasmogen - induced muscle contraction (see review Bolton, 1979). Excitation-contraction coupling is the sequence of events which must occur so that calcium is supplied to the myofibrillar elements. Since much definitive work on striated muscle contractile mechanisms preceded their elucidation in smooth muscle, the process of excitation-contraction coupling is better understood in skeletal muscle than smooth muscle, it is therefore useful to draw comparisons between the two. In skeletal muscle depolarization of the surface membrane and subsequent initiation of action potential are the initial steps which lead to activation of the contractile proteins. The depolarization is carried along the T-tubules by regenerative propagation of Na^+ (Constantin, 1975) and this signal triggers Ca^{2+} release from the terminal cisternae of the SR. It appears that influx of calcium into the SR and/or depolarization of the SR are the two important processes which cause calcium release from the SR (see review Endo, 1977). The calcium induced release of calcium has been proposed to be physiologically operative in cardiac muscle (see review Endo, 1977), in rectal smooth muscle of mice (Cheng, 1976), rat portal vein (Sigurdsson, Uvelius and Johansson, 1975) and in

turtle aorta (Bozler, 1969). In smooth muscle the T-system is poorly developed and the SR is scanty (see Bohr, 1964; Prosser, 1974) but the membrane contains numerous "impocketings" (caveolae) which serve to increase the surface area of the membrane and are speculated to be important Ca storage sites (Gabella, 1973; Devine et al, 1973). Unlike skeletal muscle where the calcium comes mainly from the SR, there is currently a consensus of opinion that the Ca^{2+} that activates the contractile protein in smooth muscle can originate from two different sources. The first is the Ca present in the extracellular fluid which includes the Ca loosely bound to superficial sites in the muscle fibre, and the second consists of a tightly bound pool of Ca that is sequestered in intracellular sites possibly the SR and mitochondria (Bohr, 1964; Lüllman, 1970; Hurwitz and Suria, 1971; Devine, Somlyo and Somlyo, 1972; Prosser, 1974).

In taenia coli of the guinea pig the action potential (spike) is carried by calcium and the tension in the muscle is related to the frequency of the spike which in turn is dependent on the degree of membrane depolarization (Bülbring, 1955). However contraction of several vascular smooth muscles to neurohormones like noradrenaline and angiotensin II has been observed to be without spikes and sometimes not to be associated with membrane depolarization (Somlyo and Somlyo, 1968a;

Speden, 1970) and to persist in tissues depolarized by high K^+ solution. Evans, Schild and Thesleff (1958) and Edman and Schild (1962) showed that the K^+-depolarized uterus contracted to the neurohormones noradrenaline and acetylcholine. It has been reported that the K^+-depolarized guinea pig taenia coli contracts to carbachol (Durbin and Jenkinson, 1961a) and in both tissues the presence of calcium was always needed. This suggested that in addition to influx of calcium due to membrane depolarization, spasmogens may cause calcium influx in smooth muscle by a process independent of membrane potential.

(iv) Calcium Antagonists

(a) Effect on Ion Currents

As early as 1968, Fleckenstein and colleagues recognised a group of substances able to suppress the contraction of heart muscle without producing significant changes in the action potential. This effect was interpreted as inhibition of calcium activation in the process of electromechanical coupling, and these substances were called "calcium antagonists". The main representatives in this group are verapamil (iproveratril) D600 (methoxy-verapamil) and nifedipine (BAY a 1040). These drugs have achieved prominence in recent years being classified,

largely on the basis of electrophysiological evidence in cardiac preparations, as specific calcium channel antagonists (Fleckenstein, 1971, 1972, 1976, 1977; Fleckenstein Tritthart, Döring and Byon, 1972). In the concentration range 10^{-6} - 10^{-8}M these agents produce a selective antagonism of the slow calcium current (inhibit the plateau phase of the action potential) and thus produce electromechanical decoupling of the heart in the activity sequence nifedipine > D600 > verapamil (Fleckenstein, 1972, 1976; Kohlhardt et al, 1972, 1973a, b; Tritthart, Volkmann Weiss and Fleckenstein, 1973). The effects of these agents are overcome by increased extracellular calcium. In addition to affecting calcium fluxes in cardiac muscle verapamil and D600 inhibit inward Ca^{2+} currents in squid axon (Baker, Meves and Ridgway, 1973) and in uterine muscle (Fleckenstein, Grün, Tritthart and Byon, 1971). At the fairly high concentration, verapamil and D600 do reduce the fast Na^{+} current in the squid axon and have some effect on both the Na^{+} and K^{+} currents in cardiac fibres (Cranefield, Aronson and Wit, 1974; Kass and Tsien, 1975). Verapamil blocks impulse conduction in desheathed frog sciatic nerve (Singh and Vaughan-Williams, 1972) and in skeletal muscle (Van der Kloot and Kita, 1975) without affecting the resting membrane potential. In this respect at high concentrations SKF525A (Suarez-Kurtz and Bianchi, 1970) and the calcium antagonists resemble procaine (Inoue and Frank, 1962) a tertiary amine local

anaesthetic-like compound whose membrane stabilisation involves blockade of Na^+ permeability (Shanes, 1958; Skou, 1961; Inoue and Frank, 1962).

(b) Calcium Antagonists and Smooth Muscle

Since the present study was confined to the use of SKF525A and the calcium antagonist verapamil as organic inhibitors of calcium movement in excitation-contraction in smooth muscle, a summary of the current knowledge of the action of these agents in smooth muscle will be presented.

The calcium antagonists are also effective inhibitors of a variety of smooth muscles (Peiper, Griebel and Wende, 1971; Fleckenstein et al, 1971; Haeusler, 1972; Massingham, 1973; Bilek, Laven, Peiper and Regnat, 1974) where they suppress not only contraction, but also the spike discharges (Golenhofen and Lammel, 1972). It has usually been assumed that their mechanism of action in smooth muscle was similar to that of heart muscle by blocking the slow calcium channels (Kohlhardt et al, 1972). This has led to the use of these agents in the study of excitation-contraction in smooth muscles. The calcium antagonistic effect of verapamil, D600 and prenylamine in smooth muscle was first reported by Grün et al (1969) and was thought to be responsible for the relaxation

produced by the drugs. Using the sucrose gap method, Fleckenstein et al (1971) showed that verapamil, D600 and prenylamine suppressed KCl , acetylcholine , carbaminocholine and oxytocin-induced contractions in uterine smooth muscle without affecting the accompanying depolarization. As in cardiac muscle, the activity of verapamil and D600 were inversely correlated with the concentration of extracellular calcium. Haeusler (1972) has found a competitive antagonism by verapamil of contractions of K^+-depolarized strips of rabbit main pulmonary artery contracted by addition of calcium ions. Although verapamil antagonized noradrenaline induced contractions of the pulmonary artery, Haeusler (1972) found that the antagonism was non-competitive. In this tissue the verapamil had no effect on the spike free depolarization of 6.3mV caused by the addition of 10^{-6}M noradrenaline. Voltage clamp studies have shown that D600 blocks the inward calcium current in rat uterine smooth muscle (Anderson, Ramon and Snyder, 1971; Mironneau, 1973).

(c) Potassium-induced Contractions and Calcium Antagonists

When rabbit aortic strips (Hudgins and Weiss, 1968; Van Breemen, 1969) and rat ventral artery (Hinke, Wilson

and Burnham, 1964; Hinke, 1965) are exposed to calcium free solution the contractile responses to potassium decline more rapidly than responses to noradrenaline suggesting that responses to potassium ions are dependent on extracellular calcium ions. Similarly in the rat uterus contractile responses to potassium decline more rapidly than responses to acetylcholine when the tissue is exposed to calcium free solution (Edman and Schild, 1962). Consequently the effect of calcium antagonists has been widely studied on the contractile responses of vascular smooth muscle to potassium ions as a means of investigating excitation-contraction mechanisms.

SKF525A blocks K^+-induced contractions of the rabbit aortic strips while exerting little effect on the contractile responses to noradrenaline or angiotensin II(Kalsner, Nickerson and Boyd, 1970). However, in these experiments, the responses to 5-hydroxytryptamine in the presence of SKF525A declined as rapidly as the responses to potassium. With rat aorta, SKF525A (Massingham, 1973) did not display selective abolition of the responses to potassium compared to the contractile responses induced by NA or Ba^{2+}. Verapamil on the other hand has been demonstrated to cause selective blockade of the contractile responses to potassium in the rat isolated aorta (Peiper et al, 1971; Massingham, 1973; Bilek et al, 1974) and rabbit pulmonary artery (Haeusler, 1972) with little

effect upon responses to noradrenaline. These differential inhibitory effects of verapamil and SKF525A have been explained by their ability to reduce membrane permeability to calcium (Mayer, Van Breemen and Casteels, 1972; Deth and Van Breemen, 1974) although no ^{45}Ca flux measurements were carried out. Verapamil abolishes spontaneous mechanical activity in guinea pig portal vein, abolishes responses to potassium and reduces responses to noradrenaline to 40% of control (Golenhofen, Hermestein and Lammel, 1973). However Bilek et al (1974) found that verapamil reduced contractions of the rat portal vein induced by noradrenaline as well as that to potassium. These observations are in agreement with those of Voth and co-workers (1971) who observed a non-selective abolition by verapamil of the contractile responses to angiotensin, noradrenaline, acetylcholine and calcium ions. This has been explained by the assumption that the noradrenaline - induced intracellular increase of calcium ion concentration in portal vein was mainly caused by depolarization and a concomitant increase in transmembrane calcium influx.

Whatever the mechanism involved in the selective inhibition of responses of vascular muscle to potassium, evidence points to the possibility that muscle contraction dependent on membrane depolarization is susceptible to the action of calcium antagonists. Thus pulmonary vasoconstrictive responses to alveolar hypoxia have been

found to be more susceptible to inhibition by verapamil and SKF525A than responses to angiotensin and $PGF_{2\alpha}$ (McMurtry, Davidson, Reeves and Grover, 1976). This is consistent with the idea that hypoxia acts directly (Lloyd, 1970; Detar and Gellai, 1971) to depolarize smooth muscle cell membrane (Bergofsky and Holtzman, 1967) and initiate transmembrane calcium influx (Bohr, 1973). Similarly contractile responses of the cat papillary muscles to electrical stimulation are readily antagonised by verapamil and D600 (Bayer, Hennekes, Kaufmann and Mannhold, 1975a). Moreover, the effectiveness of inhibiting the extracellular calcium dependent contractile responses to the guinea pig ileum to either ionophore A23187 or K^+ by D600 (see Rosenberger and Triggle, 1978) provides further evidence of calcium flux inhibition.

(d) Calcium Antagonists and Calcium Mobilisation in the Aortic Muscle

To appreciate the significance of the many reports of selective blockade of responses to K^+ compared to noradrenaline (NA) by calcium antagonists in aortic preparations it is essential to review the current knowledge of calcium mobilisation in this tissue.

In 1963, Bohr showed that there were two components to the noradrenaline induced contraction of the rabbit

aorta, an initial fast component which was suppressed by increasing extracellular Ca^{2+} and a slow component which was stimulated by the same procedure. Van Breemen (1969), Godfraind and Kaba (1972) and Sitrin and Bohr (1971) subsequently showed that the slow phase is dependent upon access of smooth muscle cells to the extracellular Ca^{2+} whereas the initial fast response persisted in calcium free solutions. Direct evidence that intracellular calcium release is involved in the response to NA was demonstrated by Van Breemen et al (1970, 1972, 1973). These workers used La^{3+} as a calcium flux inhibitor and found that K^{+} depolarizations caused a large increase in cellular calcium influx over control values but that no such increase was demonstrated during NA stimulation. However, in a later study Deth and Van Breemen (1974) found that like NA, responses to angiotensin and histamine showed an initial fast response followed by a slow tonic response. In tissues previously exposed to calcium free solutions, they showed that the delayed NA response was accompanied by cellular ^{45}Ca uptake. This slow phase was abolished by La^{3+} or SKF525A. There was an important difference between La^{3+} and SKF525A in that La^{3+} prevented all but the first release contractions. Since it was possible to obtain a number of consecutive contractions in the presence of SKF525A it was apparent that SKF525A did not prevent the replenishment of the calcium release store which is possibly

supplied from the caveolae (Gabella, 1971; Prosser, 1974). It was therefore assumed that La^{3+} displacement of Ca^{2+} from the surface membrane prevents replenishment of the "Ca^{2+} release store". It was further observed that SKF525A and La^{3+} at concentrations which abolished the second slow response, La^{3+} caused a greater reduction in ^{45}Ca influx than SKF525A. These observations pointed to the possibility of calcium influx mobilised by NA but not by K^{+} that was resistant to SKF525A.

(e) Selective Inhibition of Calcium Influx by Calcium Antagonists

Both contractions of the rabbit aorta to K^{+} and the convulsant barbiturate 5-(2-cyclohexylidene ethyl)-5-ethylbarbituric acid (CHEB) were reduced when the tissue was exposed to calcium free solution or when the tissue was treated with lanthanum (Edney and Downes, 1976). This indicated that the contractile effects to both agonists were dependent upon extracellular calcium. However, in the presence of verapamil (10^{-4}M), CHEB contracted the tissue whereas the responses to potassium were abolished. This suggested that CHEB induced calcium influx was resistant to verapamil. The observations were in agreement with the postulate of Golenhofen and Hermstein (1975) of the existence of a verapamil resistant system for transmembrane calcium fluxes which would account

for the persistence of the late tonic component of the NA response in aortic smooth muscle. ^{45}Ca flux measurements in the guinea pig fundus muscle (Lammel, 1977) have indicated that acetylcholine, like K^+ contracture, was connected with increased transmembrane calcium influx. However nifedipine (10^{-5}M) selectively inhibited the ^{45}Ca and contractile response to potassium without much effect on the contractile response and ^{45}Ca influx to acetylcholine. Since the contractile response of this muscle was dependent upon external calcium (Boev, Golenhofen and Lukanow, 1976) Lammel's results confirmed the existence of a calcium antagonist-resistant calcium influx.

(f) Theory of P and T Systems

Simultaneous measurements of membrane potential and tension have shown that NA induced contractions of guinea pig portal veins was accompanied by membrane depolarization and increased spike frequency whereas the contraction of the rat aorta was accompanied by spike free membrane depdarization (Golenhofen, 1976). Addition of verapamil to guinea pig portal vein contracted by NA resulted in complete suppression of spike discharges and phasic contractions though NA could still induce a contraction which was reduced to 40 to 50% of control. Such "spike-free" activation was also elicited in stomach smooth muscle by acetylcholine (Golenhofen and Wegner, 1975). Since this spike-free activation was

as sensitive to calcium deprivation and to application of lanthanum as was the normal activation, this indicated that in addition to "verapamil sensitive activation mechanism" another calcium activation mechanism exists in smooth muscle, which is similarly dependent on trans-membrane calcium fluxes (Golenhofen and Wegner, 1975; Golenhofen and Hermstein, 1975). Further evidence of the dual nature of calcium activation in smooth muscle has been provided by the observation that the verapamil and D600 resistant tonic activation of stomach fundus (Boev, Golenhofen and Lukanow, 1976) and rat aorta (Golenhofen, 1976) could be selectively blocked by sodium nitroprusside. On the basis of this evidence Golenhofen (1976) has classified calcium activation mechanisms in smooth muscle into P and T mechanisms (Phasic and Tonic) predominantly antagonised by verapamil-like drugs and nitroprusside respectively. Golenhofen's classification theory shows great potential although there are reservations in its applicability since nitro-prusside is without effect on the D600 resistant tonic contractions of tissues like vas deferens and rat uterus (see Kreye and Gross, 1977). Moreover, the distribution of the two activation mechanisms in different smooth muscles is not yet clearly established although current evidence indicates that they may occur together or singly. (Golenhofen, 1976; Golenhofen and Wegner, 1975). The distinction of the two systems would also depend on the

calcium antagonist used since the selectivity of blockade of the P system has been suggested as Nifedipine> D600 > verapamil (Golenhofen, Wagner and Weston, 1977). SKF525A is even less specific than verapamil (Massingham, 1973; Golenhofen and Hermstein, 1975) and in very high concentration the calcium antagonists would also block the tonic component (Golenhofen et al, 1977).

(g) Intracellular action of Calcium Antagonists

It has been observed that in cat papillary muscles verapamil and D600, unlike calcium depletion, produce a frequency dependent inhibition of contractions, with the greatest effect at higher stimulation frequencies. This inhibition of the staircase phenomena has pointed to the possibility that the calcium antagonists may inhibit intracellular calcium release (Bayer et al, 1975a, b). It is unlikely that these agents directly affect the contractile elements since in coronary smooth muscles skinned by either glycerin-water or according to the Winegrad's technique (1971) the calcium antagonistic compounds and nitrites lack inhibitory effect when the contractile system was activated directly by the addition of ATP and Calcium ions (Weder and Grün, 1973; Fleckenstein, Nakaya, Fleckenstein-Grun and Byon, 1975).

In addition to its effect on transmembrane Ca^{2+} currents verapamil (4.4×10^{-5}M and 1.1×10^{-4}M) reduces Ca^{2+} binding to the plasma membrane of the dog trabecular muscle without effect on Ca^{2+} uptake by isolated microsomes (Nayler and Szeto, 1972). In higher concentrations verapamil and D600 (10^{-3}M) causes a 50% reduction of Ca^{2+} binding and active reuptake by fragments of sarcoplasmic reticulum (SR) from dog cardiac muscle (Entman, Allen, Bornet, Gillette, Wallick and Schwart, 1972; Watanabe and Besch, 1974) and also reduces Ca^{2+} binding by SR from rabbit skeletal muscle (Bazler, 1972). Similarly in subcellular fractions of the rat myometrium verapamil and D600 inhibit active ^{45}Ca uptake only in concentrations well above those that cause smooth muscle relaxation (Crankshaw, Janis and Daniel, 1977). Blockade of Ca^{2+} uptake by SR would be expected to cause contraction rather than relaxation. At high concentration, verapamil causes relaxation of K^{+}-induced contracture of the frog sartorius muscle (Bondi, 1976) while in the absence of an agonist it causes a contracture by mobilising intracellular calcium. There have not been any reports in the literature (see Crankshaw et al, 1977) of smooth muscle contractures of tissues treated with verapamil, it is therefore unlikely that inhibition of intracellular Ca uptake in SR plays any significant role in the action of calcium antagonists in smooth muscle.

(h) Nitroprusside and Smooth Muscle

Nitroprusside has been reported to have a relaxant effect on various smooth muscle preparations (Thienes, 1926; Johnson, 1929) which is more pronounced in the smooth muscle of the tonic type such as vascular and tracheal smooth muscle, than in the smooth muscle of the phasic type from splanchnic regions (Kreye, Baron, Lüth and Schmidt-Gayk, 1975). Unlike verapamil, nitroprusside preferentially relaxes noradrenaline induced contractions of the rat aorta and rabbit isolated pulmonary artery but is less effective when the tone is induced by depolarizing concentrations of potassium (Kreye et al, 1975; Kreye and Lüth, 1976). The finding of an "antagonism" between calcium and nitroprusside in contraction studies with isolated preparations of vascular smooth muscle has suggested that nitroprusside might interfere directly with the handling of calcium ions by the cell (Kreye et al, 1975; Haeusler and Thorens, 1976). Conflicting results have been reported on the question whether nitroprusside, like verapamil, inhibits calcium influx into the cells of vascular smooth muscle. Kreye et al (1975) found that nitroprusside inhibited the ^{45}Ca influx induced by potassium depolarization but Haeusler and Thorens (1976) failed to observe an effect on calcium uptake by K^+ depolarized preparations, whereas the ^{45}Ca uptake to NA was reduced. On the other hand, in the presence of

nitroprusside an augmented calcium uptake by K^+-depolarized rabbit aortic strips has been reported by Van Breemen (1975). Other possible mechanisms of smooth muscle relaxant effects of nitroprusside have been reviewed by Kreye and Gross (1977). This has excluded mediation by cyclic AMP or direct effect on the contractile elements although the possibility remains that nitroprusside may cause relaxation by inhibiting intracellular calcium mobilisation or through a de novo release of cyanide (Kreye and Lüth, 1976; Kreye et al, 1975).

(v) The Ionic Basis of Electrical Activity in Smooth Muscle

(a) Ionic Basis of Membrane Potential

The most widely accepted theory proposed to explain the potential difference across the cell membrane originated from the work of Bernstein (1912). Applying the physicochemical concepts of Nernst and Ostwald Bernstein put forward that the resting cell membrane was selectively permeable to potassium and that this selectivity was lost during excitation, resulting in an indiscriminate penetration by other small ions such as sodium and chloride. While upholding the fundamental idea of a membrane with selective permeability for ions, it has been observed that the membrane is not only permeable to potassium ions but also to chloride ions

(Boyle and Conway, 1941) and that there is also a slight permeability to sodium ions (Levi and Ussing, 1948; Keynes, 1954). This means that external sodium ions diffuse into the cells under the combined influence of concentration gradient and the membrane potential. To maintain a steady state of ion distribution there is an active mechanism that extrudes Na ions in exchange for K ions continuously across the membrane which has been identified in giant squid axon (Hodgkin and Keynes, 1955) other nerve and skeletal fibres (see Caldwell, 1968) and in smooth muscle cells (Casteels, 1966; Casteels, Droogmans and Hendrickx, 1973; Widdicombe, 1975). The membrane potential results from the ionic gradients and the relative conductances for sodium, potassium and chloride. Assuming that the voltage gradient through the membrane is constant and that ions only move under the influence of diffusion and electric field, Goldman (1943) and later Hodgkin and Katz (1949) have obtained the following expression for the membrane potential:

$$E = -\frac{RT}{F} \ln \frac{Pk\,[K]_i + PNa\,[Na]_i + PCl\,[Cl]_o}{Pk\,[K]_o + PNa\,[Na]_o + PCl\,[Cl]_i}$$

Where R is the gas constant, T the absolute temperature, F Faraday's constant:

$(K)_i$, $(Na)_i$ and $(Cl)_i$ are activities of the ions inside the cells, and where the same symbols with the subscript o are activities outside the cells, Pk, PNa

and PCl are permeability constants of the membrane (Cm/Sec) for the individual ions.

In smooth muscle cells, the distribution of chloride ions is not passive (Casteels and Kuriyama, 1966) and hence the voltage profile must be determined in order to make an application of the Goldman equation possible. Modification of external solutions is an important tool in the study of membrane permeabilities, since it is possible to eliminate an ion species in the Goldman equation. However, modification of the concentration of one ion in the extracellular solution can also change the permeability of the membrane to other ions (see Casteels, 1970).

(b) Action Potentials in Smooth Muscle Cells

According to the ionic hypothesis the action potential is due to a specific increase in conductance for Na ions which then move down their electrochemical gradient so carrying the membrane potential to a value near the Na equilibrium potential (ENa). This is followed by a delayed increase in conductance for K ions which returns the membrane potential to its resting level (Hodgkin, 1958; Shanes, 1958). In the rat uterus

substitution of sucrose for sodium chloride causes a decrease of action potential as expected from the ionic theory (Goto and Woodbury, 1958). More recent investigations (see Kuriyama, Ito and Suzuki, 1977) have led to the suggestions that the action potential in this tissue and in cat (Kobayashi and Irisawa, 1964; Kobayashi, 1965) and guinea pig ureter(Kuriyama and Tomita, 1970) may involve both Na and Ca influx. However, spontaneous action potentials in several gastro-intestinal smooth muscles and rabbit uterus (see review Burnstock, Holman and Prosser, 1963) have been found to persist during removal of external sodium when calcium was left unaltered. This has suggested that calcium may carry the inward current during the rising phase of the action potential as has been observed in barnacle muscle in which the spike is due to calcium influx (Hagiwara and Takahashi, 1967). The spikes in guinea pig taenia coli are abolished when the tissue is exposed to calcium free solution (Brading, Bulbring and Tomita, 1969) and tetrodotoxin lacks effect on the spikes (Kao, 1966; Kuriyama, Osa and Toida, 1966) although manganese ions readily abolish them (Nonomura, Hotta and Ohashi, 1966). In addition the calcium antagonists verapamil and D600 abolish the spikes in the rat myometrium (Fleckeinstein et al, 1971), guinea pig taenia coli (Golenhofen and Lammel, 1972) and portal vein (Golenhofen, 1976). These observations favour the hypothesis that the spikes in smooth muscle

are mainly due to calcium entry. In addition to carrying the current, calcium also stabilizes the membrane in squid axon (Frankenhauser and Hodgkin, 1957) in which the spike is due to Na influx and in barnacle muscle in which the spike is due to calcium influx (Hagiwara and Takahashi,1967).

Bülbring (1955), using intracellular recording has found a close correlation between development of tension and spike activity of smooth muscle of taenia coli. The frequency of spike discharge was inversely related to the transmembrane potential within a limited range. Numerous investigations have since confirmed and extended the observations of Bülbring, both for taenia coli and other types of smooth muscle (See Bülbring, Brading, Jones and Tomita, 1970).

(c) Electrical Recording in Smooth Muscle: The Sucrose-gap Technique

The methods for electrical recording in smooth muscle have been reviewed by Burnstock et al (1963). These include:

(a) intracellular electrodes

(b) extracellular methods with wick electrodes

(c) pressure electrodes and

(d) sucrose-gap technique.

In this thesis the sucrose gap technique was used to record electrical activity in smooth muscle, an account of this method will therefore be presented.

The Sucrose-Gap Technique

Many difficulties may be encountered when intracellular microelectrodes are used to study the membrane activity of smooth muscle cells. Owing to the small size and inherent mobility of these cells, the electrodes tend to dislodge which makes continuous measurements difficult (Bülbring, 1955). Stämpfli (1954) has described a relatively simple method for measuring the full value of the resting membrane potential in bundles of nerve fibres with external electrodes. His method is based on the theoretical calculation that the full value of the membrane potential can be measured with external electrodes on a core conductor, when the short circuiting is negligible (Hodgkin and Rushton, 1946). Stämpfli obtained this condition by increasing the outside resistance of the preparation in the interpolar region by replacing most of the ions in the interstitial fluid with a nearly ion-free sucrose solution. Thus in his experiment Stampfli introduced a bundle of myelinated nerve fibres of a frog into a horizontal hole of slightly greater diameter through which an isotonic sucrose solution, of at least $2 \times 10^6 \Omega$ cm specific resistance,

flowed at constant rate. The inflow of sucrose was made in the middle of the hole and the outflowing sucrose was spilled away by Ringer or test solutions flowing through vertical channels at both ends of the horizontal hole (see Fig. 1). The nerve fibres were bent upwards into the two vertical channels, thus being in contact with the inflowing solutions. Since the resistance of the liquid in the vertical channels was very small each of them could be considered as equipotent. As there was a sharp increase in longitudinal resistance at the entry of the horizontal hole, the potential difference between chlorided silver electrodes at the lower ends of the vertical channels, with Ringers solution on one side and an isotonic potassium chloride solution on the other was therefore equal to the resting potential of the fibres of 70mV. Later this method was applied to bundles of myelinated nerve fibres (Stämpfli and Straub, 1954) and to non-medulated fibres (Ritchie and Straub, 1956) where action potentials were also recorded. In 1958, Burnstock and Straub successfully used the technique to measure continuously resting membrane potential and spike activity in the guinea pig taenia coli smooth muscle. The method has since been adapted to measure simultaneously membrane potential and tension changes in guinea pig taenia coli and in other smooth muscles (Bülbring and Tomita, 1969b; Shimo and Holland, 1966; Marshall and Csapo, 1961; Somlyo and Somlyo, 1968a).

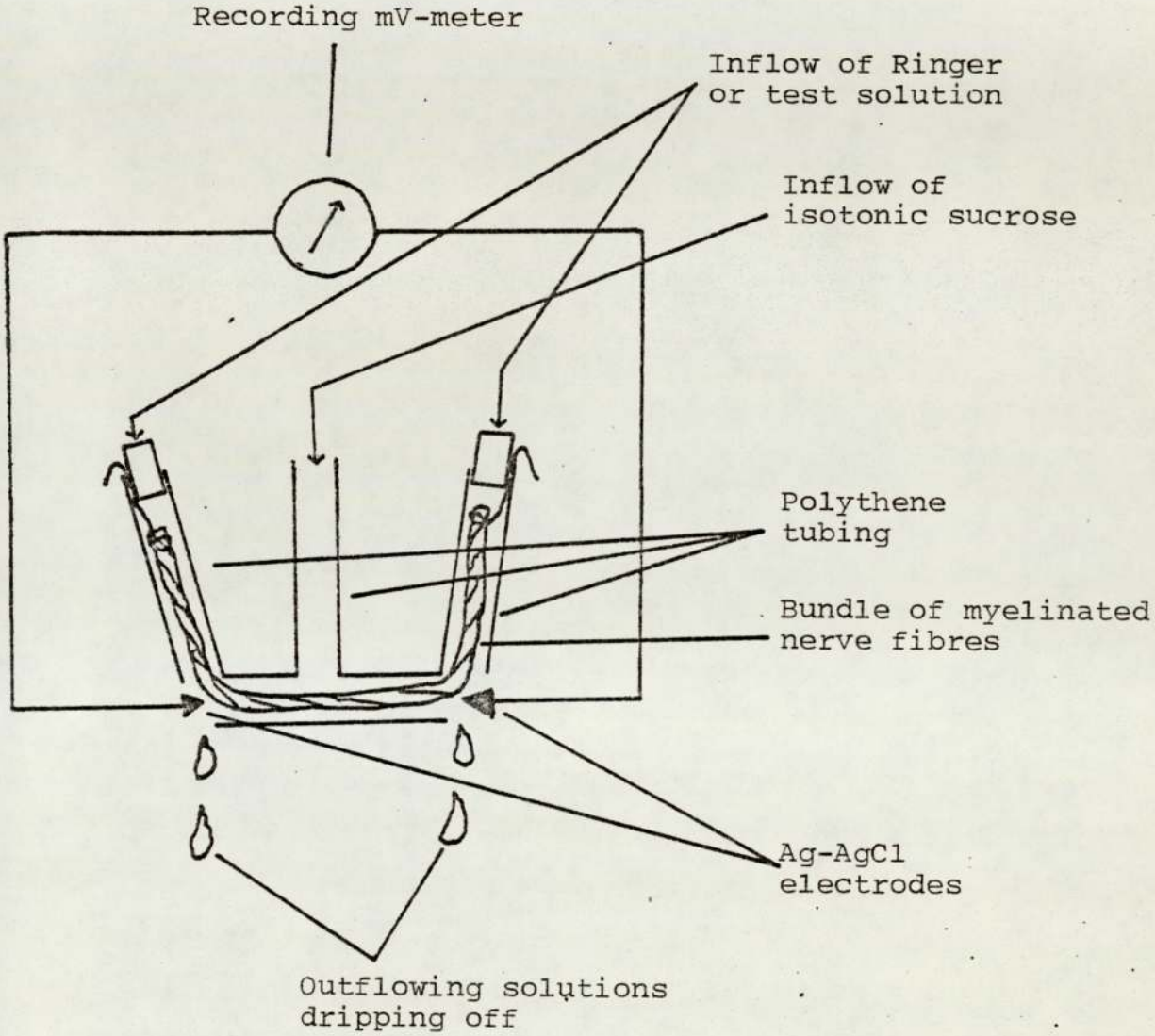


Fig. 1

Arrangement of polythene tubes for recording membrane potentials (Simplified after Stämpfli, 1954).

The fact that the sucrose gap technique has been successfully used to record from smooth muscle with external electrodes several millimetres apart when the muscle between the electrodes is bathed in a high resistance sucrose solution suggests that the muscle cells which are 50-100 μ long for taenia coli (see Burnstock and Straub, 1958) and 70-100μ long for uterine muscle (see Marshall and Csapo, 1961) are connected by some internal low resistance path. The precise manner in which smooth muscle cells are connected is unknown, although suggestions of low impedance pathways (Prosser and Sperelakis, 1956), intercellular bridges (see Prosser, Burnstock and Kahn, 1960) and even direct protoplasmic continuity (Thaemert, 1959), have been made. The suggestion that smooth muscle cells are linked electrically has been further supported by Bozler (1948) who observed propagation of action potentials which could not be explained by a nervous mechanism. This hypothesis has also been confirmed on nerve free preparations by Evans and Schild (1953 and 1956) and furthermore Burnstock and Prosser (1960b) found from their electrophysiological and microscopical measurements in different smooth muscles that electrical conduction velocity in smooth muscles decreases with the gap size between the individual cells.

A second consideration is that of junction potentials which occur at interfaces between solutions

composed of ions differing in mobility or concentration (Blaustein and Goldman, 1966), this may give rise to artefacts that may complicate the interpretation of results obtained with this technique when measuring changes in resting potential produced by addition of different ion concentrations. Attempts have been made to avoid artefacts which can arise from tissue movements and from liquid junctional potentials (l.j.p.) by the introduction of rubber membranes at the junctions between the sucrose solution and the potassium sulphate or potassium chloride solution and the test solution in the sucrose gap apparatus. Rubber membranes were first introduced by Berger (1963) who regulated the diameter of the membrane by controlled stretch. In a later modification (Berger and Barr, 1969) the stretch of the rubber membranes was adjusted by a low pressure system. However, in 1971, Boev used a closed chamber with non-adjustable rubber membranes. In the present study a modification of the sucrose gap apparatus of Boev and Golenhofen (1974) has been used which consists of a closed chamber with rubber membranes where the size of the hole can be adjusted by concentric pressure for simultaneous measurement of electrical and mechanical activity in smooth muscle. This apparatus is described in detail in the methods section. The magnitude of the potential difference recorded between electrodes will depend, among other things, on the completeness of

replacement of interstitial fluid with sucrose and on the amount of cell damage within the muscle strip. Incomplete washout with sucrose, i.e. a high external circuiting factor, and an excessive amount of fibre damage will tend to lower the measured values (Marshall and Csapo, 1961). For these reasons it is best to consider the measured potential difference not as an absolute value for the membrane potential, but only as a relative potential change under various experimental conditions.

(vi) Cyclic AMP and Smooth Muscle Contraction

It is generally accepted that cyclic AMP may act as a second messenger to mediate the events following a drug-receptor interaction (Sutherland, Robison and Butcher, 1968; Robison, Butcher and Sutherland, 1971; Sutherland and Robison, 1966). The role of cyclic AMP in smooth muscle contraction has been the subject of numerous reviews (Bar, 1974; Andersson, 1972; Rasmussen and Goodman, 1977). The smooth muscle relaxant effects of β-adrenoceptor stimulation by catecholamines of intestinal (Bueding et al; 1966), uterine (Marshall and Kroeger, 1973; Triner et al, 1971) tracheal (Murad, 1973) and vascular smooth muscles (Triner et al, 1971) as well as of drugs that inhibit phosphodiesterase such as papaverine and theophylline (Triner, Vulliemoz, Schwatz and Nahas, 1970; Poch and Kukovetz, 1971; Andersson, 1972) is mediated by an increase in cyclic AMP. The smooth muscle relaxant effect of cyclic AMP is related to its ability to reduce the free myoplasmic Ca concentration (Andersson et al, 1975). The nucleotide increases calcium uptake by isolated smooth muscle microsomes (Andersson and Nilsson, 1972; Angles d'Auriac and Meyer, 1972; Carsten, 1969) and causes cellular calcium efflux (Marshall and Kroeger, 1973) possibly by activation of protein kinase enzymes (see Andersson et al, 1975; Sands et al, 1973). Membrane hyperpolarization

also contributes in the relaxant effect of cyclic AMP (Takagi, Takayagi and Tomiyama, 1971; Ohkawa, 1976). However, cyclic AMP does not directly affect the contractile machinery (Takagi et al, 1971). Since an increase in cyclic AMP is associated with smooth muscle relaxation, a decrease in intracellular cyclic AMP has therefore been proposed to mediate smooth muscle contraction (see Andersson, 1972). However, direct measurements of cyclic AMP concentration during agonist induced contraction has yielded conflicting results varying not only with the agonist and type of muscle but also with the phase of the contraction (see Andersson, 1972; Rasmussen and Goodman, 1977). In some smooth muscles the intracellular level of cyclic AMP has been found to be reciprocally related to the intracellular content of cyclic GMP a nucleotide that causes release of Ca^{2+} from isolated microsomes and it has been suggested that this nucleotide may mediate contraction of smooth muscle (Lee, Kuo and Greengard, 1972). This has led to the theory of biological regulation through opposing influences of cyclic GMP and cyclic AMP; "The Yin Yang Hypothesis" (Goldberg, Haddox, Nicol, Glass, Sanford, Kuehl and Estensen, 1975).

(C) Angiotensin and Smooth Muscle

(i) Angiotensin and Isolated Smooth Muscle Preparations

Angiotensin contracts a variety of isolated smooth muscles. Isolated guinea pig ileum is sensitive to the myotropic action of angiotensin. It responds to angiotensin in a linear dose response relationship in the range of 1 to 10 nmol (Picarelli, Kupper, Prado, Prado and Valle, 1954; Bisset and Lewis, 1962; Regoli and Vane, 1964). Khairallah and Page (1961) have shown that morphine and atropine antagonized the responses of the guinea pig ileum to angiotensin. They concluded that angiotensin acts partly in an indirect manner by release of acetylcholine from parasympathetic nerve endings. Using isometric recording the guinea pig ileum response to angiotensin could be dissociated into two components (Godfraind, Kaba and Polster, 1966a, b) an initial fast component which was attributed to an indirect action (mediated by acetylcholine) followed by a slow component that was attributed to the direct action of angiotensin. However, more recently Paiva et al (1976b) have provided evidence against cholinergic mediation of the effect of angiotensin II in the guinea pig ileum. At concentrations of atropine used by Khairallah and Page (1961) Paiva and colleagues could not block the initial fast response to angiotensin although it disappeared during tachyphylaxis to angiotensin. At

that concentration atropine had no effect on the maximal response to angiotensin and caused a non-specific shift to the right of the dose response curves to angiotensin, histamine, bradykinin and $BaCl_2$. Nicotine, hemicholiniums, or destruction of intramural ganglia by incubation at $4^{o}C$ for 48 - 56 hours was without effect on the maximal responses to angiotensin and did not affect the phasic component.

The uterus from an oestrous rat or one pretreated with oestrogen or diethyl stilboestrol, is another tissue that has been used for the bioassay of angiotensin. There is a linear dose response relationship in the concentration range of 0.1 to 1.0 nMol and the contractions are regular (Paiva and Paiva, 1960; Gross and Turrian, 1960; Bisset and Lewis, 1962). Attempts to identify an acetylcholine mediated affect of angiotensin on the rat uterus (Khairallah and Page, 1963), rat stomach strip and rabbit aortic strip (Rioux, Park and Regoli, 1973) have not been successful.

The rat colon, both ascending and descending, is extremely sensitive to angiotensin and has been used for the assay of this polypeptide (Regoli and Vane, 1964). It responds to angiotensin as low as 10^{-10} g/ml. Regoli and Vane (1964) have demonstrated that the contractile responses are unaffected by antagonists hyoscine, mepyramine,

hexamethenium, morphine, phenoxybenzamine and reserpine in their specific concentration range. It was concluded that angiotensin exerts its myotropic effect directly on the smooth muscle. The relative potencies of twelve analogues of angiotensin tested corresponded well with their relative potencies on the nephrectomized rat blood pressure and this was taken as evidence that in the rat, the receptors for angiotensin in the colon are similar to the pressor receptors in the same animal. In order to improve assay specificity for angiotensin, in addition to the humoral blockers used by Regoli and Vane (1964), Gagnon and Sirois (1972) suggested the use of the prostaglandin blocker polyphloretin phosphate (PPP) (Eakins, Karim and Miller, 1970; Eakins, Miller and Karim, 1971; Bennett and Posner, 1971) and a further characterization of the contractile responses by the specific inhibitor of angiotensin 8-L-ala-angiotensin II (Gagnon, Park and Regoli, 1971; Turker, Yamamoto, Khairallah and Bumpus, 1971).

Guinea pig taenia coli is contracted by angiotensin and its action is not mediated by parasympathetic nerve elements (Ohashi, Nonomura and Ohga, 1967).

Vascular smooth muscle, both arteries and veins, are generally contracted by angiotensin (see reviews Somlyo and Somlyo, 1966; Gross, 1971; Regoli, Park and

Rioux, 1974; Callingham, 1976). There have been reports that noradrenaline release from sympathetic nerve endings may serve to mediate angiotensin contractile responses, but most evidence suggests that the contraction of isolated vascular smooth muscle elicited by angiotensin does not depend to any significant degree to the ability of the agent to release noradrenaline from the sympathetic nerve endings (see Regoli et al, 1974; Bohr, 1974; Callingham, 1976). The nature of the contractile response to angiotensin varies with the type of smooth muscle, for instance, in the spirally cut rabbit aortae (Furchgott and Bhadrakom, 1953) angiotensin produces a slowly developing contraction, which is followed by a slow relaxation. The onset of action is delayed 2 to 4 minutes and maximum contraction may not be reached until after 10 to 20 minutes (Bohr and Uchida, 1967) unlike rat portal vein where the muscle exhibits spontaneous activity and the response reaches a maximum in less than 5 minutes (Carruba, Mandelli and Mantegazza, 1973).

The repetitive addition of angiotensin on isolated tissues results in a decline of subsequent responses called tachyphylaxis. This occurs in most isolated smooth muscle preparations with the exception of rabbit and guinea pig aorta (Khairallah, Page, Bumpus and Turker, 1966); rat portal vein (Carruba, Mandelli and Mantegazza,

1973) and rat stomach strip (Rioux, Park and Regoli, 1973). Several investigators (Khairallah et al, 1966; Bohr and Uchida, 1967; Walter and Bassenge, 1969) have shown that in vascular muscle tachyplaxis to angiotensin was diminished by exposure of the tissue to a solution containing angiotensinase activity or with Dowex-50 which adsorbs angiotensin. It has therefore been suggested that tachyphylaxis occurs as a result of angiotensin molecules occupying the receptor sites and thereby prevent their activation by other angiotensin molecules to which the tissue is subsequently exposed. Both guinea pig ileum and rat uterus show tachyphylaxis to angiotensin especially when exposed to high concentrations at frequent intervals (Godfraind, Kaba and Polster, 1966a; Freer, 1975a, b). This tachyphylaxis is reduced by an increase in the time between successive additions of angiotensin. In both rat uterus and guinea pig ileum, the polarized tissues are more predisposed to tachyphylaxis in conditions of low pH (< 6.8) and low calcium in the bathing medium (< 0.18 mM) (Paiva, Juliano, Nouailhetas and Paiva, 1974; Freer, 1975a, b) conditions which have been suggested to interfere with the drug receptor interaction. The possible release of vasodilator prostaglandins has been implicated in the tachyphylaxis to angiotensin in the isolated coeliac and mesenteric arteries of the rabbit since tachyphylaxis to angiotensin in this tissue was partially reversed by prostaglandin synthesis

inhibitors indomethacin, aspirin and meclofenamic acid (Aiken, 1974). In contrast to tachyphylaxis, potentiation of responses to low concentrations of angiotensin has been observed in longitudinal muscle of guinea pig ileum at a time when the response to high doses of angiotensin were depressed (Godfraind, 1968). This change in responsiveness has been postulated to be related to the effect of angiotensin on the Na-Ca exchange system (Godfraind, 1970).

Small renal arteries of the dog (Bohr and Uchida, 1967), guinea pig and rabbit portal veins (Carruba, Mandelli and Mantegazza, 1973), rabbit saphenous, femoral, axillary vein and vena cava (Somlyo and Somlyo, 1966), guinea pig vas deferens (Bell, 1972) are insensitive to the myotropic effect of angiotensin. The cat tracheal smooth muscle contracted by 5-hydroxytryptamine and acetylcholine is relaxed by angiotensin (Turker and Ercan, 1976).

(ii) Calcium and Angiotensin Induced Contractions

Changes in extracellular calcium concentration have widely been used in the study of the role of calcium in the contractile action of angiotensin in a variety of smooth muscle tissues. Elimination of calcium in bathing solutions abolished the contractions to angiotensin of the rat uterus and guinea pig ileum (Khairallah, Vadaparampil and Page, 1965). In their study, Khairallah et al (1965) also found abolition of responses to acetylcholine and bradykinin during removal of external calcium. In addition, Freer (1975a), has observed total abolition of contractions of the rat uterine muscle to angiotensin in conditions of calcium depletion while acetylcholine maintained approximately 20% of the control response obtained in normal calcium. Perfusion of the dog mesenteric artery (Burks, Whitacre and Long, 1967) and rat tail artery (Hinke, Wilson and Burnham, 1964) with calcium free media resulted in a gradual decline in the vasoconstrictor effect to angiotensin and in the case of the rat tail artery the recovery of the responses to angiotensin on readmission of calcium was more rapid and complete than of responses to potassium. This has led to the suggestion that part of the response to angiotensin in vascular muscle may be due to mobilisation of intracellular calcium.

The contractile responses of the rabbit aorta to angiotensin and noradrenaline (NA) have also been found to be resistant to calcium depletion (Deth and Van Breemen, 1974; Sullivan and Briggs, 1968). Pattern analysis of the contractile responses of this tissue to angiotensin and NA has revealed two components, an initial fast response resistant to calcium depletion followed by a slow tonic response dependent on external calcium (Deth and Van Breemen, 1974; Van Breemen, Farinas, Gerba and McNaughton, 1972). However, recently (St Louis, Regoli, Barabe and Park, 1977), it has not been possible to identify two components of the angiotensin response, although like NA the angiotensin response persisted in calcium free solution. An analysis of the rate of decline of angiotensin and NA contractions of the rabbit aorta during exposure to calcium free solution has revealed that angiotensin responses decline more rapidly than NA responses (Paiva, Paiva, Miyamoto and Nakaie, 1977; St-Louis, Regoli, Barabe and Park, 1977). Decline of contractions to a spasmogen in calcium free solution has been used as evidence of mobilisation of external calcium for contraction. In the case of angiotensin, several reports have suggested that calcium may also be necessary for the angiotensin receptor interaction (Freer, 1975a, 1975b; Paiva, Aboulafia, Nouailhetas and Paiva, 1976a; Stewart, 1974). Total tissue ^{45}Ca uptake measurements have demonstrated an increase of calcium uptake into rat uterus (see Goodfriend, Fyhrquist and

Allman, 1974), guinea pig taenia coli (Shibata, Carrier and Frankenheim, 1968) and rabbit aorta (Shibata and Carrier, 1967) during exposure to angiotensin and increases both total and cellular ^{45}Ca uptake in the longitudinal muscle of the guinea pig ileum (Godfraind, 1973).

Studies have also been carried out on the effects upon angiotensin responses of drugs and ions whose primary pharmacological effects on smooth muscle are thought to be due to their ability to antagonize Ca^{2+} binding or movements through membranes. These include verapamil (Fleckenstein, Grün, Tritthart and Byon, 1971), SKF525A (Deth and van Breemen, 1974; Hodgson and Daniel, 1973; Kalsner, Nickerson and Boyd, 1970), Mn^{2+} (Anderson, Ramon and Synder, 1971; Mironneau, 1973; Hagiwara and Nakajima, 1966; Nonomura, Hotta and Ohashi, 1966), La^{3+} (Deth and Van Breemen, 1974; Hodgson and Daniel, 1973; Mironneau, 1973; Van Breemen, Farinas, Casteels, Gerba, Wuytack and Deth, 1973; Van Breemen, Farinas, Gerba and McNaughton, 1972) and tetracaine (Feinstein, 1966; Feinstein, Paimre and Lee, 1968). SKF525A and the calcium antagonist verapamil block the angiotensin induced contractions of the polarized and depolarized rat uterus (Freer, 1975a). La^{3+}, Mn^{2+} and tetracaine also exert an antagonist effect on the myotropic effect of angiotensin of the rat uterus (Freer, 1975a). The rabbit aorta contractile responses to angiotensin are resistant to SKF525A and the calcium

antagonists (Kalsner et al, 1970; Freer, 1975a, 1975b). Sullivan and Briggs (1968) have studied the effect of Mn^{2+} on the rabbit aorta. They found that reduction in external calcium had little effect on angiotensin induced contractions but observed a marked antagonism of the responses elicited in low calcium solution by Mn^{2+}. Lanthanum also inhibits angiotensin responses of the aorta. Thus when a rabbit aortic strip exposed to calcium free buffer was treated with La^{3+}, angiotensin as well as NA and histamine induced only a single contraction (Deth and Van Breemen, 1974; Van Breemen et al, 1972).

Although less well understood, current evidence suggests that the adrenergic blocking agents chlorpromazine, dibenamine and phenoxybenzamine may have calcium antagonistic properties. These agents antagonised the tissue ^{45}Ca uptake and contraction induced by K^+ in taenia coli (Shibata et al, 1968). The inhibitory effects of these agents were antagonized by an increase in external calcium. Similarly these agents inhibited responses to angiotensin in this tissue but had no effect on contractions induced by barium. It is interesting to note the observation that phenoxybenzamine antagonized the contractile response of vascular muscle of the rabbit to K^+ without effecting the angiotensin induced contraction (Bevan, Osher and Su, 1963). In addition, Shibata and Carrier (1967) have reported that dibenamine and chlorpromazine inhibited

the potassium induced ^{45}Ca uptake and contraction of the rabbit aorta but not the contractile response to angiotensin.

The possible mobilisation of intracellular calcium by angiotensin has been studied on isolated microsomes of rabbit aorta (Baudouin, Meyer, Fermandjian and Morgat, 1972). The vesicles appeared to be formed from membranes, but the original location of the membranes in the aorta before homogenization was not known. The affect of angiotensin was to decrease binding of calcium by these vesicles and to increase the rate at which trapped calcium was released. It was assumed that the release of calcium from the microsomes may represent intracellular mobilisation by angiotensin in intact vascular muscle.

(iii) Sodium and the Action of Angiotensin in Smooth Muscle

Sodium has been implicated in the contractile action and membrane effects of drugs in smooth muscle (see review by Van Breemen, Aaronson and Loutzenhiser, 1979) and several studies have been designed to investigate the role of sodium ions in the contractile action of angiotensin on smooth muscle.

Reduction of external sodium concentration from 140 meq/1 to 75 meq/1 with isomolar sucrose substitution had no effect on the contractile response of the rat uterus to angiotensin (Khairallah, Vadaparampil and Page, 1965). When the concentration was further reduced to 25 meq/1, the response was reduced to 50 ± 5% of control and at Na^+ concentration of 15 meq/1 the response was completely inhibited. Responses elicited with bradykinin and acetylcholine were similarly affected by sodium depletion which suggested that the changes in responses were due to a non-specific effect of the muscle to the decrease in external ion concentration. In the same study it was shown that angiotensin contractile responses of guinea pig ileum were more sensitive to sodium depletion than contractions of the rat uterus. Reduction of external sodium to 100 meq/1 caused a 35% inhibition of angiotensin responses of the guinea pig ileum and at 75 meq/1 there was an inhibition of 90 ± 2%. The corresponding changes

in external sodium were less effective on responses to acetylcholine and bradykinin. Reduction of sodium to 75 meq/l had no effect on the responses to acetylcholine and bradykinin and at 25 meq/l Na^+ the responses to these spasmogens was still maintained at 50 ± 3% of control in normal sodium solution. Since it had been previously suggested by Khairallah and Page (1961) that part of the angiotensin response is mediated by acetylcholine, Khairallah and co-workers (1965) attributed the sensitivity to sodium depletion of the angiotensin response of the guinea pig ileum to parasympathetic ganglia blockade in the cholinergically mediated component. However using the same tissue Blair-West and McKenzie (1966) observed a decrease of the response to angiotensin during reduction of external sodium by 30 meq/l and an increase when the sodium in the medium was increased by 30 meq/l. The corresponding responses to acetylcholine were reduced by both increases and decreases in external sodium. Atropine 5×10^{-8} g/ml abolished the submaximal contractions to acetylcholine and reduced the effect of angiotensin to 40%. In a later study (Blair-West, Harding and McKenzie, 1967) it was confirmed that the effect of changes in external sodium on angiotensin responses was similar in the presence or absence of atropine and tetrodotoxin. These workers provided unequivocal evidence that changes in external sodium affected the direct effects of angiotensin on guinea pig ileum.

More recently Freer and Smith (1976) have investigated the effect of changes in external sodium concentration in conditions of reduced calcium (0.2 mM) on the contractions of the rat uterus to angiotensin. Reduction of Na^+ in the medium by half with Li^+ substitution completely abolished the contractile response to angiotensin and acetylcholine whereas when tris or sucrose were used as substitutes, there was no change in the responses and in some cases the tissue was more responsive to angiotensin. The inhibitory effect of Li^+ was antagonized by increases in calcium in the bathing solution and was absent in potassium depolarized tissues contracted by addition of angiotensin. Freer and Smith (1976) have suggested that the inhibition is due either to competition by Li^+ for calcium transport sites utilized in excitation-contraction coupling or interference with the receptor binding site to angiotensin.

Blair-West et al (1968) extended their study of effect of changes in extracellular Na^+ to angiotensin vasoconstrictor action of the rabbit ear artery. They established that the pressor effect of injected angiotensin was not reduced by α and β-adrenergic blocking agents, guanethidine, atropine or antagonists to serotonin or histamine, this was taken as evidence for a direct action of angiotensin on the vascular smooth muscle cell in this preparation. The vasoconstrictor effect of angiotensin

of the rabbit ear artery was reduced when the sodium concentration of the perfusion medium was either increased or decreased by 40 meq/l. These changes in external sodium had no effect on the vasoconstrictor effect of noradrenaline which suggested that sodium played a specific role in the action of angiotensin. Similarly a 50% reduction in sodium in perfusion medium abolished the vasoconstrictor effect of the isolated rat ventral artery to angiotensin without affecting the response to noradrenaline (Hinke and Wilson, 1962). Later, in similar experiments, Hinke and colleagues (1964) observed potentiation of responses to potassium during reduction of sodium in perfusion medium, this they attributed to a reduction in the competition by Na^+ for Ca^{2+} diffusion sites or carriers as has been proposed in cardiac muscle (Niedergerke and Lüttgau, 1957).

A 25% reduction in sodium in bathing medium potentiated the contractile responses of the rabbit aorta to angiotensin and a similar increase in sodium had the converse effect (Napodano, Caliva, Lyons, De Simone and Lyons, 1962). These effects were attributed to the additive effect due to change in sodium gradient between the inside and outside of the muscle cell during acute changes in external sodium which has been shown to change muscle tone (Friedman, Jamieson and Friedman, 1959).

The role of sodium in the vascular reactivity to angiotensin has been extended to the pressor action in whole animals. Current evidence indicates that sodium depletion, in animals and humans, maintained on sodium deficient diet, markedly impairs pressor reactivity to angiotensin (Reid and Laragh, 1965; Baraclough, Jones, Marsden and Bradford, 1967; Laragh, 1962; Davis, Hartroft, Titus, Carpenter, Ayers and Spiegel, 1962; Blair-West, Coghlan, Denton, Goding, Munro and Wright, 1963; Kaplan and Silah, 1965). Conversely, when sodium is given in excess especially if a mineralocorticoid is also administered, the pressor response to angiotensin infusion is enhanced (Reid and Laragh, 1965; Cowley and McCaa, 1976). The reasons for this are not clear, some investigators believe that sodium depletion activates the renin angiotensin system which leads to increases in circulating angiotensin that dampens (i.e. tachyphylaxis) the receptor response to infused hormone (Kaplan and Silah, 1965). This idea has been supported more recently by Thurston and Laragh (1975) who observed normal pressor reactivity to angiotensin in sodium depleted rabbits treated with the converting enzyme inhibitor SQ20881. Other workers (Brunner, Chang, Wallach, Sealey and Laragh, 1972) have suggested that changes in the vascular response to and the avidity for angiotensin results from a more direct action of the sodium ion on the receptor per se since similar changes in reactivity to angiotensin have been demonstrated

in fresh preparations of aortic strips taken from sodium depleted rabbits (Strewler, Hinrichs, Guiod and Hollenberg, 1972). In addition to changes in extracellular sodium concentration the involvement of Na^+ in the action of angiotensin has been studied by use of ouabain and by flux measurement of sodium. Electrophysiological studies have been considered elsewhere, they will therefore not be cited in this section.

Antagonism between angiotensin and ouabain has been reported (Turker, Page and Khairallah, 1967). It was observed by these workers that angiotensin caused sodium efflux in the rat uterus and dog carotid artery. Since ouabain blocked both the sodium efflux and the contractile action of angiotensin of the rat uterus it was concluded that angiotensin stimulated $Na^+ - K^+$ ATPase. This was in agreement with the previous observations of Turker (1965) of inhibition by angiotensin of ouabain induced cardiac arrhythmia in the guinea pig. In contrast to Turker and colleagues' observations it has since been reported that prolonged incubation in 10^{-3}M ouabain had no effect on the contractile responses of the rat uterus to angiotensin (Freer and Smith, 1976). In the same tissue Hamon and Worcel (1973) have shown an increase in ^{24}Na influx to angiotensin in both the polarized and depolarized tissue and sometimes an increased efflux of ^{24}Na in the depolarized muscle. These investigators also observed an increase

in ^{45}K and ^{36}Cl effluxes in polarized muscles, an effect which could be suppressed by potassium depolarization; and they concluded that this phenomenon was a consequence of the depolarization induced by angiotensin. Measurement of total ionic content in longitudinal muscle of guinea pig ileum and rat aorta by Godfraind (1973) has revealed that incubation with angiotensin 10^{-9} M for 30 min resulted in an increased calcium uptake accompanied by a fall in sodium content. Since he had formerly observed an antagonism by angiotensin of acetylcholine induced uptake in tissue sodium in the guinea pig longitudinal muscle (Godfraind, 1970), these observations led him to speculate that these changes induced by angiotensin could occur through direct stimulation of Na-Ca exchange or indirectly by modification of Na^+-K^+ ATPase activity.

In vascular smooth muscle angiotensin increased sodium influx and potassium efflux (Friedman and Friedman, 1964, 1965) and has been reported to increase the total tissue Na and Ca content (Villamil, Nachev and Kleeman, 1970). Sodium flux measurements have to be interpreted cautiously since a considerable portion of a slowly exchanging sodium may be extracellular and indistinguishable from true efflux by simple compartmental analysis (Keatinge, 1968).

(iv) Angiotensin and Membrane Potential

Angiotensin causes contraction of spontaneously active tissues of guinea pig taenia coli (Ohashi, Nonomura and Ohga, 1967), rat myometrium (Hamon and Worcel, 1977) and rabbit (Cuthbert and Sutter, 1965; Somlyo and Somlyo, 1968a) and dog (Somlyo and Somlyo, 1968a) mesenteric veins that is accompanied by membrane depolarization and an increase in spike frequency. Cuthbert and Sutter (1965) showed that the action potential discharge (spikes) induced by angiotensin or K^+ occurred in parallel with the contractile response and that angiotensin was less effective than noradrenaline in contracting the K^+ depolarized tissue. However, Somlyo and Somlyo (1968a) did not see any correlation between increase in spike frequency and tension development. Furthermore when the membrane potential was held constant by voltage clamp (Hamon and Worcel, 1977) angiotensin still caused contraction of the rat myometrium thus casting doubts as to the role of membrane depolarization on contraction in these tissues.

In quiescent tissues of rabbit and dog pulmonary arteries and aorta, angiotensin, noradrenaline and histamine produce a graded depolarization without spikes, somewhat proportional to the amplitude of contraction (Somlyo and Somlyo, 1968a). Conflicting results have been obtained on sheep carotid arteries by Keatinge (1966). Angiotensin,

acetylcholine, noradrenaline and bradykinin caused depolarization and simultaneous contraction of these tissues. Contractions could be maintained with each of these drugs, despite the fact that repolarization had started. Shibata and Briggs (1966) using the sucrose gap technique have reported that angiotensin contracts rabbit aortic strips independently of any electrical phenomenon and produced the same effect on polarized or depolarized tissues. In addition Somlyo and Somlyo (1968a) have reported that angiotensin contracts potassium depolarized dog aorta. Other workers have found that angiotensin II contracts vascular muscle (Keatinge, 1966; Shibata and Briggs, 1966; Freer, 1975a) and uterine muscle (Freer, 1975a) in the presence of depolarizing concentrations of potassium and have therefore suggested that the action of angiotensin II is independent of transmembrane potential. The variation in the electrical activity to vasoconstrictors, including angiotensin, has led Somlyo and Somlyo (1968a) to suggest three mechanisms of inducing contraction of vascular muscle: the first by increasing the frequency of spike potentials, the second, through graded depolarization of the cell membrane and the third by "pharmacomechanical coupling".

Where angiotensin may exert an inhibitory or relaxant effect, as in guinea pig portal vein, some hyperpolarization and a decrease in frequency of action potential bursts had been observed (Weston and Golenhofen, 1976).

(v) Angiotensin and Cyclic AMP

Volicer and Hynie (1971) have reported that angiotensin causes a decrease of cyclic AMP levels in the rat tail artery. It also reduced the adenyl cyclase activity in rat aorta and antagonized theophylline induced increase in cyclic AMP in this tissue. These workers therefore suggested that a decrease in cyclic AMP may mediate angiotensin contraction although causal relationships of decrease in cyclic AMP and contraction were not established. Angles d'Auriac and Meyer (1972) have also investigated the possibility that contractions due to angiotensin in the rat uterus may be related to an effect by the peptide on adenyl cyclase system. They measured cyclic AMP formation after radioactive prelabelling of adenine nucleotide pool and by direct determination of cyclic AMP content. It was found that angiotensin did not affect the rate of cyclic AMP formation although it reduced the stimulatory effect on epinephrine on cyclic AMP formation. Other studies have concentrated on the effect of angiotensin on the activities of adenyl cyclase and phosphodiesterase, two enzymes which control respectively the production and degradation of cyclic AMP (Robison, Butcher and Sutherland, 1966). In experiments with rat uterus angiotensin has been found not to affect the activity of phosphodiesterase (Angles d'Auriac and Meyer, 1973) and to have no effect on the levels of cyclic

AMP and cyclic GMP. Murad and Kimura (1974) in their incubation studies of guinea pig trachea rings have observed an increase in cyclic AMP to epinephrine that was blocked by propranolol and an increase of cyclic GMP to acetylcholine and carbachol but angiotensin had no effect on the levels of either nucleotide.

METHODS

SECTION I: CONTRACTION STUDIES

(1) Procedure

Male Wistar rats (150-300 g) previously fasted overnight were killed by a blow on the head and cervical dislocation. The abdomen was opened and 5cm of the terminal colon adjacent to the rectum was excised and quickly placed into Tyrode's solution at 32°C gassed with air. It was then cleaned and freed of mesentery before being cut into two equal parts.

The paired pieces of the terminal colon were mounted vertically under isotonic conditions in 20ml muscle baths and allowed to equilibrate for 1.5 hours under a resting tension of 2g. The Tyrode's solution (pH 7.4) was gassed with air continuously and maintained at 32°C. During equilibration the incubation media was changed every 15 minutes to provide fresh glucose and to avoid accumulation of toxic metabolites of glucose oxidation. Spasmogens were predissolved in physiological saline and were added to the bath in volumes not exceeding 0.5ml. Initially a contact time of 90 seconds was used for each spasmogen with an interval of 10 minutes between addition of spasmogens but this was later changed to 120 seconds at an interval of 15 minutes. The preparation was washed five times between addition of spasmogens to minimize interference. Control responses to spasmogens were

sometimes elicited after experimental treatments to ascertain that the muscle preparation did not show tachyphylaxis or non-specific changes in sensitivity.

(2) Solutions

Tyrode's solution was made up in double distilled water as follows (mM):

NaCl, 137; KCl, 2.7; $MgSO_4$, 1.1; $CaCl_2$, 1.8; NaH_2PO_4, 0.3; $NaHCO_3$, 11.9; D-glucose, 5.6. In experiments where the calcium concentration of the Tyrode's solution was increased, the $NaHCO_3$ and NaH_2PO_4 were replaced with 5mM tris chloride. The stock trischloride solution used was made up at pH 7.6 at room temperature to buffer at pH 7.4 at 32°C. In this case the solution was freshly prepared just before use to minimize precipitation and was gassed with pure oxygen.

(3) Recordings

Isotonic recordings were made with a Devices 2LD01 transducer coupled to a Devices two channel recorder. The contractile responses to the spasmogens were measured as the maximum sustained deviation from the baseline. In most of the experiments paired preparations from the same animal were used in each experiment. Hence the response of one preparation to angiotensin was compared with the

response of the other preparation to either PGE_2 or to potassium. This protocol yielded two groups of similar results for angiotensin, so unless there was a marked difference in the responses to the two groups due to variation in preparations the results from only one group of tissues will be quoted in this thesis.

(4) Evaluation of Results

The effect of experimental treatments on responses to a spasmogen was determined after recording three successive control responses of equal size. The control response for each spasmogen was approximately equivalent to 50% of the maximal response to angiotensin of a preparation determined in normal Tyrode's solution at the beginning of every experiment. The subsequent responses have been expressed as a percentage of the control response. For each treatment, the results are expressed as the mean ± standard error of the mean for values obtained from at least six preparations.

However, in experiments involving theophylline and imidazole, the effect of experimental treatments have been evaluated by comparison of the shift on the x-axis caused by either agent on the dose response (concentration response) curves to each spasmogen.

Unless otherwise stated results have been analysed by Student's t-test for paired observations. A p-value of 0.05 or less was considered to be significant.

SECTION II: ELECTROPHYSIOLOGICAL STUDIES

(1) The Sucrose-gap Apparatus

The sucrose-gap chamber is shown in Fig. 2. It consisted of three cylindrical perspex sections with a central canal of diameter 1.5mm which contained the preparation. The sections were held in position by two screws which would allow adjustment of the distance between them and in this way allow regulation of the pressure on the rubber membranes between the sections. Thin circular rubber (Tambour rubber supplied by Bioscience, Sheerness, Great Britain) plates of 12mm diameter and 0.5mm thickness were used as rubber membranes. The central hole in the rubber plate was made with a heated wire and had a diameter of 1.5mm. The length of the sucrose chamber was 5mm and that of the chambers for K_2SO_4 and for physiological saline (Krebs' or Tyrode's solution) were each 20mm. Each of the three perspex pieces had a hole (2mm diameter) drilled for influx of perfusion solution. Whereas the pieces for the sucrose and active chamber each had a hole for the efflux of the perfusion solution, the K_2SO_4 solution drained away via the central canal. The two end sections had an extra aperture for insertion of the recording electrodes.

(2) Dissection and Experimental Procedure

Experiments were performed on smooth muscle strips of the longitudinal muscle of the rat descending colon and guinea-pig taenia coli.

(a) The Longitudinal Muscle of the Rat descending colon

Whole pieces of rat descending colon could not be used since the sucrose gap technique requires very small pieces of muscle (see Boev and Golenhofen, 1974), thus the longitudinal muscle of the rat colon was used in this study.

The longitudinal smooth muscle was dissected from the rat descending colon by a modification of the method described by Ambache (1954) for removal of the longitudinal muscle layer from the rabbit intestine.

The terminal 5cm length of rat descending colon was cleaned of the mesentery and was washed in Tyrode's solution gassed with air. The preparation was drawn over a hollow perspex rod of 5mm external diameter with a tapered end. The preparation was then anchored at both ends with cotton thread so that the muscle was under gentle tension. The rest of the dissection procedure was performed under bright light. A circular incision

was made with a scalpel at each end through the longitudinal muscle down the circular muscle. A similar incision was made longitudinally along the line of the mesenteric attachment. The longitudinal muscle receded slightly at the second incision and was detached gently over several millimetres with fine forceps. The edges at one end of the longitudinal muscle were grasped with forceps and removed longitudinally as an entire sheet.

The 5cm sheet of longitudinal muscle of rat colon was trimmed longitudinally to produce a preparation 2-3mm wide that could be folded longitudinally to form a quasi cylindrical preparation. A cotton thread was tied at each end of the strip. The muscle strip was then carefully threaded into the central channel of the sucrose gap apparatus. The middle portion of the muscle strip lay in the portion perfused with isotonic sucrose at room temperature (20-25^{o}C). One end of the muscle preparation was fixed to a holder whereas the "test" end was attached by a thread to an isometric transducer (Devices UFI) which was attached to a 2-channel *y*-t recorder (Teckman). A resting tension of 0.5g was applied to the whole muscle. Initially each end of the muscle was perfused with Tyrode's solution for 30 minutes. Tyrode's solution perfusing the "test" (or "active") end of chamber was maintained at 32^{o}C by passing through a preheating coil. At the same time Tyrode's solution from a reservoir bottle at room

temperature flowed continuously through the opposite chamber (chamber 1, Fig 2) which contained the other end of the muscle. This end remained "inactive" and thus maintained a stable potential against which changes in potential at the active end could be compared. The rubber membranes were then closed by gradually tightening the two screws until the recorded resting membrane potential between the inactive and active side was zero. This was regarded as the disappearance of the liquid junction potential between the Tyrode and sucrose solutions. A two-way tap was inserted into the lines perfusing each end of the muscle preparation. Then, with the rubber membranes closed, the inactive end of the muscle preparation was switched to either isotonic potassium chloride or potassium sulphate at room temperature. The recorded resting membrane potential gradually rose to a stable value before addition of any spasmogen or switching to a solution of different ion concentration.

(b) The Guinea-pig Taenia Coli

Male guinea pigs (300-400g) previously starved overnight were killed by a blow on the head and cervical dislocation. Strips of taenia coli (taenia caecum), 5cm long (at in situ length) and 0.5-1mm in diameter were dissected out from the surface of the colon (Bulbring, 1954). They were similarly mounted in the sucrose chamber as

already described for longitudinal muscle of the rat descending colon but Krebs' solution was used instead of Tyrode's solution and the solution was warmed to 37°C before reaching the "test" chamber.

(3) Solutions

The composition of the Tyrode's solution has been described previously under contraction experiment methods (page 68). The composition of the Krebs' solution for the guinea-pig taenia coli was (mM):

NaCl, 94.1; KCl, 4.7; $CaCl_2$, 2.4; KH_2PO_4, 1.1; $MgSO_4$, 0.5; $NaHCO_3$, 25.0; D-glucose, 11.1.

Throughout the whole course of the experiment the Tyrode's solution was gassed with air whereas Krebs' solution was gassed with a mixture of 95% O_2 - 5% CO_2. As described earlier HCO_3^- and $H_2PO_4^-$ were omitted and the solutions were buffered throughout in 5mM tris buffer and gassed with pure oxygen in experiments which involved high extracellular Ca^{2+} concentrations. The isotonic KCl and K_2SO_4 solutions were 154 mM (1.15% $^W/v$) and 103 mM (1.79 $^W/v$) respectively. These solutions were isotonic on the basis of freezing point data. The isotonic sucrose flowing through the "sucrose" chamber was 308 mM (10.54% $^W/v$) solution made by dissolving the sucrose in double distilled dionized water that had been passed through an Elgastat dionizer

and had a resistivity of not less than 1.5 MΩcm.

(4) Electrical Recordings

(a) Preparation of Ag-AgCl Electrodes

Ag-AgCl electrodes were used for recording electrical activity of smooth muscle in the sucrose gap apparatus. These electrodes consisted of cylindrical pellets (3mm diameter, 4mm long) made by compressing a mixture of one part reagent grade powdered silver to two parts powdered silver chloride in a small mould around a loop on the end of a silver wire 20mm long and 0.5mm diameter (see Martin, Wickelgren and Beranek, 1970). The end of the silver wire was joined to a copper wire by soldering. This junction was insulated by wire sheath using an adhesive, "Araldite", which was allowed to dry before mounting the electrode in plastic capillaries (drawn out of a polypropylene tubing of 3mm internal diameter) containing 3M KCl-Agar.

(b) Electrical Recording Using the Sucrose Gap Apparatus

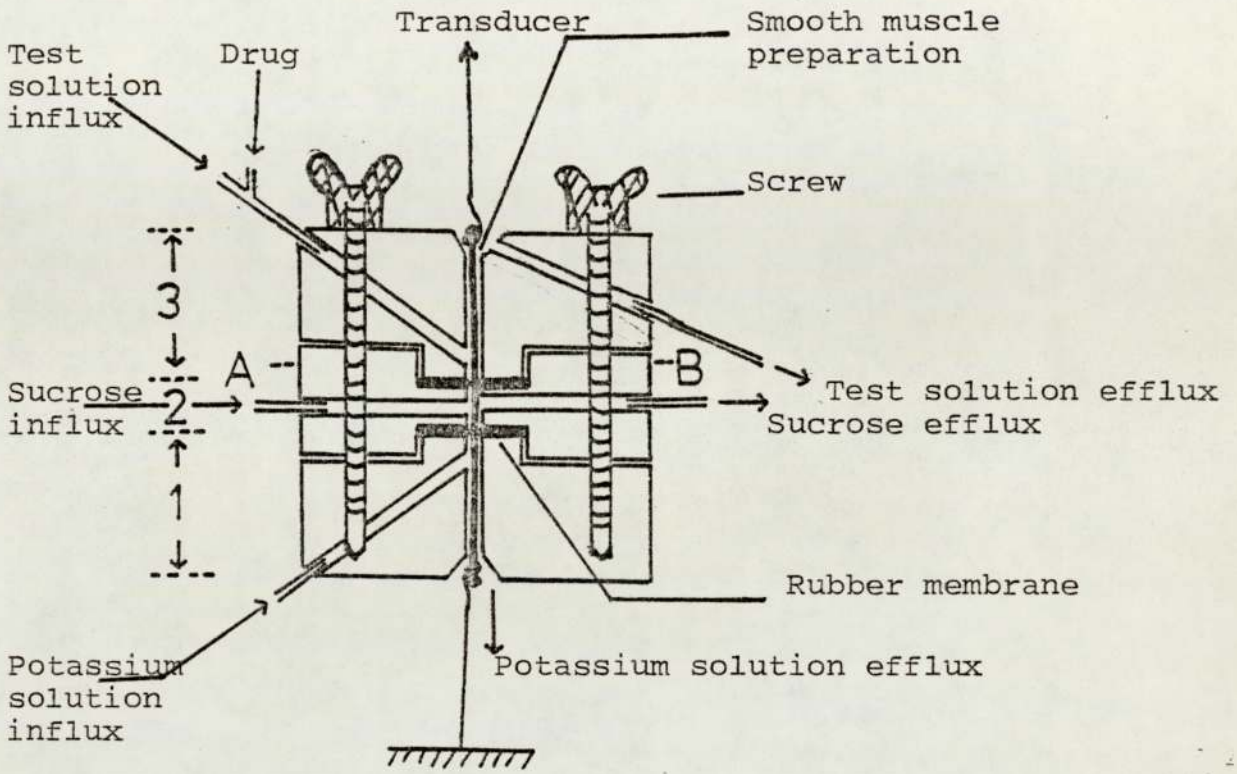
A pair of the Ag-AgCl electrodes mounted in plastic capillaries containing 3M KCl-Agar were used to record the potential difference between the "active" and "inactive" chambers (Fig 2), the inactive side being at earth potential. The two electrodes were each inserted into the holes directly connected with the central canal until the KCl-agar

bridge tip was just touching the surface of the tissue. Vaseline was applied to the sides of the plastic capillary electrode holders to ensure electrical insulation and prevent leakage of perfusate. The whole apparatus was enclosed in a metal box connected to earth to minimize electrical interference. The electrodes were connected to a High Imput Impedance Millivoltmeter with a digital display which had an imput impedance of 10^{12} ohms and a frequency response of greater than 100 KH_z. This was constructed in the department by Mr. David Briggs (Fig 3). The millivoltmeter was connected to the second channel of the y-t recorder (Techman). Sometimes the millivoltmeter was simultaneously connected to an M_2 Devices chart recorder which could be run at a faster speed. Together with the greater frequency response of the Devices recorder, this allowed a more detailed analysis of the electrical events.

(5) Tension Recording

Muscle movement during contraction is more limited during isometric recording of muscle tension than with isotonic measurement of contraction. In order to minimize the possibility of electrical artifacts that may arise due to movement of muscle preparation during contraction in the sucrose gap apparatus, in all these electrophysiological experiments mechanical activity of the muscle

(a)



(b)

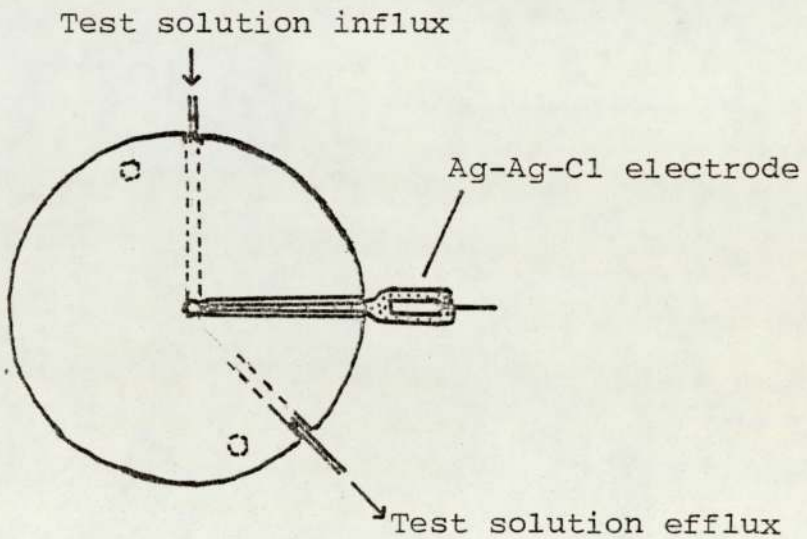
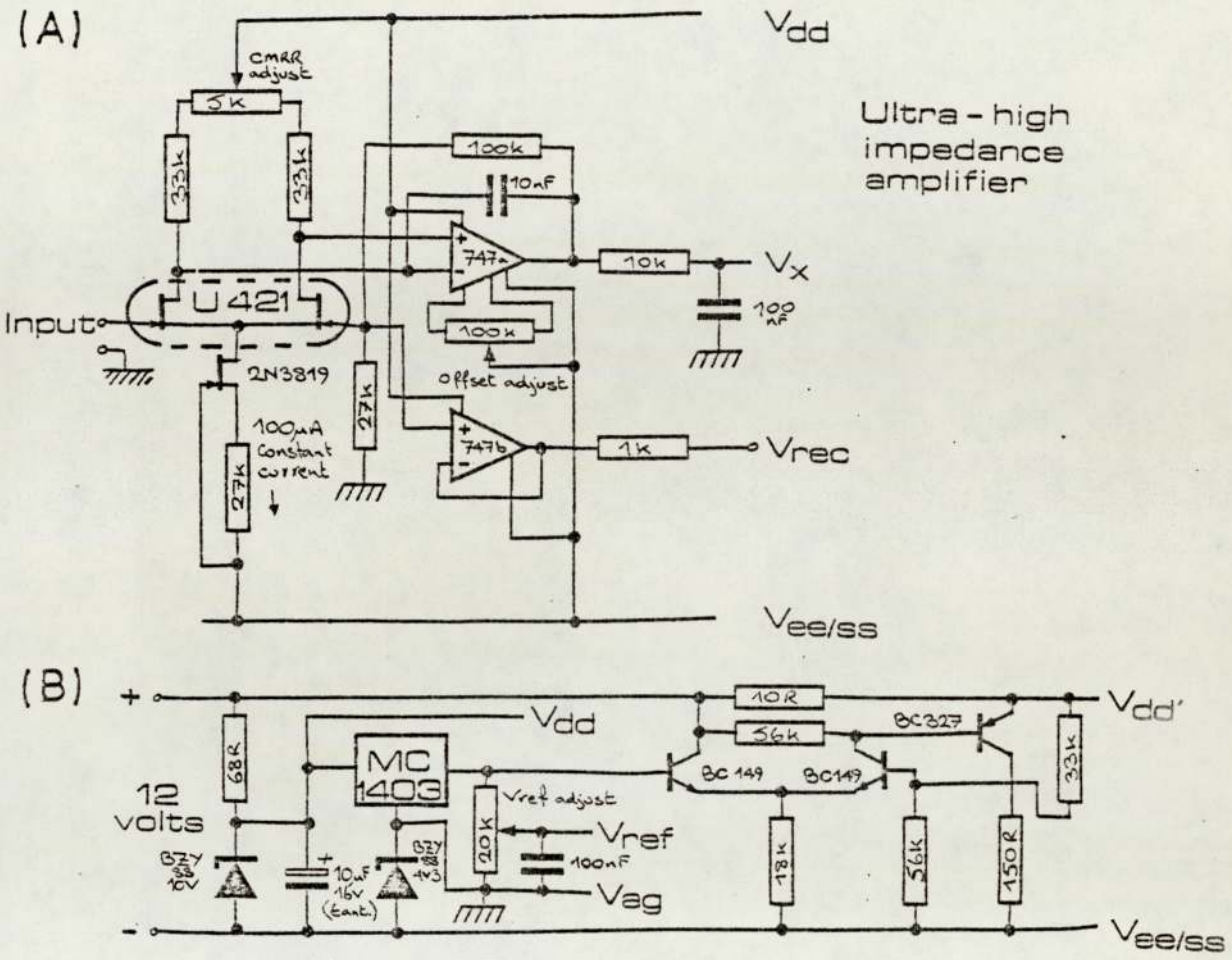


Fig. 2

(a) Diagram of the chamber used for measuring membrane potential with the sucrose-gap technique.
(1) Inactive chamber 20mm
(2) Sucrose chamber 5mm
(3) Test or active chamber 20mm.

(b) Cross-section through A-B, cross section diameter 45mm.

Scale 1:1



Digital millivolt meter

preparations was measured simultaneously with an isometric Devices UFI transducer and the tension changes were recorded alongside the electrical effects on the same y-t recorder.

(6) Addition of Drugs

Drugs were dissolved in either Tyrode's or Krebs' solution depending upon the fluid that was perfusing the active side of the muscle preparation. The stock drug concentrations used were angiotensin (2-8 x 10^{-7}M) and KCl (0.25 - 1.0M). At first spasmogens were dissolved to the required concentration in the appropriate physiological solution to form the test solution. The preparation was then brought in contact with the spasmogen by changing to the test solution by means of a tap. This initial method was abandoned because of artefacts which arose both from switching of the tap and from slight changes in flow rate which could not be differentiated from the effects of the drug. Thus, the spasmogens were added in 20μl volumes from a microlitre syringe by injecting into the perfusion solution just before the solution reached the preparation in the test chamber. Because the solution was constantly flowing, the final drug concentration in contact with the preparations cannot be exactly determined. However, the rate of each perfusion fluid was 1.5-3.0 millitres per minute and was maintained constant throughout any one experiment.

SECTION III: RADIOCHEMICAL STUDIES: ^{45}Ca UPTAKE BY LONGITUDINAL MUSCLE OF RAT DESCENDING COLON

(1) Procedure

The longitudinal muscle of the rat descending colon was dissected as described previously in the methods for the electrophysiological experiments. The terminal 0.5cm ends of the 5cm sheet of longitudinal muscle were trimmed off and the muscle was cut into pieces of approximately 1cm length. Each preparation was mounted on a steel tissue holder and suspended in a 10ml jacketed organ bath containing warm aerated Tyrode's solution at 32°C. Tension was measured by an isometric Devices UFI transducer connected to an M_2 Devices recorder. Initially these muscle preparations were equilibrated in the muscle baths under a resting tension of 0.3g for at least 1 hour before the experimental treatment. During this initial incubation period the bathing solution was changed periodically as already described (pp 67).

(2) Cellular and Total ^{45}Ca Uptake by Muscle Preparation during Incubation

After the initial one hour equilibration time, paired pieces of longitudinal muscle from the same rat descending colon were mounted into two parallel muscle baths and incubated in ^{45}Ca uptake medium for 2.5, 5, 10

and 20 minutes. After the incubation period one piece of muscle was washed for 30-40 seconds in calcium-free Tyrode's solution to remove adhering calcium ions from the external surface. It was then prepared for total counting of ^{45}Ca uptake (to be described later). The other piece of muscle preparation was similarly washed in Ca^{2+} free Tyrode's solution followed by lanthanum treatment (as will be described later in the lanthanum method) before the muscle was prepared for scintillation counting of the intracellular ^{45}Ca uptake.

(3) Effect of Verapamil on Cellular ^{45}Ca Uptake during Muscle Contraction

The uptake of ^{45}Ca by the longitudinal muscle of the rat colon was studied during activation by a supra-maximal concentration of either angiotensin II or potassium chloride both in the presence and in the absence of verapamil (10^{-5} molar). In this case three pieces of longitudinal muscle from the same animal were mounted into three parallel muscle baths. After the initial 1 hour equilibration in normal Tyrode's solution, the preparations were exposed to ^{45}Ca uptake solution for 10 minutes to allow the extracellular space to equilibrate with the applied solution (see Fig 34). Then the spasmogen was added to the first two preparations and left in contact with the preparations for 5 minutes. In addition,

verapamil was introduced to the second preparation 2 minutes before the addition of either spasmogen. Changes in muscle tension were recorded isometrically as described earlier. The third preparation was a control and therefore a volume of Tyrode's solution was added which was comparable with the volumes of spasmogen added to the first two preparations. All the preparations were then treated with lanthanum, as will be described in the lanthanum method, prior to preparation for scintillation counting of the intracellular ^{45}Ca.

(4) The Lanthanum Method

It has been shown by Van Breemen et al (1972) that in the rabbit aorta measurement of total content of the muscle lacks correlation with contraction due mainly to a relatively large extracellular calcium exchange. Therefore in this study cellular ^{45}Ca uptake was determined by the lanthanum method (Van Breemen and McNaughton, 1970; Van Breemen, Farinas, Gerba and McNaughton, 1972; Mayer, Van Breemen and Casteels, 1972). The principle of the method is that after the muscle has taken up ^{45}Ca, and before counting, all the extracellular ^{45}Ca is washed out and displaced from binding sites by lanthanum, while membrane bound lanthanum blocks the loss of ^{45}Ca from within cells. The measured radioactivity will then give unidirectional calcium flux into the cells during the

experimental time period. Thus after the experimental treatment, the ^{45}Ca uptake solution was drained away and was replaced with tris buffered Tyrode's solution of the same specific activity containing 10mM La^{3+} (gassed with oxygen, 32°C) for 3 minutes. The purpose of this treatment was to block the loss of ^{45}Ca from the cellular compartment prior to the washing procedure. Later, the muscle preparation was washed in a calcium-free tris Tyrode's solution containing 10mM La^{3+} for 60 minutes. The solution in the organ bath was changed at 15 minute intervals. The muscle preparations were dismounted from their holders and blotted; they were then placed between two sheets of Whatman filter paper and pressed four times with a metal roller weighing 90g, this procedure was sufficient to remove all residual moisture from the preparation. Each preparation was then weighed on a Class A analytical balance to give the muscle wet weight.

(5) Solutions

(a) Physiological Saline

The composition of Tyrode's solution was the same as described earlier under contraction experiment methods. Since lanthanum precipitates in media which contains HCO_3^- and $H_2PO_4^-$, tris chloride buffered Tyrodes solution was used in all experiments involving lanthanum treatment. Because the solution contained a high concentration of

La^{3+} (10 mMolar), the solution was freshly prepared (gassed with 100% oxygen) just before use to minimise precipitation.

(b) ^{45}Ca Uptake Solution

The ^{45}Ca used was supplied as an aqueous calcium chloride solution of specific activity 2.03 mCi per ml by the Radiochemical Centre, Amersham. Small quantities of this solution were diluted x 100 with deionised double distilled water to make a stock solution A. 100 µl of solution A was added to 10ml of normal Tyrode's solution to make the uptake solution (32°C, gassed with air).

(6) Scintillation Counting

After blotting and weighing each preparation was transferred to a scintillation vial, each preparation was dissolved in 0.5ml protosol (NEN) tissue solubilizer overnight at 50°C. During this incubation period the vials were tightly capped to minimise escape of solvent and possible precipitation. Occasionally at the end of an overnight incubation, precipitates appeared in the scintillation vials and sometimes the samples were discoloured to a yellowish colour. In such case the precipitate was redissolved by addition of 100 µl of methanol followed by agitation and in case of colour formation the samples

were decolourized to minimize colour quenching by addition of 100μl hydrogen peroxide 30% $^{w}/_{v}$ (BDH) followed by incubation at 50^{o}C for an extra 1 hour. The samples were allowed to cool down to room temperature, this was followed by addition of 5ml of Dimilume-30 Packard Scintillation fluid. They were then left in the dark at room temperature for at least 2 hours to minimize chemiluminescence before they were counted in a Beckman LS-230 liquid scintillation counter.

The sample vials were counted together with control vials (containing 0.5ml protosol and 5ml Dimilume-30) for background count correction. In addition, the radioactivity of ^{45}Ca uptake Tyrode's solution was estimated by addition of 100μl of the uptake solution to vials containing 0.5ml protosol and 5ml of Dimilume-30. These vials were included in triplicates and were incubated and treated like sample vials prior to the addition of scintillant.

(7) Quench Correction

The efficiency of counting in each vial was determined by using an internal standard. Thus after an initial counting of the sample vials, a known amount of ^{45}Ca was added to each vial and the counting of the vials was repeated.

$$\text{Efficiency} = \frac{\text{cpm during 2nd count - cpm during 1st count}}{\text{dpm of } ^{45}\text{Ca added}} \times 100$$

The cpm obtained in the initial count for each vial were corrected for efficiency and background counting and were converted in dpm for calculation of cellular or total ^{45}Ca uptake by the muscle preparation.

8. Calculation of ^{45}Ca Uptake

The muscle ^{45}Ca content was calculated by dividing the disintegrations per minute (dpm) of the muscle preparation per gram by the specific activity of the uptake medium. Where the specific activity of the uptake medium was expressed as dpm per micromole of calcium in the uptake medium. For calculation purposes the final concentration of calcium in the uptake solution was regarded the same as that in normal Tyrode's solution since the contribution by the very small quantities of ^{45}Ca added was negligible. Thus uptake has been expressed as apparent muscle tissue content of ^{45}Ca (Godfraind, 1976) according to the formula:

$$^{45}\text{Ca}(\mu\text{moles/g tissue}) = \text{dpm/g tissue} \div \text{dpm per } \mu\text{mole of calcium in uptake medium}$$

SECTION IV: DRUGS AND CHEMICALS

All chemicals used were reagent grade, in addition the following drugs and chemicals were used.

Drugs and Chemicals	Supplier
* Angiotensin II: "Hypertensin, Ciba" as Val 5-angiotensin II asp-B-amide	CIBA Limited
^{45}Ca, as an aqueous calcium chloride solution of specific activity 2.03 mCi per ml	The Radiochemical Centre, Amersham
Cyclic GMP as Guanosine 3^1: 5^1 cyclic monophosphoric acid	SIGMA Limited
+ Dantrolene sodium	Norwich Pharmacal Co
Dibutyryl cyclic AMP as N^6 O^2 dibutyryl adenosine 3^1: 5^1 - cyclic monophosphoric acid	SIGMA Limited
Dimilume-30, a complete liquid scintillation cocktail with chemiluminescence inhibitor	Packard Limited
2-4- Dinitrophenol	SIGMA Limited
Ethylenediamine tetraacetic acid disodium salt, Na_2EDTA, Analar grade	BDH Limited

	Hydrogen Peroxide as a 30% $^{w}/_{v}$ solution, Aristar grade	BDH Limited
	Imidazole	SIGMA Limited
	Indomethacin	MSD Limited
	Isoprenaline, as DL-isoprenaline sulphate	SIGMA Limited
	Lanthanum Chloride	BDH Limited
	Methanol	BDH Limited
	Potassium Chloride, Analar	BDH Limited
+*	Prostaglandin E_2	The Upjohn Company
	Protosol, tissue and gel alkaline solubilizer as a 0.5 molar solution	NEN
+	SKF 525A, (Proadifen hydrochloride) as B-diethylamino ethyldiphenyl-propylacetate	SK & F Limited
	Sucrose, Analar grade	BDH Limited
	Theophylline	SIGMA Limited
	Tris (hydroxymethyl) aminomethane (Tris)	SIGMA Limited
	Tris (hydroxymethyl) aminomethane hydrochloride	SIGMA Limited

+ Verapamil hydrochloride ABBOT Limited

*Angiotensin (10^{-5}M in distilled water) and PGE_2 (10^{-3}M in absolute alcohol) were stored at -18°C and were diluted in physiological saline just before use.

+Drugs supplied as gifts, for which I am very grateful.

R E S U L T S

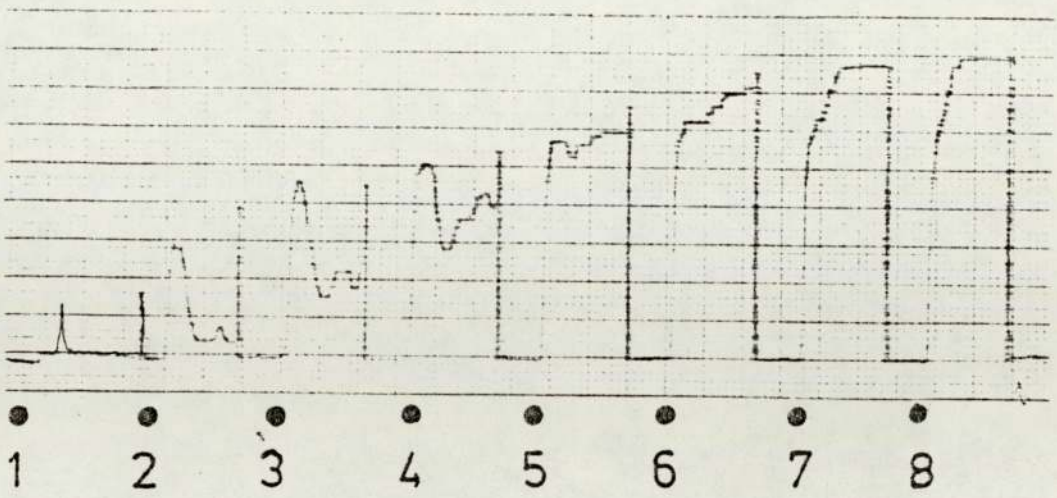
SECTION I: CONTRACTION STUDIES

A Comparative Study of the Contractile Action of Angiotensin II of PGE_2 and of Potassium Chloride on the Rat descending Colon

A. Determination of Equieffective Concentrations of Spasmogen

Concentration-response curves for angiotensin II, for PGE_2 and for potassium chloride were determined on the rat descending colon in a consecutive manner (see Fig 4a). Complete concentration-response curves to all three spasmogens were determined on each preparation, the order of addition of spasmogens (but not the doses) was randomised. The isotonic responses to each of the spasmogens were expressed as a percentage of the maximal response to angiotensin. It can be seen from Fig 4b that the maximal responses to both angiotensin and potassium chloride were equal, but PGE_2 was less effective, its maximal response was $56.9 \pm 7.9\%$ (n=6) of the maximal angiotensin response. The arrows in Fig 4b indicate the approximate log spasmogen concentrations that elicited contractile responses equivalent to 50% of angiotensin maximal response.

In later experiments these spasmogen concentrations were redetermined at the beginning of each experiment and used to elicit the control responses in normal Tyrode's solution. The subsequent responses were then expressed as a percentage of this control response obtained in normal Tyrode.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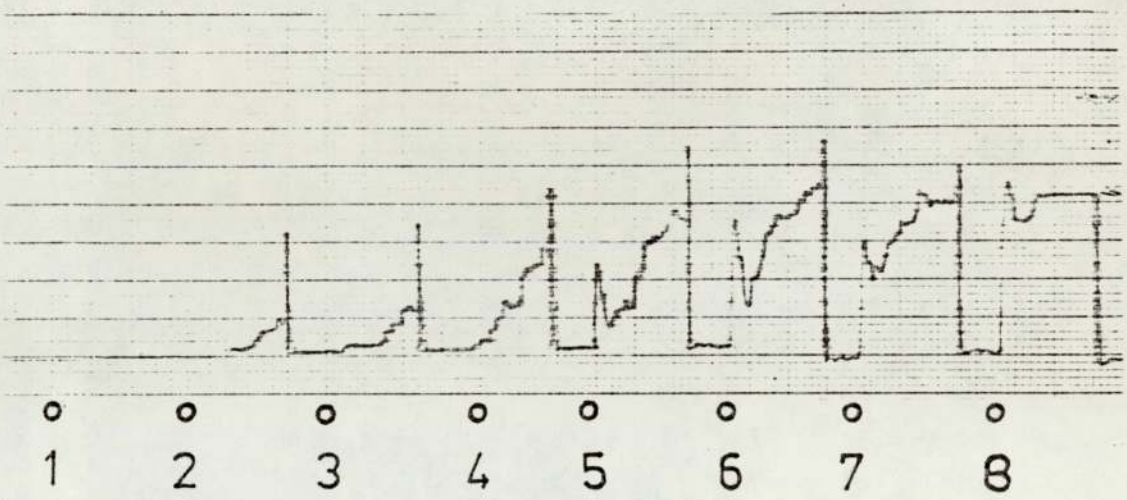
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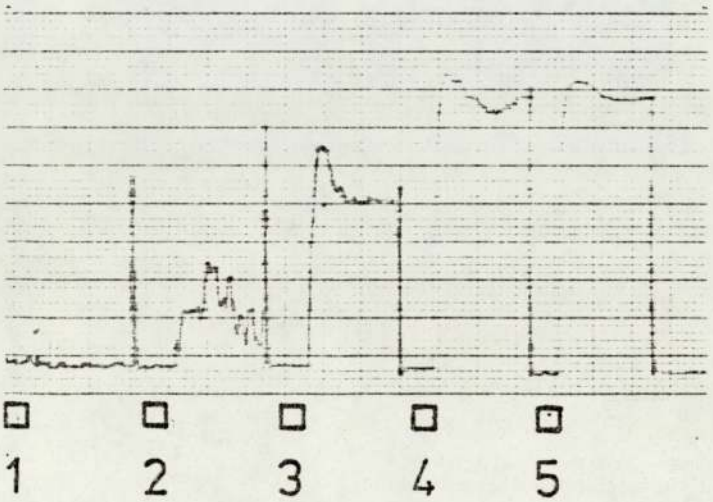
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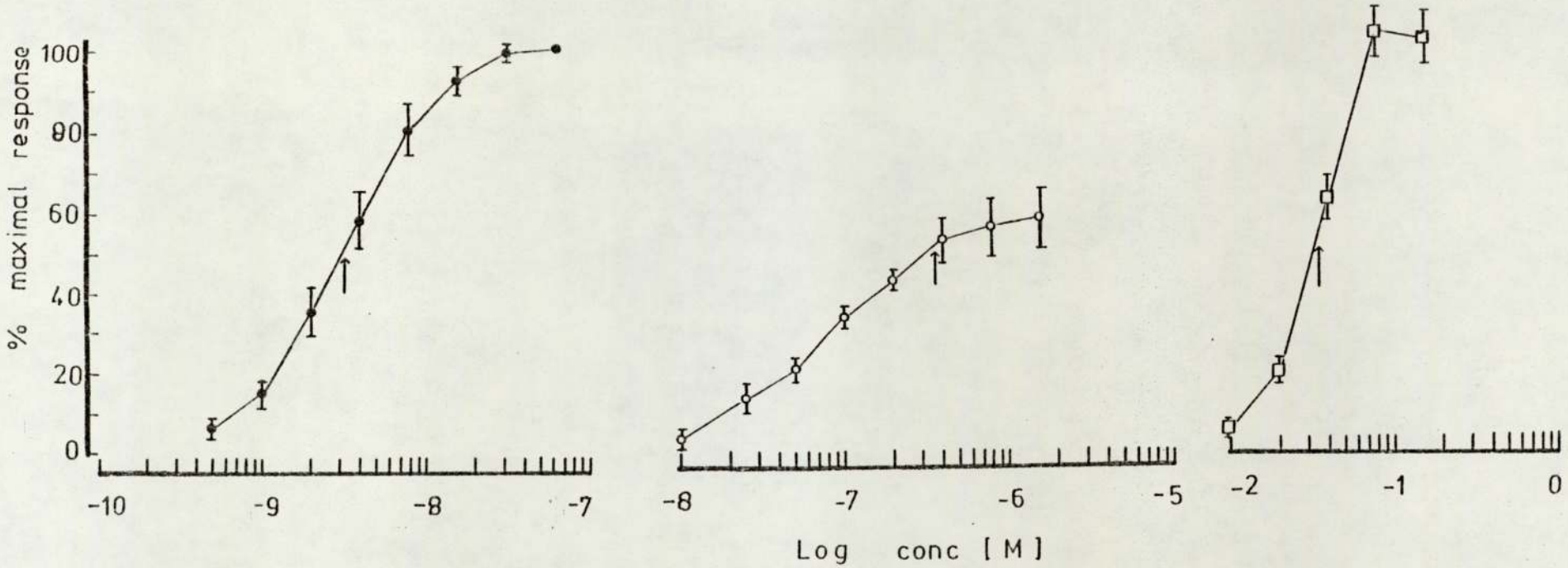


Fig. 4b

Log concentration response curves of the rat descending colon to Angiotensin (●); PGE_2 (O) and Potassium Chloride (KCl) (□).

Responses are expressed as a percentage of the maximal angiotensin response.

Arrows indicate the log-concentrations of spasmogens eliciting a contraction equivalent to 50% maximal angiotensin response.

Each point represents the mean and the vertical bars denote the S.E.M. (n=6).

B. Role of Calcium Ions in the Contractile Responses of Rat Descending Colon to Angiotensin

(1) Effect of Low $[Ca^{2+}]_e$ on the Responses of Rat Descending Colon to Angiotensin, PGE_2 and to KCl

Calcium deprivation of muscle preparations has been previously used in the study of the role of extracellular calcium ions in the contractile action of angiotensin in the guinea-pig ileum and rat uterus (Khairallah et al, 1965). In this study the role of $[Ca^{2+}]_e$ in the contractile action of angiotensin was studied by exposure of the rat descending colon to Ca^{2+}-free Tyrode. Fig 5 shows the effect of a 45 minute exposure of the rat descending colon to Ca^{2+}-free Tyrode containing 25μM Na_2EDTA to which sucrose (1.8mM) was added to maintain osmolarity. There was a decrease in the responses to all three spasmogens but throughout the exposure responses to angiotensin were reduced significantly more than the responses to either PGE_2 or KCl ($p < 0.001$, n=6 in each case). Thus after 25 minutes the responses to PGE_2 and to KCl were reduced to $42.6 \pm 3.8\%$ and $27.8 \pm 3.2\%$ (n=6) of control respectively whereas the response to angiotensin was abolished. These results suggested that all three spasmogens require external Ca^{2+} to elicit their responses and furthermore the responses to angiotensin were more dependent upon extracellular calcium ions than the responses to either PGE_2 or KCl.

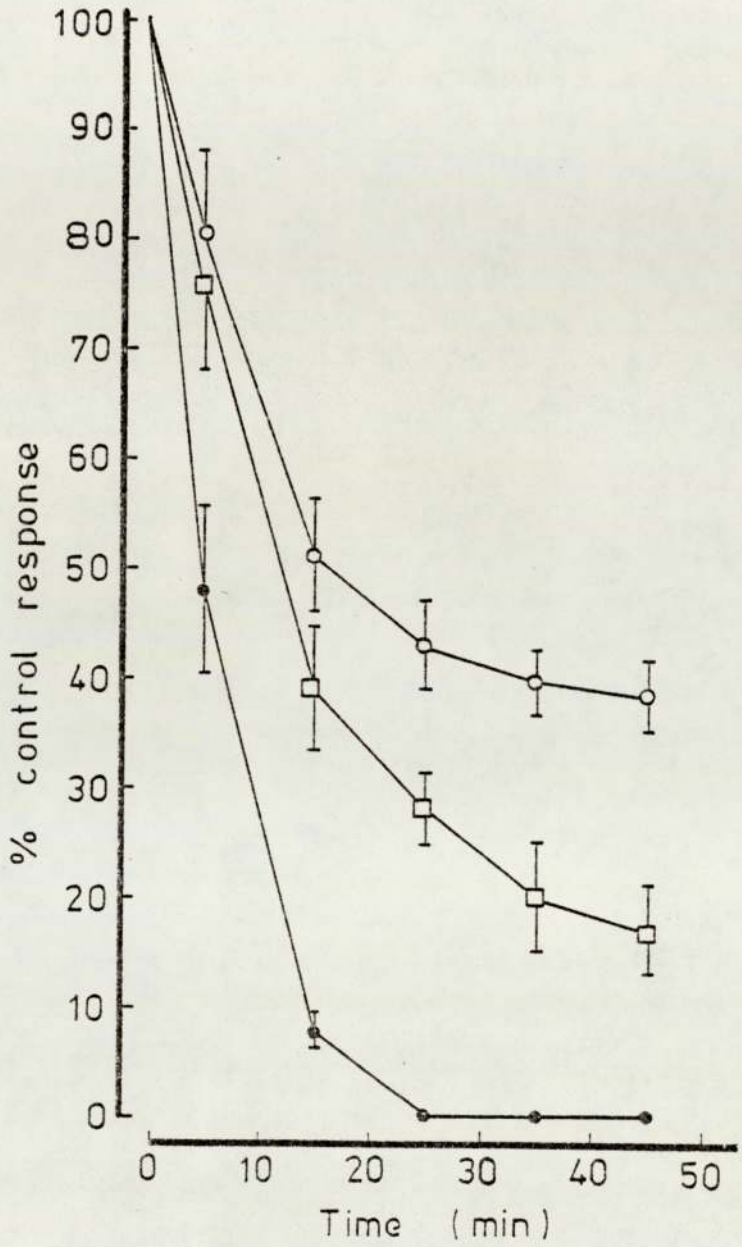


Fig. 5

The effect of exposure to Ca^{2+}-free Tyrode's solution containing Na2EDTA 25μM on the responses of the rat descending colon to angiotensin (●), PGE_2 (O) and KCl (□). Results are expressed as a percentage of control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode. Each point represents the mean and vertical bars denote the S.E.M. (n=6).

(ii) Effect of Dantrolene Sodium and Low $[Ca^{2+}]_e$ on the Responses of Rat Descending Colon to Angiotensin and to PGE_2

The apparent difference on responses of the rat descending colon to angiotensin and PGE_2 during exposure of the preparation to Ca^{2+}-free Tyrode in the previous study was investigated in order to determine whether it was due to differences in the mobilisation of intracellular Ca^{2+} by PGE_2. It was therefore thought that the addition to the Ca^{2+}-free bathing solution of a drug reputed to inhibit intracellular Ca^{2+} release might abolish the difference in response of the preparation to the two spasmogens if it was due to mobilisation of intracellular Ca^{2+} by PGE_2. Dantrolene sodium is a skeletal muscle relaxant thought to act by inhibition of a release of Ca^{2+} from the sarcoplasmic reticulum (Putney and Bianchi, 1974; Brocklehurst, 1975). As in the preceding experiment the preparations were exposed to Ca^{2+}-free Tyrode containing 25 μM Na_2EDTA to which sucrose (1.8mM) was added to maintain osmolarity. In addition dantrolene sodium 2.5×10^{-5}M (maximum solubility in physiological saline: 3.5×10^{-5}M, Ellis and Bryant, 1972) was added to the Ca^{2+}-free Tyrode. The results are shown in Fig 6 . There was a decrease in the responses to both spasmogens but throughout the exposure responses to angiotensin were reduced significantly more than the responses to PGE_2 ($p < 0.001$, n=6 in each case). Thus after 25 minutes the

responses to PGE_2 were reduced to 47.3 ± 7.0%, (n=6) of control whereas the angiotensin responses were reduced to 2.8 ± 1.6%, (n=6). Since responses to PGE_2 were significantly greater than the responses to angiotensin ($p < 0.001$) throughout the exposure of the rat descending colon to either Ca^{2+}-free Tyrode and dantrolene sodium (in this study) or to Ca^{2+}-free Tyrode alone (in preceding study) the results show that dantrolene sodium was without effect. The possible significance of this observation will be discussed in detail later in the 'Discussion'. Since other investigators have reported dantrolene sodium at concentrations effective in skeletal muscle to be ineffective in cardiac and smooth muscle (see Nott and Bowman, 1974) further experiments with dantrolene sodium were abandoned.

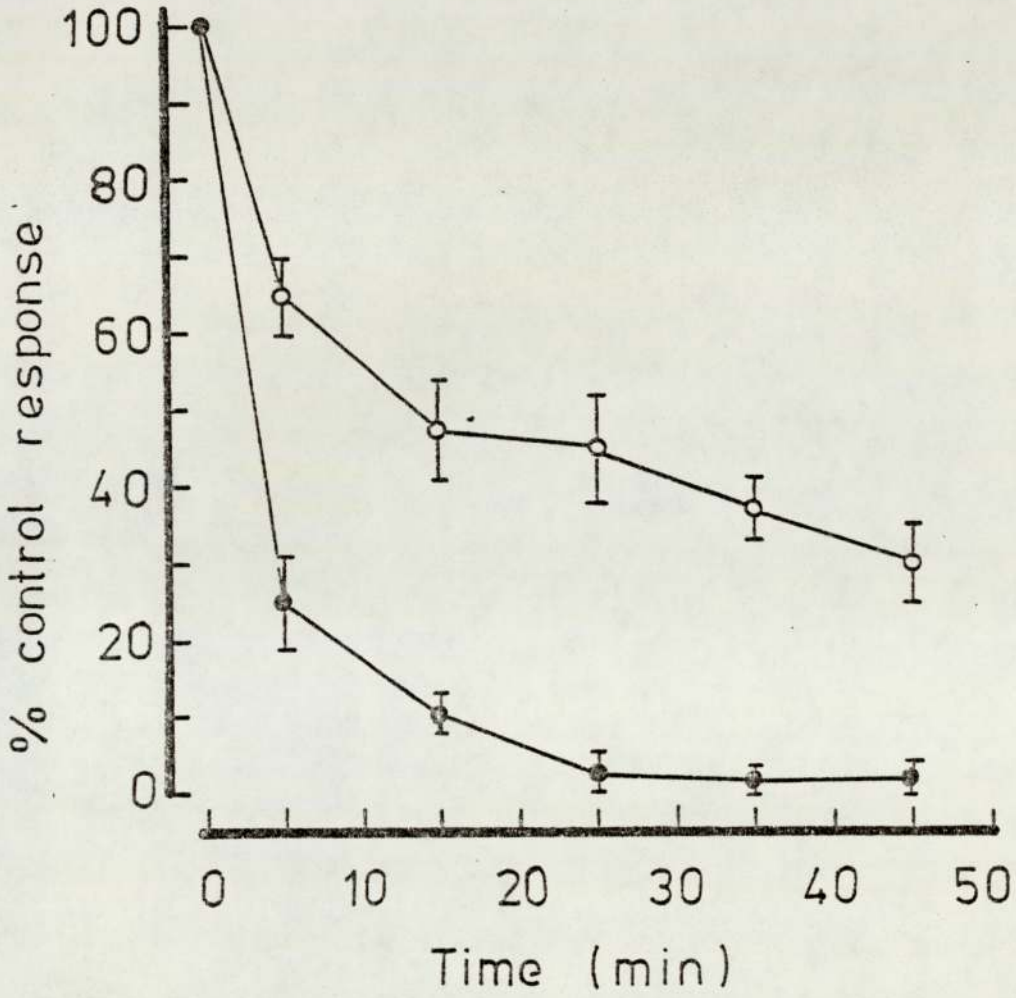


Fig. 6

The effect of exposure to Ca^{2+}-free Tyrode containing dantrolene sodium 2.5 x 10^{-5} M and Na_2EDTA 25μM on the responses of the rat descending colon to angiotensin (●) and PGE_2 (○). Results are expressed as percentage of control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode's solution. Each point represents the mean and vertical bars denote the S.E.M. (n=6).

(iii) Effect of increased $[Ca^{2+}]_e$ on the responses of Rat descending Colon to Angiotensin, PGE_2 and to KCl

Since deprivation of Ca^{2+} had a profound effect on the responses of the rat descending colon to angiotensin, the effect of increasing external $[Ca^{2+}]$ was studied. Fig 7 shows the effect of increasing the calcium ion concentration of the Tyrode's solution (tris buffer, gassed with oxygen) from 1.8mM to either 3.6mM, 7.2mM or 10.8mM. There was no change in the muscle tone of the preparation following the change in extracellular Ca^{2+} concentration. The average of three responses obtained after changing the Ca^{2+} concentration was expressed as a percentage of the control response obtained in normal Tyrode. An increase in Ca^{2+} concentration to 3.6mM caused a significant increase in the responses to angiotensin and to KCl to $186.2 \pm 4.6\%$ ($p < 0.001$, $n=6$) and $118.2 \pm 4.6\%$ ($p < 0.01$, $n=6$) respectively, compared to the control responses obtained in normal Tyrode, but the response to PGE_2 was unaffected. The increase in the angiotensin response was significantly greater than that of the KCl response ($p < 0.001$). A further increase in Ca^{2+} concentration to 10.8mM caused a significant increase ($p < 0.01$) in the response to angiotensin and PGE_2 to $178.5 \pm 4.1\%$ ($n=6$) and $125.5 \pm 15.0\%$ ($n=6$) of control respectively. However the response to KCl was significantly reduced to $54.5 \pm 4.8\%$ ($n=6$) of the control response obtained in normal Tyrode. The observed marked increase of responses to angiotensin during high

calcium ion concentration indicates a dependence of the angiotensin response on extracellular calcium ions. However, the reduction of the response to KCl at a higher extracellular Ca^{2+} concentration of 10.8mM was suggestive of membrane stabilization as reported by Holman (1958) for guinea pig taenia coli and Bohr (1963) for rabbit aortic strip. These results suggest that angiotensin exerts its contractile action by causing mobilisation of extracellular Ca^{2+} by a process independent of membrane depolarization.

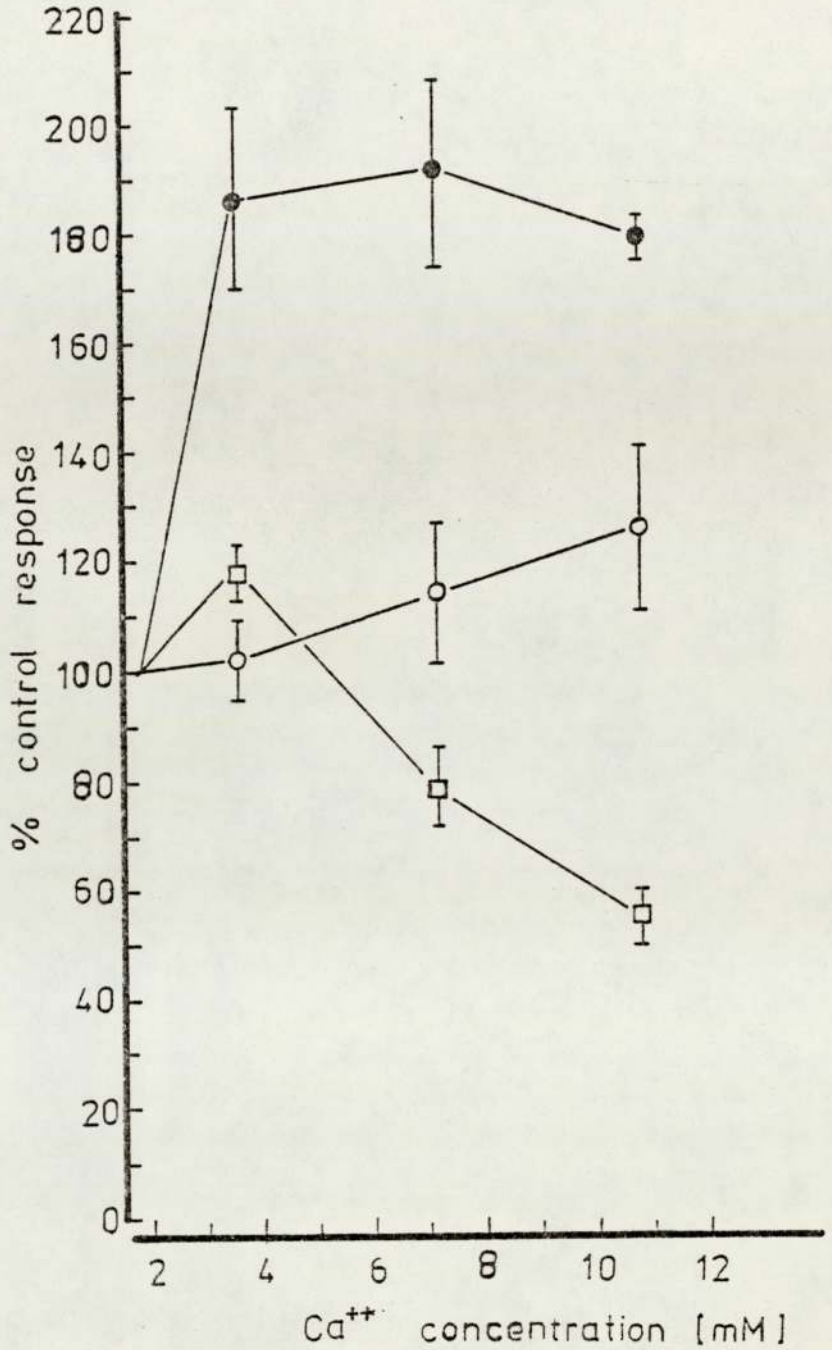


Fig. 7

The effect of increases in the Ca^{2+} concentration of the Tyrode's solution (tris buffer) on the responses of the rat descending colon to angiotensin (●), PGE_2 (○) and KCl (□). Results are expressed as a percentage of the control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode's solution containing 1.8mM Ca^{2+}. Each point represents the mean and vertical bars denote the S.E.M. (n=6).

(iv) Effect of SKF525A and Verapamil on Responses of Rat Descending Colon to Angiotensin, PGE_2 and to KCl

The role of extracellular Ca^{2+} in the contractile response of the rat descending colon to angiotensin was investigated further by the addition of either SKF525A or verapamil, agents which have been reported to inhibit calcium influx associated with membrane depolarization (Kalsner et al, 1970; Fleckenstein et al, 1975).

To eliminate the possible involvement of intracellular calcium, the tissues were deprived of extracellular Ca^{2+} by an initial incubation for 60 minutes in Ca^{2+}-free Tyrode (without addition of EDTA) containing either 2.6×10^{-5}M SKF525A (Fig 8) or 3.5×10^{-6}M verapamil (Fig 9). In both cases the responses to all three spasmogens were reduced below control more rapidly than during exposure to Ca^{2+}-free Tyrode without verapamil or SKF525A (see Table 1). At the end of the initial 60 minutes of incubation of preparations in Ca^{2+}-free Tyrode containing either SKF525A (Fig 8) or verapamil (Fig 9) the responses to all three spasmogens were abolished.

Figure 8 shows the effect of the addition of Ca^{2+} to the preparations preincubated in a Ca^{2+}-free Tyrode containing SKF525A (2.6×10^{-5}M), there was a rapid partial recovery of the responses to angiotensin (to $40.7 \pm 6.4\%$, $n = 6$) and PGE_2 (to $32.4 \pm 4.3\%$ $n=6$) within 5 mins whereas there

was no recovery of the responses to KCl. This was followed by a progressive decrease of responses to angiotensin and PGE_2. The reason for this decline is not clear but it may be due to ionic redistribution following the reintroduction of Ca^{2+} or to the progressive action of SKF525A. A similar progressive action of calcium antagonists has been reported in both cardiac muscle (Bayer et al, 1975a) and vascular muscle (Kalsner et al, 1970; Massingham, 1973; Bilek et al, 1974). Subsequent washing of the preparation with normal Tyrode without SKF525A resulted in a gradual recovery of the responses to angiotensin and PGE_2 which was greater than the recovery of the corresponding KCl responses. At 110 minutes after washing the preparation with normal Tyrode the responses to angiotensin and to PGE_2 were $86.4 \pm 4.1\%$ (n=6) and $71.7 \pm 4.9\%$ (n=6) respectively of control and were significantly greater ($p < 0.001$ in both cases) than those to KCl, $43.7 \pm 7.8\%$ (n=6) of control. The smaller recovery of the response to potassium indicated that the effect of SKF525A still persisted.

Figure 9 shows the results of a similar experiment with verapamil (3.5×10^{-6}M) and the results were similar to those obtained with SKF525A. Thus reintroduction of Ca^{2+} to muscle preparations preincubated in Ca^{2+}-free Tyrode containing verapamil resulted in rapid partial recovery of the responses of angiotensin and PGE_2 to $64.0 \pm 8.9\%$ (n=6) and $66.0 \pm 5.9\%$ (n=6) respectively within 5 minutes, whereas

the responses to KCl failed to recover. 110 minutes after re-exposure of muscle preparations to normal Tyrode by the removal of verapamil the responses to angiotensin and PGE_2 recovered to 75.0 $\pm$ 9.4% (n=6) and 66.2 $\pm$ 8.6 (n=6) respectively. In this experiment the responses to KCl recovered to 20.8 $\pm$ 3.3% (n=6) which was significantly less than the responses to angiotensin and PGE_2 ($p < 0.001$ in both cases).

The rapid partial recovery of the responses to angiotensin and PGE_2 but not to KCl (a depolarizing agent) during reintroduction of Ca^{2+} in the continued presence of either SKF525A or verapamil at a time when the muscle preparations deprived of Ca^{2+} were unresponsive to all the three spasmogens suggests that the contractile responses to angiotensin and PGE_2 may involve extracellular Ca^{2+} influx independent of membrane depolarization.

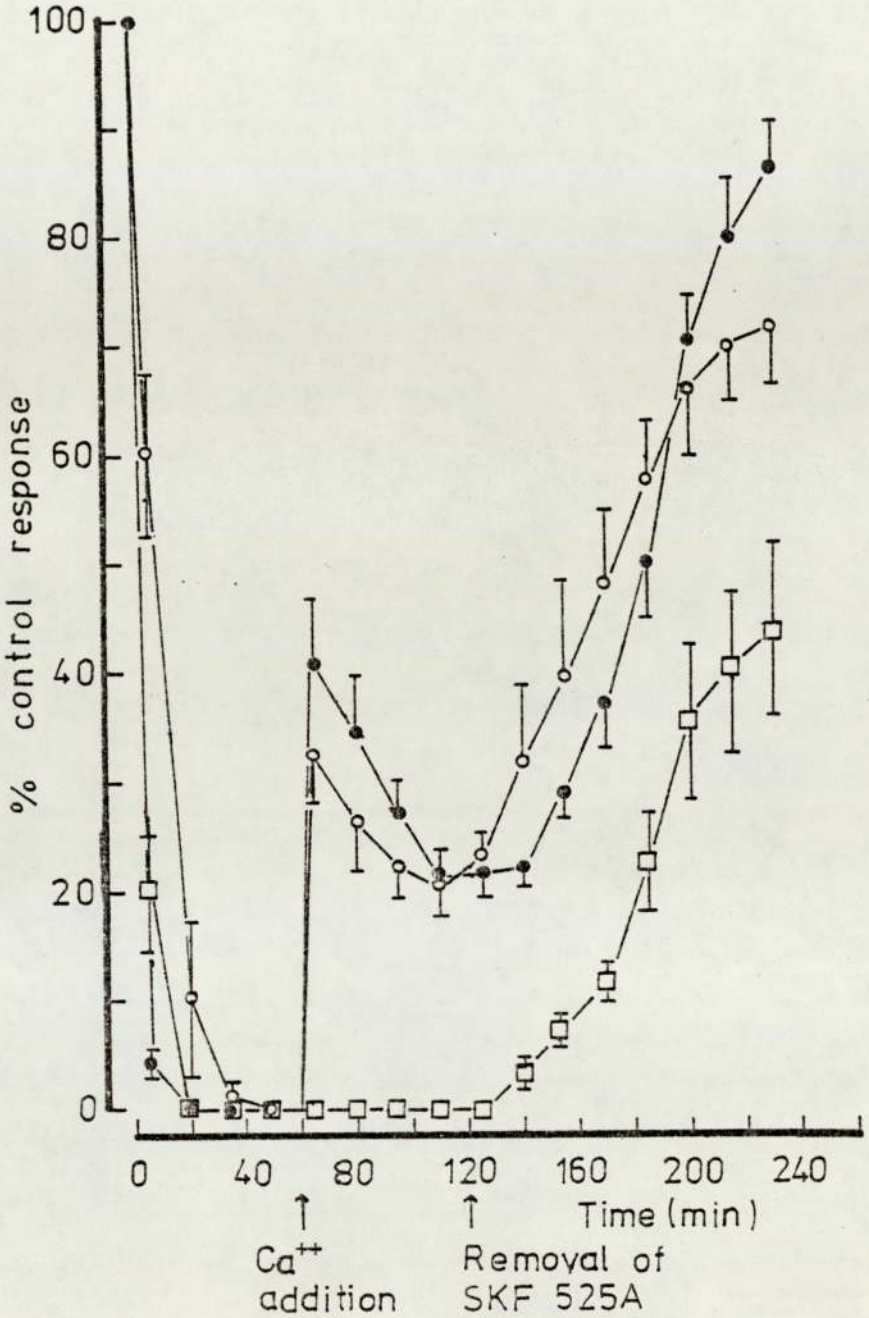


Fig. 8

The recovery of the responses to angiotensin (●), PGE_2 (○) and to KCl (□) of the rat descending colon following an initial 60 min incubation in Ca^{2+}-free Tyrode's solution containing SKF525A (2.6×10^{-5}M). The responses are expressed as a percentage of the control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode's solution. Reintroduction of Ca^{2+} at 60min and SKF525A removed at 120min. Each point represents the mean and the vertical bars denote the S.E.M. (n=6).

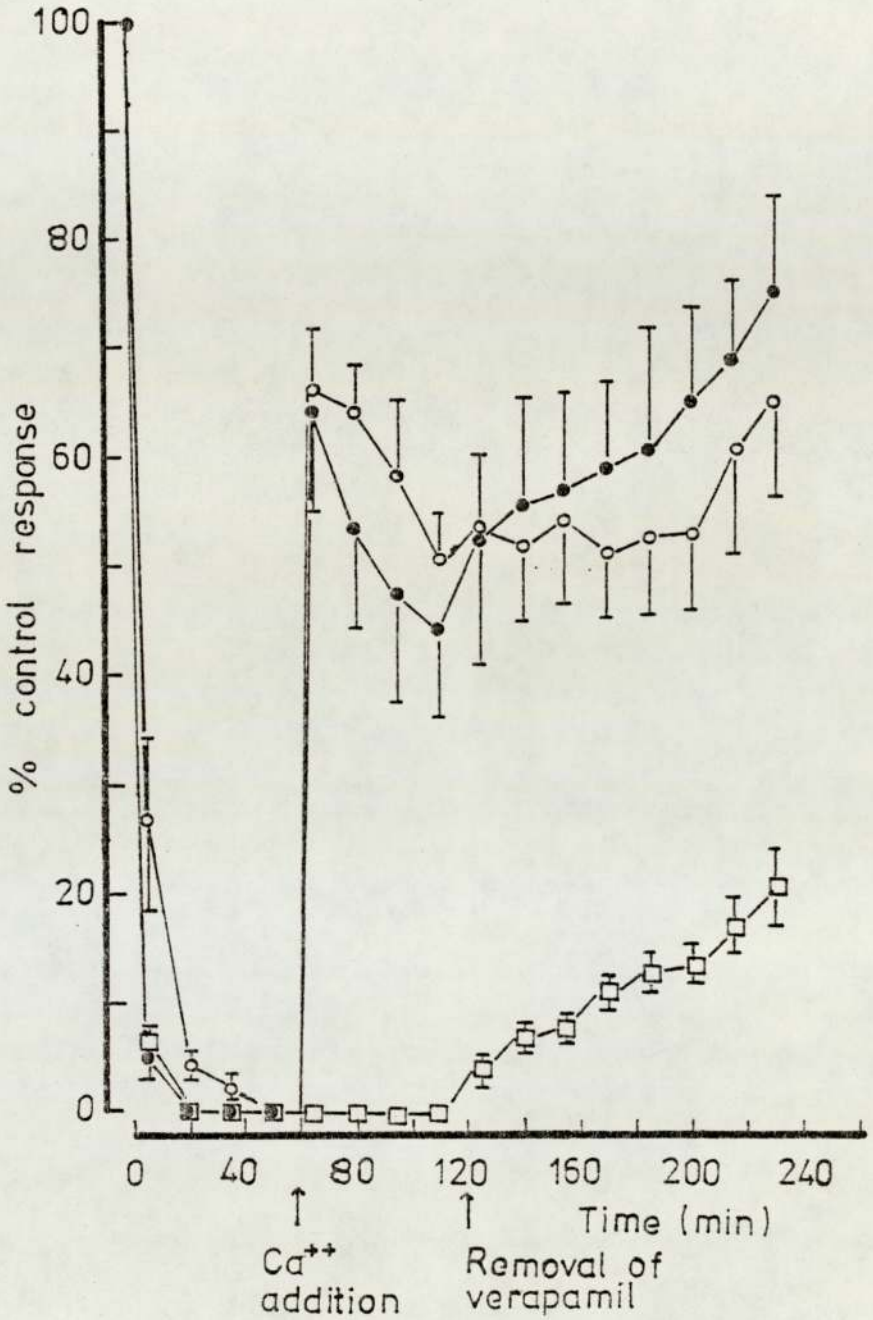


Fig. 9

The recovery of the responses to angiotensin (●), PGE_2 (○) and to KCl (□) of the rat descending colon following an initial 60 min incubation in Ca^{2+}-free Tyrode's solution containing verapamil (3.5×10^{-6}M). Results are expressed as a percentage of control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode. Reintroduction of Ca^{2+} at 60min and verapamil removal at time 120min. Each point represents the mean and the vertical bars denote the S.E.M. (n=6).

Table 1

Spasmogen	Mean time taken for the contractile responses of rat descending colon to be reduced to 50% of control values obtained in normal Tyrode's solution		
	Ca^{2+}-free Tyrode containing Na_2EDTA (25μM) Extrapolated from Fig 5	Ca^{2+}-free Tyrode containing SKF525A (2.6×10^{-5}M) Extrapolated from Fig 8	Ca^{2+}-free Tyrode containing Verapamil (3.5×10^{-6}M) Extrapolated from Fig 9
Angiotensin	5 min	3 min	3 min
PGE_2	16 min	8 min	3 min
KCl	12 min	3 min	3 min

C. Role of Sodium Ions in the Contractile Responses of Rat Descending Colon to Angiotensin

The role of sodium ions in the contractile action of angiotensin has been studied in a variety of smooth muscles (Turker et al, 1967; Blair-West et al, 1968; Godfraind, 1970, 1973). Although most investigators are of the opinion that sodium ions may be involved in the mobilisation of smooth muscle calcium by angiotensin, the exact mechanism is still in dispute. Thus in this study the effect of reduction in extracellular concentration of Na^+ was investigated on the isotonic contractions of the rat descending colon to angiotensin.

(i) Effect of Reduction in $[Na^+]_e$ on the Contractile Responses of Rat descending Colon to Angiotensin, PGE_2 and KCl

(a) Exposure to 68.5 mM Na^+-Tyrode

Fig10 shows the effect of a 50 minute exposure of the muscle preparations to Tyrode's solution in which the extracellular Na^+ was reduced from 137mM to either 68.5mM (dotted lines) or to 34.3mM (solid lines). Sucrose was added to maintain osmolarity. 5 minutes after exposure of the muscle preparations to Tyrode's solution containing 68.5mM Na^+ the responses to angiotensin, to PGE_2 and to potassium were increased to $148.7 \pm 10.1\%$, $119.2 \pm 5.6\%$ and $154.3 \pm 10.4\%$ (n=6 in each case). Subsequent responses to

angiotensin and PGE_2 were reduced whereas the responses to potassium remained potentiated throughout. Thus, after 50 minutes exposure to the Na^+-deficient solution the responses to angiotensin and PGE_2 were reduced to 74.3 ± 7.7% (n=6) and 53.3 ± 6.7% (n=6) of control respectively. On the other hand the corresponding potassium response was still potentiated to 147.8 ± 12.0% (n=6) of control responses obtained in normal Na^+-Tyrode.

(b) Exposure to 34.3mM Na^+-Tyrode

In similar experiments the muscle preparations were exposed to Tyrode's solution in which the Na^+ concentration was reduced further to 34.3mM (Fig 10) (solid lines). After 5 minutes exposure to this solution the responses to angiotensin and potassium were potentiated to 175.0 ± 23.8% (n=6) and 159.0 ± 11.4% (n=6) of control respectively. The corresponding responses to PGE_2 were reduced to 77.3 ± 8.7% (n=6) of control. After 50 minutes of exposure to this Na^+-deficient solution the responses to angiotensin and PGE_2 were diminished to 8.3 ± 1.0% (n=6) and 20.5 ± 3.4% (n=6) of control respectively. The responses to potassium were still potentiated to 131.2 ± 7.4% (n=6) of control responses obtained in normal Na^+-Tyrode's solution.

(c) Initial Angiotensin Response in 34.3mM Na^+-Tyrode

Brading (1973) has observed calcium influx linked to sodium efflux when smooth muscle preparations were exposed to Na^+-free solutions. In this study, it is possible that the potentiation of a single response to angiotensin immediately following the reduction in extracellular sodium could be attributed to calcium influx linked to sodium efflux. This proposal was tested using two experimental approaches; firstly, by varying the incubation time in low $[Na^+]_e$ before the determination of the initial angiotensin response. This was based on the hypothesis that a potentiation that is dependent on intracellular sodium to exchange with extracellular calcium would diminish with prolonged time of incubation in Na^+-deficient solution since the intracellular sodium pool would decrease due to diffusion down a concentration gradient. Fig 11 shows the effect of variation in incubation time on the initial response to angiotensin. Thus after 5 minutes of incubation in Tyrode's solution containing 34.3mM the initial responses to angiotensin were potentiated to 175.0 $\pm$ 23.7% (n=6) of control. However when the incubation time preceding the determination of the initial response was extended, the responses to angiotensin were markedly reduced. Thus the responses determined after 30 or 60 minutes of incubation were reduced to 75.5 $\pm$ 6.8% (n=6) and 30.8 $\pm$ 6.4% (n=6) of control respectively. A similar variation in incubation time in control

muscle preparations in normal Tyrode's solution showed a slight increase of the initial responses to angiotensin (Fig 11). This confirmed that the observed change in responses to angiotensin during exposure to Na^+-deficient solution was not a non-specific effect.

The decline of the angiotensin responses determined repeatedly during exposure of the rat descending colon to 34.3mM Na^+-Tyrode was more rapid than (reduced to approximately 25% of control in 30 minutes, see Fig 10) of the initial angiotensin responses (reduced to approximately 76% of control in 30 minutes, see Fig 11). This suggested that the responses to angiotensin were affected by both time of incubation of muscle preparation in Na^+-deficient Tyrode and prior exposure of the preparation to the spasmogen. The significance of the latter treatment will be considered in detail in the following experiment.

The second approach was a study of the effect of exposure of the muscle preparations to Tyrode's solution containing 34.3mM Na^+ on alternate equal responses to either angiotensin and potassium (Fig 12a,b) or angiotensin and PGE_2 (Fig 12 c,d). The spasmogens were added alternately at a 7.5 minute interval. In either case the responses studied were equivalent to the 50% maximal response to angiotensin. It can be seen from Fig 12 that exposure of the muscle preparation to Na^+-deficient

solution resulted in slight increase in the resting muscle tone of the preparation suggesting an increase in free intracellular calcium ions as a consequence of reduction of extracellular $[Na^+]$. The results in this experiment were similar to the ones described previously in that responses to potassium were potentiated throughout the period of exposure of preparations to Na^+-deficient Tyrode (Fig 12a,b). The initial response to angiotensin was enhanced (Fig 12a,c). However the increase of the initial response to angiotensin was not observed when the response was preceded by a contraction to another spasmogen (Fig 12b,d). This observation can be explained if it is assumed that the contraction preceding the angiotensin response involves calcium influx which would accelerate an already existing Ca-Na exchange process and thus lead to a quicker depletion of the intracellular sodium pool. Caution should be exercised in the interpretation of the possible effect of a contractile response which precedes the initial angiotensin response, since an outward diffusion of sodium down a concentration gradient during the interval of 7.5 minutes used between the addition of spasmogens may be a contributing factor. It should be realised that these two approaches used to test the possible involvement of Ca-Na exchange in potentiation of the initial angiotensin response following reduction in $[Na^+]e$ were indirect and need to be substantiated by direct measurement of ion flux.

In summary, these results show that extracellular sodium ions are essential for the contractile action of angiotensin, they further suggest that angiotensin may exert its contractile action by a process involving Ca-Na ion movement.

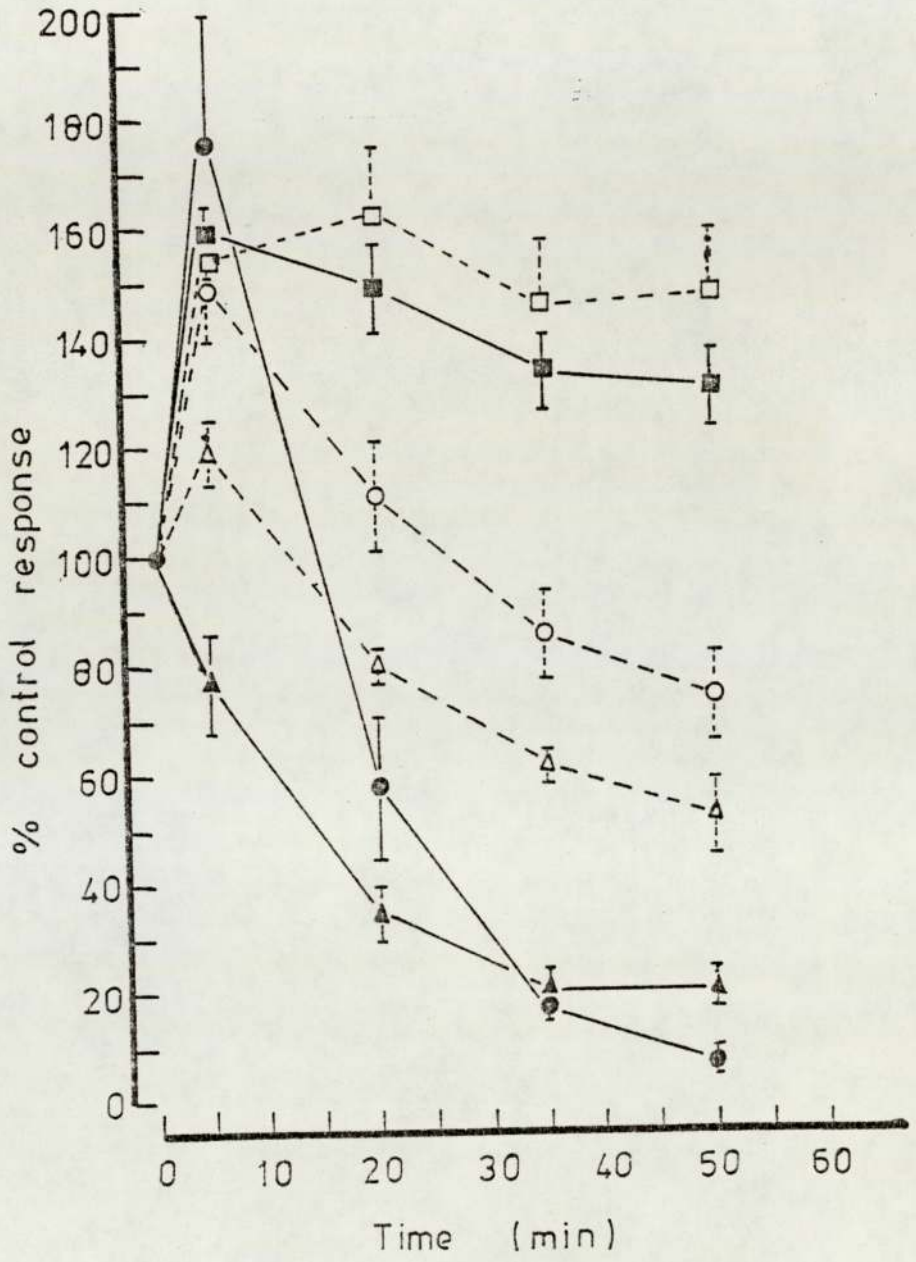


Fig. 10

The effect of exposure to Tyrode's solution containing either 68.5mM Na^+ (open symbols) or 34.3mM Na^+ (closed symbols) on the responses of the rat descending colon to angiotensin (○,●), PGE_2 (△,▲) and KCl (□,■). Results are expressed as a percentage of the control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode containing 137mM Na^+. Each point represents the mean and the vertical bars denote the S.E.M. (n=6).

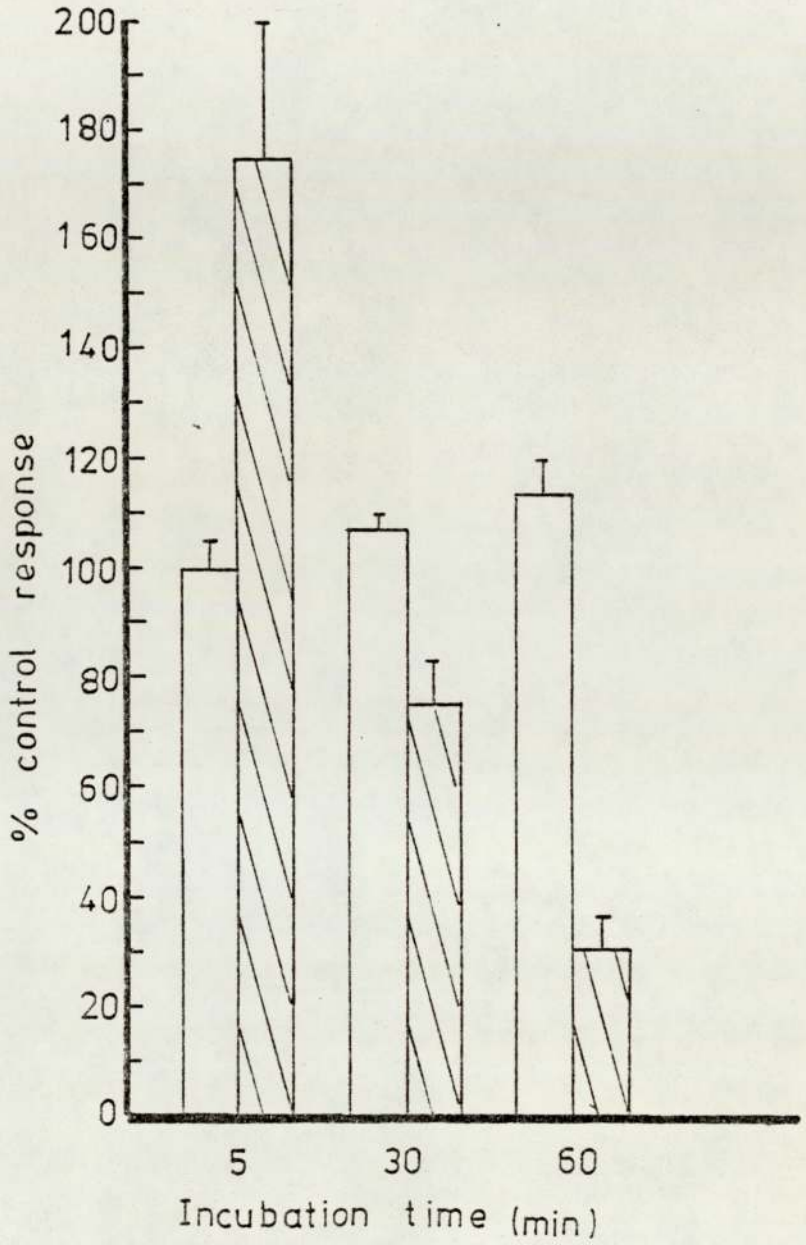
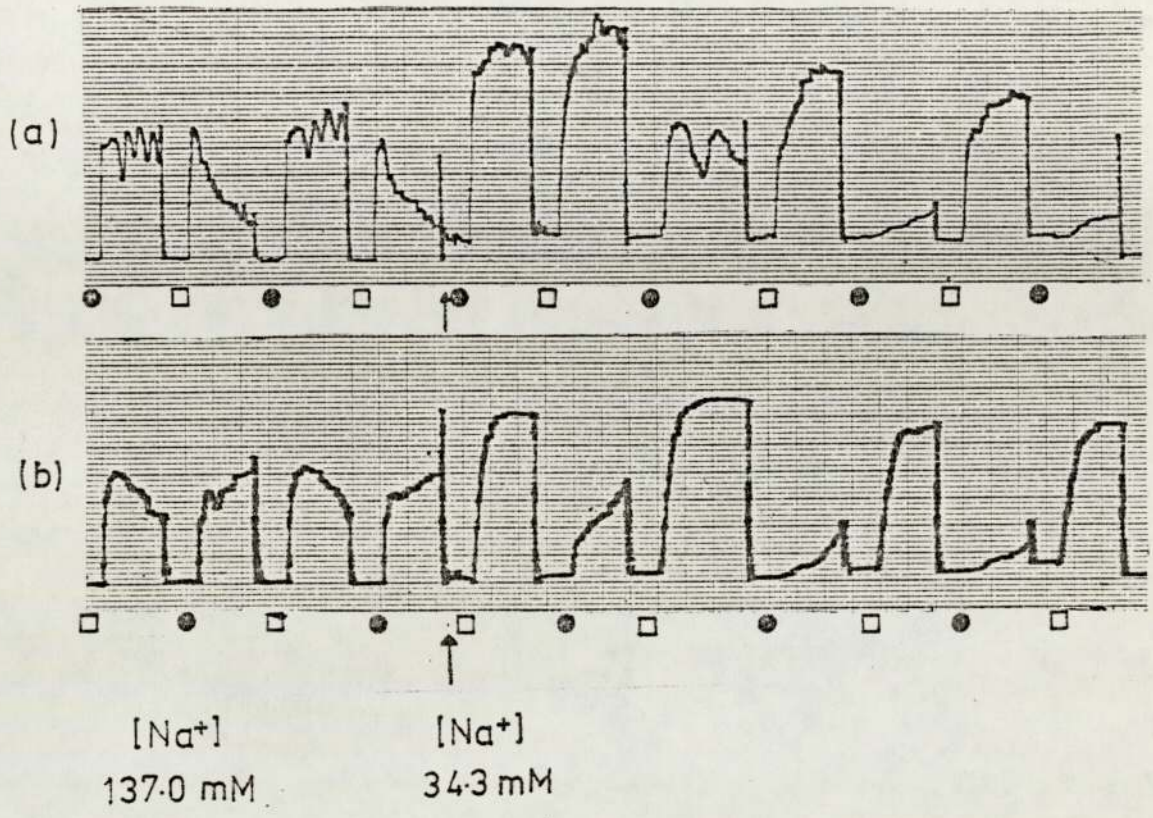
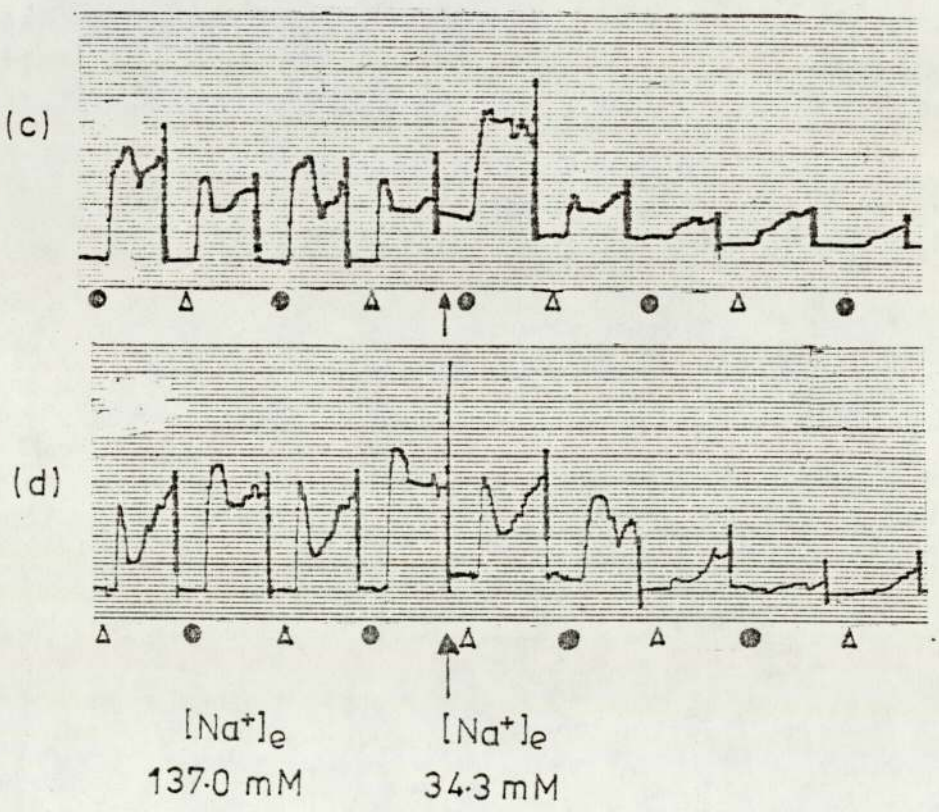


Fig 11.

The effect of variation of time of incubation on the initial response of the rat descending colon to angiotensin; Open columns: control muscle preparations incubated in normal Tyrode's solution; Crossed columns: muscle preparations incubated in Tyrode's solution containing 34.3mM Na^+. Each column represents the mean of the responses expressed as a percentage of the control (equivalent to 50% angiotensin maximum) response obtained in normal Tyrode containing 137mM Na^+ . Vertical bars denote the S.E.M. (n=6).


(a)
(b)
[Na+]
137·0 mM
[Na+]
34·3 mM


(c)
(d)
[Na+]e
137·0 mM
[Na+]e
34·3 mM

D. Role of Cyclic AMP on the Contractile Responses of Rat Descending Colon to Angiotensin

The smooth muscle relaxant effect of the β-adrenoceptor stimulant isoprenaline and the phosphodiesterase inhibitor theophylline is associated with an increase in intracellular concentration of cyclic AMP (Andersson, 1972; Marshall and Kroeger, 1973). The cyclic AMP mediates the relaxant effect by its ability to reduce free intracellular calcium (see review Bar, 1974). This observation has led to the suggestion that spasmogens may exert their smooth muscle contractile effect by decreasing intracellular levels of cyclic AMP (Robison, Butcher and Sutherland, 1966; Robison and Sutherland, 1970). The idea that contraction of smooth muscle might be related to a reduced cyclic AMP content received some support when it was reported that α-adrenergic stimulation was associated with a reduction of cyclic AMP formation in vascular tissue (Volicer and Hynie, 1971). In addition it was reported that adrenaline reduced the adenyl cyclase activity in rat uterus by stimulation of α-adrenoceptors (Triner et al, 1971). Moreover angiotensin has been reported to reduce cyclic AMP formation in vascular tissue (Volicer and Hynie, 1971). Thus in the following experiments the effect of agents which modify intracellular levels of cyclic AMP was investigated on the contractions of the rat descending colon to angiotensin, PGE_2 and potassium chloride. The rationale of this approach was that a

spasmogen action directly mediated by changes in intracellular level of cyclic AMP would be affected more than one not directly mediated by cyclic AMP changes.

(1) Effect of Theophylline and Imidazole on the Concentration-response curves of Rat Descending Colon to Angiotensin, PGE_2 and to KCl

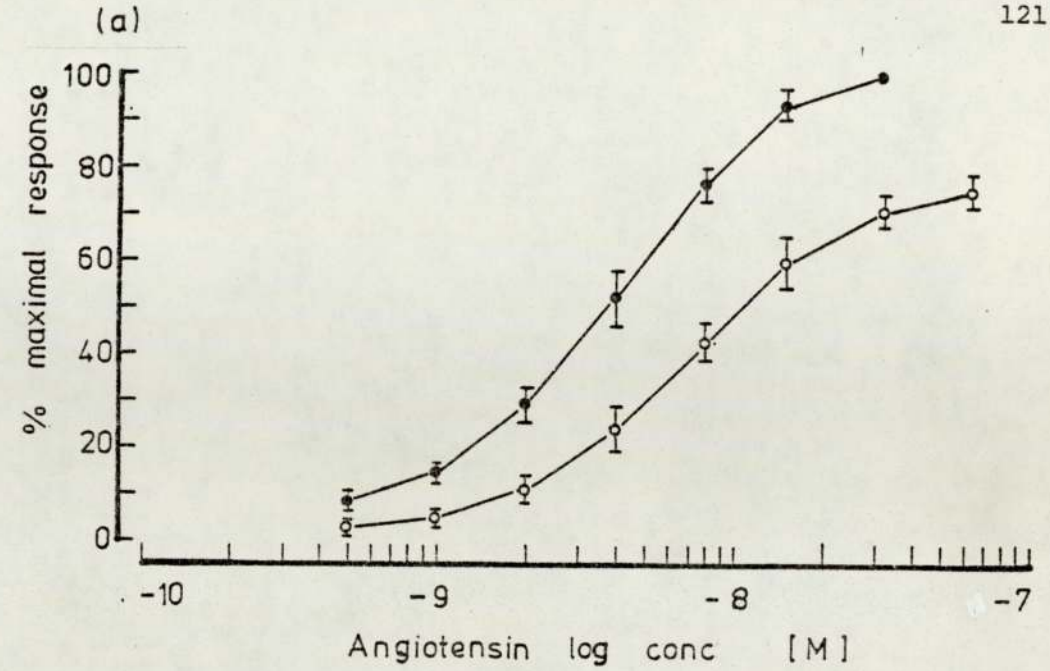
(a) Effect of Theophylline

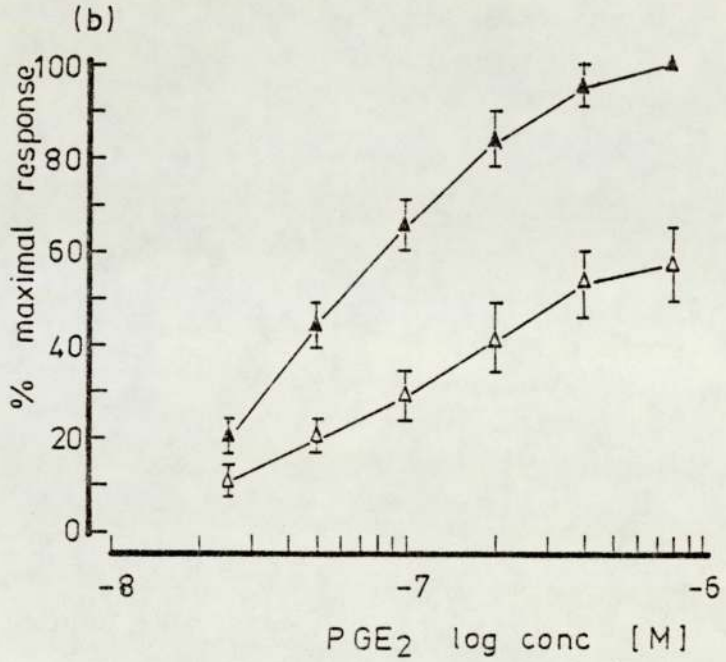
Theophylline has been shown to increase intracellular cyclic AMP levels by inhibition of phosphodiesterase (Butcher and Sutherland, 1962). It was considered that treatment of muscle preparations with this agent might have greater antagonistic effect on any spasmogen responses directly linked to changes in intracellular cyclic AMP than those causing contraction by a different mechanism. Thus dose response (concentration-response) curves to each spasmogen were determined in normal Tyrode. This was followed by an incubation in Tyrode's solution containing 0.3mM theophylline for 30 minutes to allow the drug to exert its effect. After this 30 minute incubation the concentration-response curve of each spasmogen was determined in the continued presence of theophylline. The responses were expressed as a percentage of the maximal response of the spasmogen in normal Tyrode's solution. As seen in Fig13a,b&c, theophylline caused a shift of the concentration-response curves for all three spasmogens to

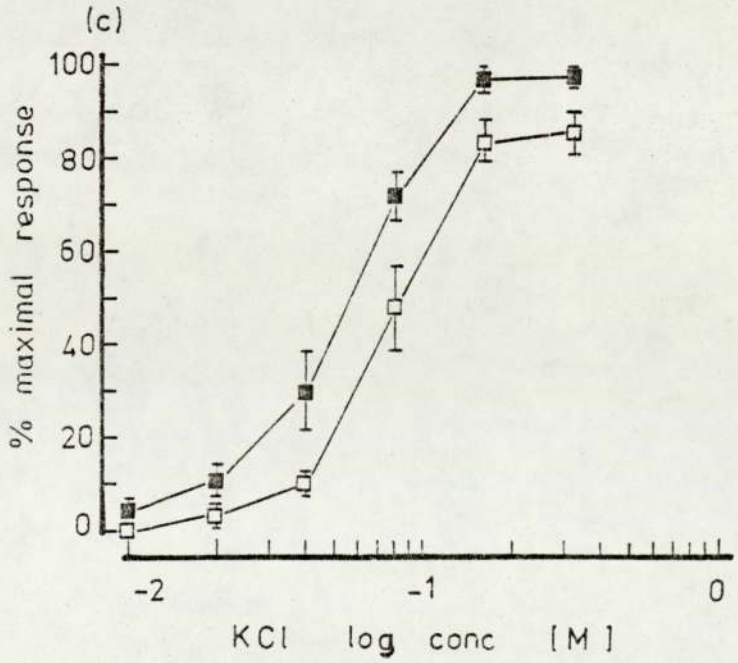
the right of those obtained in the absence of theophylline which indicated an inhibition of the responses. The inhibition by theophylline was unsurmountable since it caused a reduction of the maximal responses to angiotensin, PGE_2 and potassium chloride to 74.9 ± 3.1%, 56.8 ± 8.3% and 84.8 ± 4.6% respectively (for at least six observations in each case) of the maximal control responses. The shift of the concentration-response curves to the spasmogens were evaluated by estimation of the dose ratio (Gaddum, Hameed, Hathway and Stephens, 1955). This has been defined as the ratio of equieffective agonist (spasmogen) concentrations after and before addition of an antagonist, and has been found useful in assessing the effect of non-specific antagonists (Gaddum et al, 1955). Thus the ratio of the spasmogen concentrations that produced a 50% maximal response after and before treatment with theophylline was determined by interpolation (Table 2) of concentration-response curves for each muscle preparation as proposed by Schild (1949). It can be seen in Table 2 that the increase in the dose ratio was significantly greater for angiotensin and PGE_2 than for KCL ($p < 0.01$ in each case). This suggested that if there was an increase in the concentration of intracellular cyclic AMP, induced by theophylline, it was associated with a preferential reduction of the response to angiotensin and PGE_2 compared with potassium.

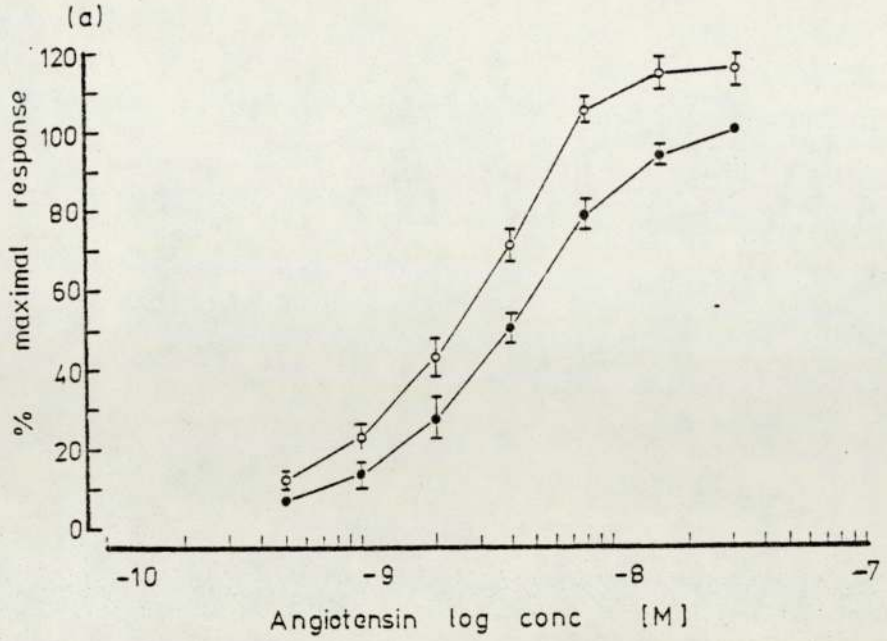
(b) Effect of Imidazole

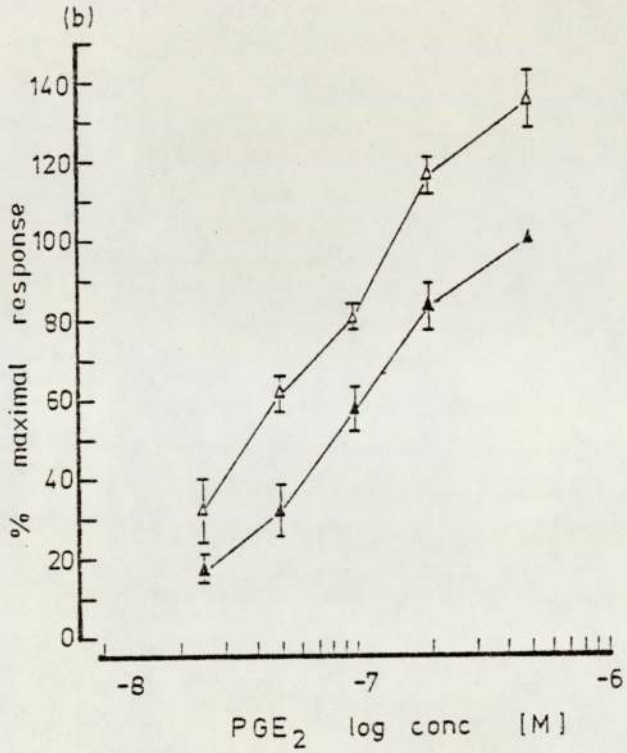
Similar experiments were performed with imidazole which has been shown to decrease intracellular concentration of cyclic AMP by activation of phosphodiesterase (Butcher and Sutherland, 1962). Responses were expressed as a percentage of the maximal response to the spasmogen obtained in normal Tyrode's solution. Treatment with 50mM imidazole caused a shift of the concentration-response curves for all three spasmogens to the left of those obtained in the absence of imidazole (Fig 14 a,b & c) which indicated a potentiation of the responses to the spasmogens. There was no significant difference in the decrease of the dose ratio (Table 2) for each spasmogen which indicated that the shift of concentration response curves to the left during treatment with imidazole was similar for all three spasmogens. Imidazole also caused an increase in the maximal responses to angiotensin, PGE_2 and potassium chloride to 115.5 $\pm$ 3.6%, 135.4 $\pm$ 7.2% and 104.2 $\pm$ 2.4% respectively (for at least six observations in each case).


(a)
% maximal response
100
80
60
40
20
0
-10
-9
-8
-7
Angiotensin log conc [M]


(b)
% maximal response
100
80
60
40
20
0
-8
-7
-6
PGE2 log conc [M]


(c)
% maximal response
100
80
60
40
20
0
-2
-1
0
KCl log conc [M]


(a)
% maximal response
120
100
80
60
40
20
0
-10
-9
-8
-7
Angiotensin log conc [M]


(b)
% maximal response
140
120
100
80
60
40
20
0
-8
-7
-6
PGE2 log conc [M]

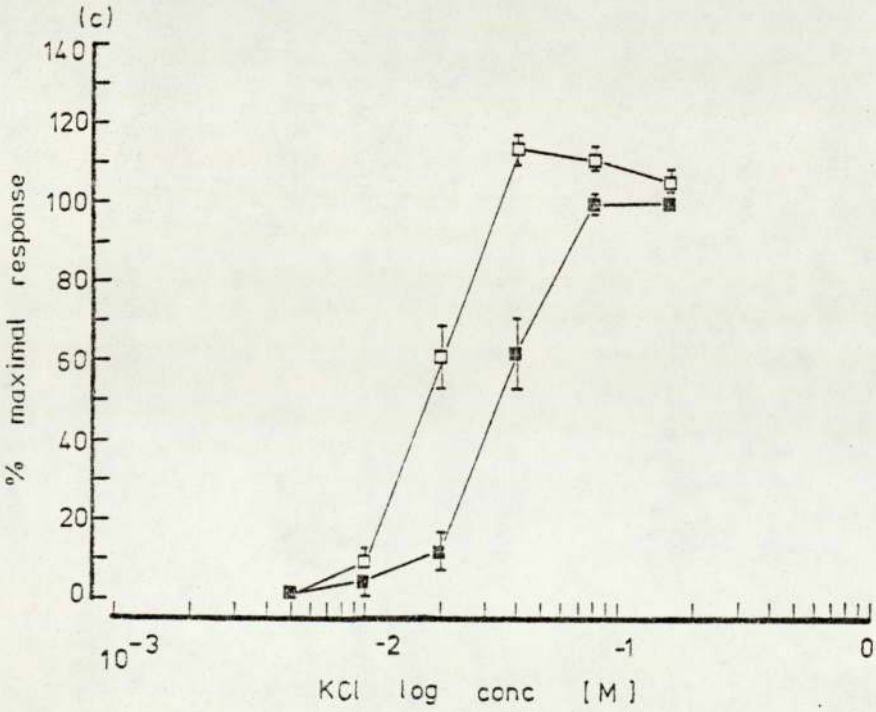

(c)
% maximal response
140
120
100
80
60
40
20
0
10-3
-2
-1
0
KCl log conc [M]

Table 2

Effects of Theophylline (0.3mM) and Imidazole (50mM) upon Isotonic responses of Rat descending Colon to Angiotensin, PGE_2 and KCl

Spasmogen	Dose Ratio[1]	
	With Theophylline (0.3 mM)	With Imidazole (50mM)
Angiotensin	3.31 ± 0.49 (n=7)^{xx}	0.58 ± 0.04 (n=10)^{NS}
PGE_2	6.20 ± 1.05 (n=6)^{xx}	0.50 ± 0.05 (n=6)^{NS}
KCl	1.60 ± 0.12 (n=6)	0.49 ± 0.02 (n=6)

[1]The ratio of the concentration of spasmogen producing 50% of the maximal response after and before treatment with either Theophylline or Imidazole.

NS $p > 0.05$; xx $0.001 < p < 0.01$ when comparing the dose ratio to that of KCl using Student's t-test for unpaired data.

(ii) Effect of Isoprenaline on the Responses of Rat Descending Colon to Angiotensin, PGE_2 and to KCl

Isoprenaline is a β-adrenoceptor stimulant that has been shown to increase intracellular cyclic AMP by activation of adenyl cylase (Marshall and Kroeger, 1973). Figure15 shows the effect of increasing concentrations of isoprenaline (5×10^{-9} - 1.6×10^{-7}M) added to the bath 30 seconds before the addition of either angiotensin, PGE_2 or potassium in concentrations that elicited contractions equal to the 50% maximal angiotensin response. There was no significant decrease in the resting tone of the muscle preparations following addition of isoprenaline. However isoprenaline caused a dose dependent decrease in the response to all three spasmogens with the potassium response significantly less affected throughout than the responses to either angiotensin ($p<0.001$) or PGE_2 ($p<0.001$) (n=6 in each case). These results are similar to those obtained with theophylline which also caused a greater reduction of responses to angiotensin and PGE_2 than of responses to potassium.

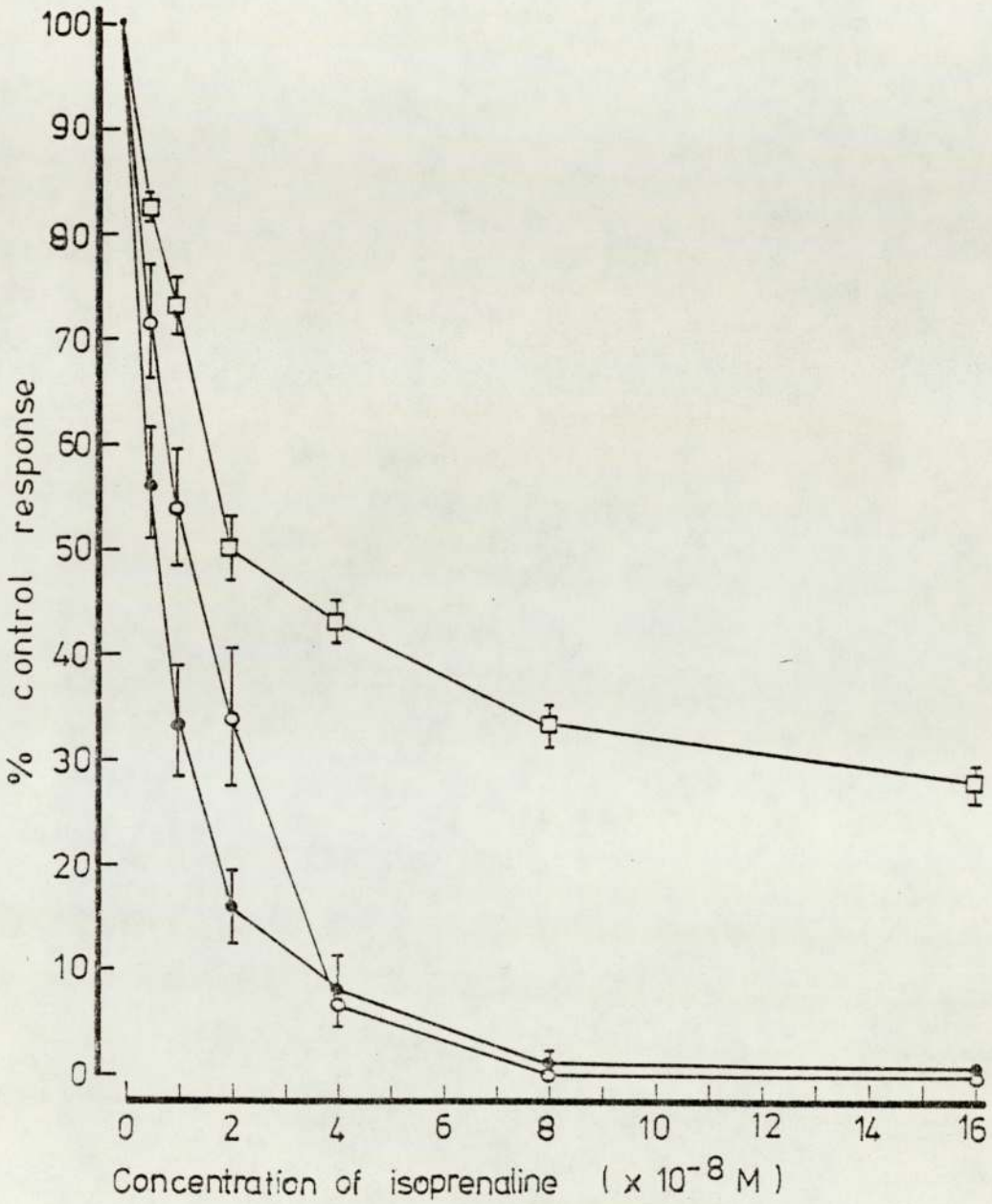


Fig. 15

The effect of isoprenaline on the responses of the rat descending colon to angiotensin (●), PGE_2 (○) and KCl (□). Results are expressed as a percentage of the control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode. Each point represents the mean and the vertical bars denote the S.E.M. (n=6).

(iii) Effect of Dibutyryl Cyclic AMP on the Contractile Responses of Rat Descending Colon to Angiotensin, PGE_2 and to KCl

Dibutyryl cyclic AMP is more stable to hydrolysis than cyclic AMP (Robison et al, 1971; Moore et al, 1968). It penetrates the cells easily (Falbriard et al, 1967) and acts by mimicking cyclic AMP at the site of action (Moore et al, 1968).

Figure16 shows the effect of increasing concentrations of dibutyryl cyclic AMP (0.01 - 0.64mM) added to the bath 5 minutes to allow penetration into the cell (exerts maximum relaxant action in ~ 5 minutes, Moore et al, 1968) before the addition of either angiotensin, PGE_2 or potassium in concentrations that elicited contractions equal to the 50% maximal angiotensin response. Dibutyryl cyclic AMP caused a dose dependent decrease in the responses to all three spasmogens with potassium responses being less affected. At the highest concentration of dibutyryl cyclic AMP added (0.64mM), the responses to angiotensin and PGE_2 were reduced to $6.6 \pm 2.5\%$ (n=5) and $11.0 \pm 3.0\%$ (n=5) of control respectively but the response to potassium was significantly less affected and was reduced to $42.8 \pm 5.4\%$ (n=5) of control ($p < 0.001$ in both cases). This showed that exogenously added dibutyryl cyclic AMP had similar effects upon the responses to the spasmogens as the treatments with both theophylline and isoprenaline.

During contraction of some smooth muscles the changes in intracellular cyclic AMP have been found to be reciprocally related to intracellular content of cyclic GMP (Goldberg, Haddox, Nicol, Glass, Sanford, Kuehl and Estensen, 1975). There have been suggestions that an increase in intracellular cyclic GMP may mediate contraction in smooth muscle (Lee, Kuo and Greengard, 1972; Goldberg et al, 1975). In three preliminary experiments similar to the one described for dibutyryl cyclic AMP, the addition of cyclic GMP ($2 - 16 \times 10^{-6}$M) was without effect on the responses to angiotensin, PGE_2 or potassium. Since the dibutyryl derivative was not used the lack of activity of cyclic GMP could be due to inability to penetrate into the cell, thus further studies with this nucleotide were abandoned.

Since the smooth muscle relaxant affects of theophylline, isoprenaline and dibutyryl cyclic AMP have been postulated to be due to an increase in intracellular levels of cyclic AMP (Marshall and Kroeger, 1973; Moore et al, 1968), the greater reduction of the responses to angiotensin and PGE_2 than KCl (a depolarizing agent) observed in this study support the hypothesis that the contractions to angiotensin and PGE_2 may be mediated by a decrease in intracellular cyclic AMP concentration. However the equipotentiation of the responses to the spasmogens when the muscle preparations were treated with

imidazole an agent which activates phosphodiesterase (Butcher and Sutherland, 1962) suggests either an involvement of cyclic AMP in the contractile responses to all three spasmogens or a non-specific potentiating effect by imidazole. The significance of this will be discussed in detail later in the Discussion chapter.

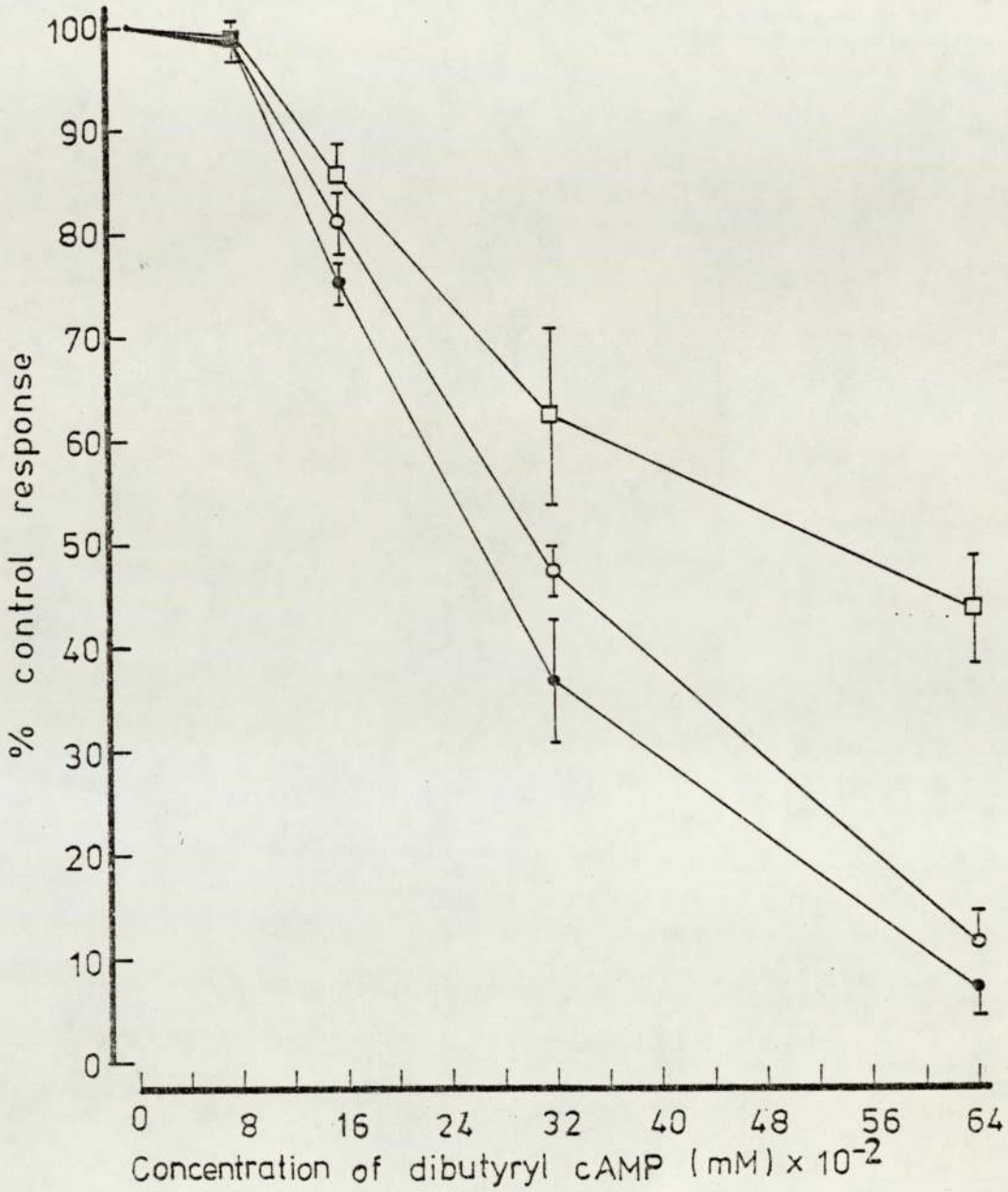


Fig. 16

The effect of dibutyryl cyclic AMP on the responses of rat descending colon to angiotensin (●), PGE_2 (○) and KCl (□). Responses are expressed as a percentage of the control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode. Each point represents the mean and the vertical bars denote the S.E.M. (n=5).

E. Role of Prostaglandins in the Contractile Responses of Rat Descending Colon to Angiotensin

It has been postulated that release of prostaglandins participates in the smooth muscle contraction induced by a number of spasmogens. Indomethacin, an inhibitor of prostaglandin biosynthesis (Vane, 1971), depresses responses of the rat uterus to bradykinin (Barabe, Park and Regoli, 1975) and angiotensin II (Baudouin-Legros, Meyer and Worcel, 1974). This has been interpreted as evidence that prostaglandins are involved in the contraction of uterine smooth muscle by these peptides, although under oestrogen domination the uterus is more sensitive to these spasmogens but less sensitive to prostaglandins (Weeks, 1972).

In vascular smooth muscles, in which prostaglandins usually produce relaxation (Weeks, 1972) prostaglandin release has been implicated in tachyphylaxis to angiotensin because this phenomenon was partly suppressed by indomethacin (Aiken, 1974).

On the other hand, Chong and Downing (1973, 1974) described a selective inhibition by indomethacin of angiotensin-induced contractions of the guinea-pig ileum, which was reversed by prostaglandin. They interpreted this as evidence that prostaglandin mediates angiotensin action. Aboulafia et al (1976) have not been able to demonstrate selective inhibition of the response to angiotensin of guinea-pig

ileum by indomethacin. In view of the above observations an investigation into the possible role of prostaglandins was undertaken on the contractile effects of angiotensin in the rat descending colon.

(i) The Effect of Indomethacin on the Contractile Responses of Rat Descending Colon to Angiotensin, PGE_2 and to KCl

In concentrations of 3-6 μM indomethacin inhibits prostaglandin synthesis in smooth muscle preparations (Ferreira, Moncada and Vane, 1972; Eckenfels and Vane, 1972; Flower, 1974).

Exposure of the muscle preparations to Tyrode's solution containing 5μM indomethacin for 50 minutes had no effect on the responses to angiotensin, PGE_2 and potassium compared to control obtained in normal Tyrode. Thus the concentration of indomethacin was increased to 50μM. Fig 17 shows the effect of a 50 minute exposure of the rat descending colon to a Tyrode's solution containing 50μM indomethacin. Exposure to this solution reduced the responses to all the three spasmogens and after 5 minutes exposure, the responses to angiotensin, PGE_2 and potassium were reduced to 85.2 $\pm$ 2.0%, 86.5 $\pm$ 7.1% and 53.9 $\pm$ 4.3% of control obtained in normal Tyrode respectively (n=6 in each case). After 5 minutes exposure to indomethacin,

there was a significantly greater reduction of the response to potassium than of the responses to angiotensin and PGE_2 ($p<0.001$ in each case), but at all other times the responses were equally reduced. This preferential reduction of responses to potassium 5 minutes after exposure of the preparation to a solution containing indomethacin (50μM) suggests that indomethacin was exerting a superficial effect, possibly by blocking ion fluxes at the muscle membrane (Northover, 1971, 1972). The subsequent non-selective reduction of responses to the three spasmogens does not support a specific role of prostaglandin release in contractions elicited by angiotensin.

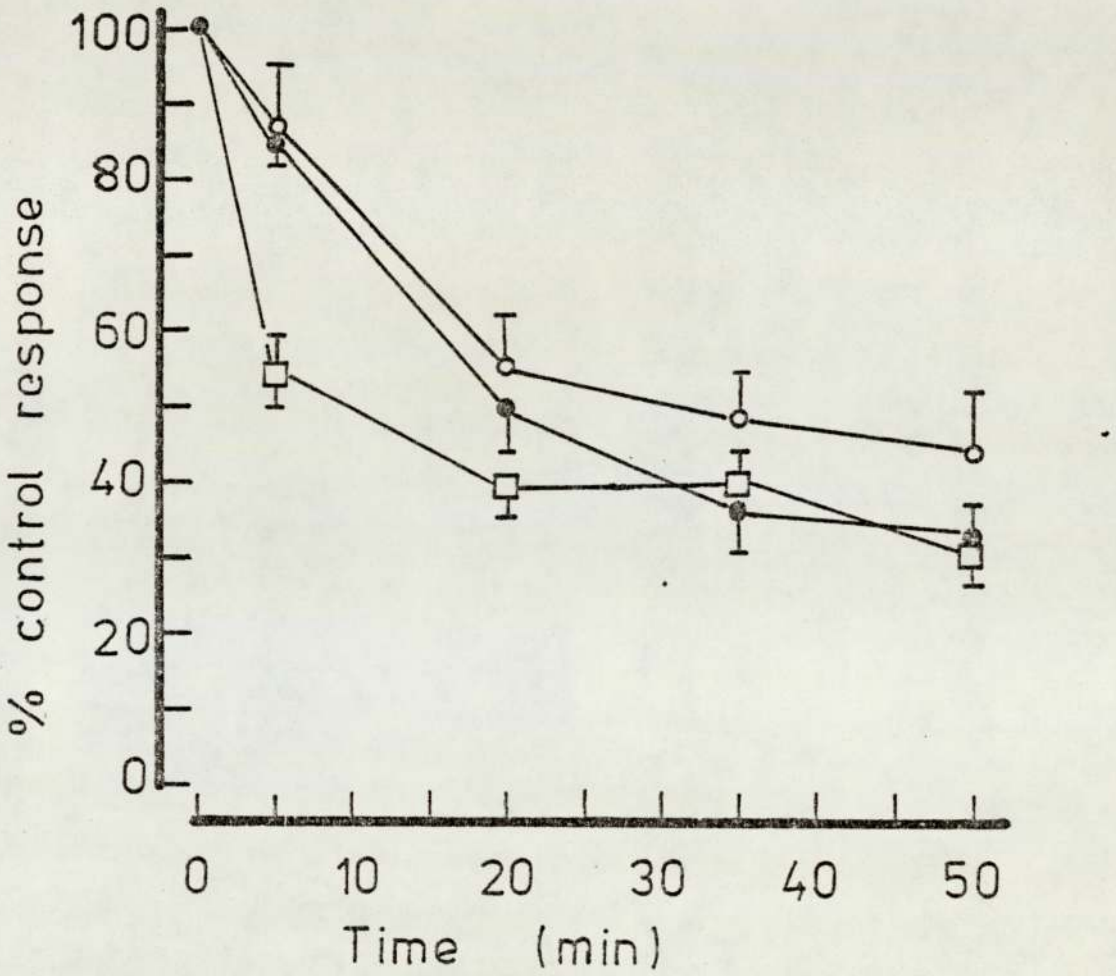


Fig. 17

The effect of exposure to Tyrode's solution containing indomethacin 50μM on the responses of the rat descending colon to angiotensin (●), PGE_2 (○) and KCl (□). Results are expressed as a percentage of the control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode. Each point represents the mean and vertical bars denote the S.E.M. (n=6).

F. Role of Metabolism in the Contractile Responses of the Rat Colon to Angiotensin

Substrate depletion and/or anoxia in a variety of smooth muscles has revealed that contractions induced by spasmogens differ in their metabolic requirements (Gross and Clark, 1923; Coceani and Wolfe, 1966; Crocker and Wilson, 1974, 1975). Thus, the contractile actions of angiotensin, barium and histamine upon the rabbit aortic strip but not those of catecholamines or potassium are dependent upon a continuous supply of exogenous glucose (Altura and Altura, 1970). The reasons for these differences are unclear but it has been suggested to be due to a difference in either the substrates used to yield ATP or the amount of energy required by the agonist to elicit its action. In the following experiment the role of metabolism was studied in the contractile responses of the rat descending colon to angiotensin, PGE_2 and potassium chloride during exposure to 2, 4-dinitrophenol, which has been shown to inhibit oxidative phosphorylation (Lardy and Elvehjem, 1945; West, Hadden and Farah, 1951; Rangachari, Paton and Daniel, 1972).

(i) Effect of 2,4-dinitrophenol on the Contractile Responses of Rat descending Colon to Angiotensin, PGE_2 and to KCl

Figure 18 shows the effect of a 65 minute exposure of the rat descending colon in Tyrode's solution containing 2,4 - dinitrophenol (0.05mM) on the isotonic responses to angiotensin, PGE_2 and potassium chloride. Exposure of the muscle tissues to this solution caused a progressive reduction of the responses to all three agonists. Throughout the period of exposure the reduction of responses to angiotensin and PGE_2 was significantly greater than that of the corresponding responses to potassium chloride ($p<0.001$, $n=6$ in each case). On the other hand there was no significant difference between the reduction of responses to angiotensin compared with PGE_2. These results show that 2,4 - dinitrophenol reduces the responses to angiotensin and PGE_2 more than responses to potassium. They suggest that the responses to angiotensin and PGE_2 either require more energy or are more dependent on ATP formed by oxidative phosphorylation than responses to potassium.

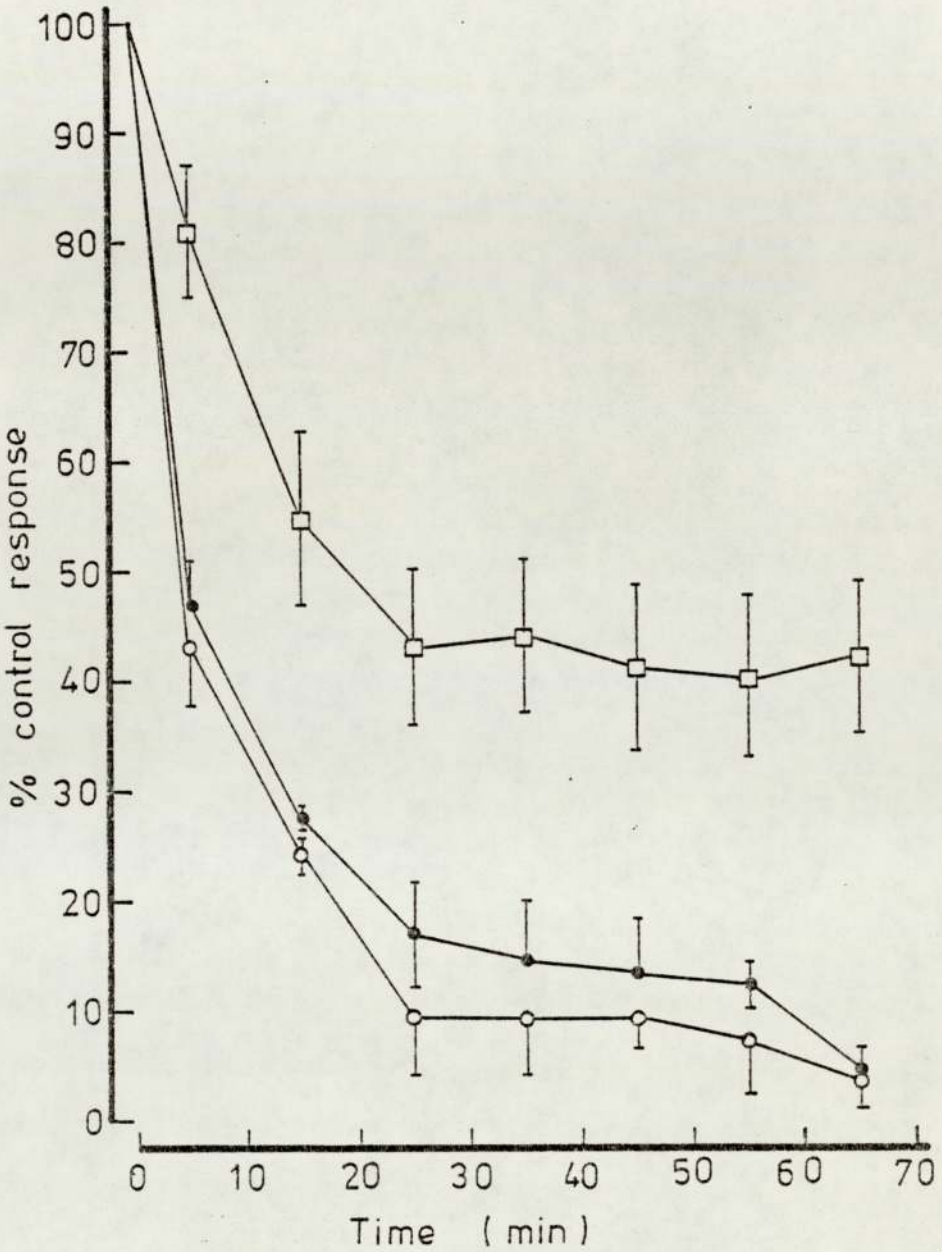


Fig. 18

The effect of exposure to Tyrode's solution containing 2,4-dinitrophenol 0.05mM on the responses of the rat descending colon to angiotensin (●), PGE_2 (○) and KCl (□). Results are expressed as a percentage of the control (equivalent to 50% angiotensin maximum) responses obtained in normal Tyrode. Each point represents the mean and vertical bars denote the S.E.M. (n=6).

SECTION II: ELECTROPHYSIOLOGICAL STUDIES

An Electrophysiological Study of the Action of Angiotensin II compared to Potassium Chloride on the Longitudinal Muscle of the Rat Descending Colon and the Guinea-pig Taenia Coli

The role of membrane excitation in the contractile effect of angiotensin of the longitudinal muscle of the rat descending colon was studied by the sucrose gap method. The investigation was extended to determine the role of calcium and sodium ions on the mechanical and electrical effects of angiotensin on both the longitudinal muscle of the rat descending colon and the guinea-pig taenia coli. In addition to an investigation of the effect of altered extracellular sodium and calcium ion concentration upon responses to angiotensin and potassium chloride the study has involved the use of verapamil and sodium-nitroprusside, drugs which have been reported by Golenhofen (1976) to antagonize respectively spike and spike-free calcium mobilisation in smooth muscle.

The guinea-pig taenia coli (a longitudinal muscle; Bulbring, 1954) was included in this study as a control, because like rat descending colon the contractile effect of angiotensin in this prepartion is wholly direct and not mediated by nerves (Ohashi, Nonomura and Ohga, 1967). These workers have also showed that the contractile response of the guinea-pig taenia coli to angiotensin is associated

with membrane depolarization and an increase in spike frequency. Potassium chloride has been used as a control spasmogen since its mechanism is reasonably well established (Burnstock and Straub, 1958; Shimo and Holland, 1966; Kuriyama, 1963).

1. Normal Electrical and Mechanical Activity of Smooth Muscle and the Action of Angiotensin and of Potassium Chloride

A. Resting Membrane Potential

(i) Longitudinal Muscle of Rat Descending Colon

Depolarization of the "inactive" end of the preparation in the sucrose gap apparatus by isotonic potassium chloride resulted in a gradual increase of the membrane potential over a period of 15-25 minutes to a constant level of 24.5 ± 2.5mV (n=6). Higher values of resting membrane potential of 58.6 ± 2.8mV (n=8) were obtained when isotonic potassium sulphate was used as the depolarizing agent.

(ii) Guinea-pig Taenia Coli

When the inactive end of guinea-pig taenia coli was perfused with isotonic potassium chloride the resting membrane potential increased to a maximum value of 23.0 ± 3.5mV (n=5) over a period of 20-30 minutes. However

when isotonic potassium sulphate was used, a higher resting membrane potential of 55.7 ± 2.8mV (n=8) was obtained. The resting membrane potential values obtained in this study were comparable to the values of 21.3mV and 56.1mV obtained by Burnstock and Straub (1958) when isotonic potassium chloride or potassium sulphate were used as the depolarizing agents. The higher values of resting membrane potential obtained when isotonic potassium sulphate was used as the depolarizing agent were close to the resting membrane potential of 60mV measured with internal electrodes for in situ length taenia coli (Bülbring, 1954), therefore in all subsequent experiments isotonic potassium sulphate was used as the depolarizing agent for the inactive end of the muscle.

B. Normal Electrical and Mechanical Muscle Activity

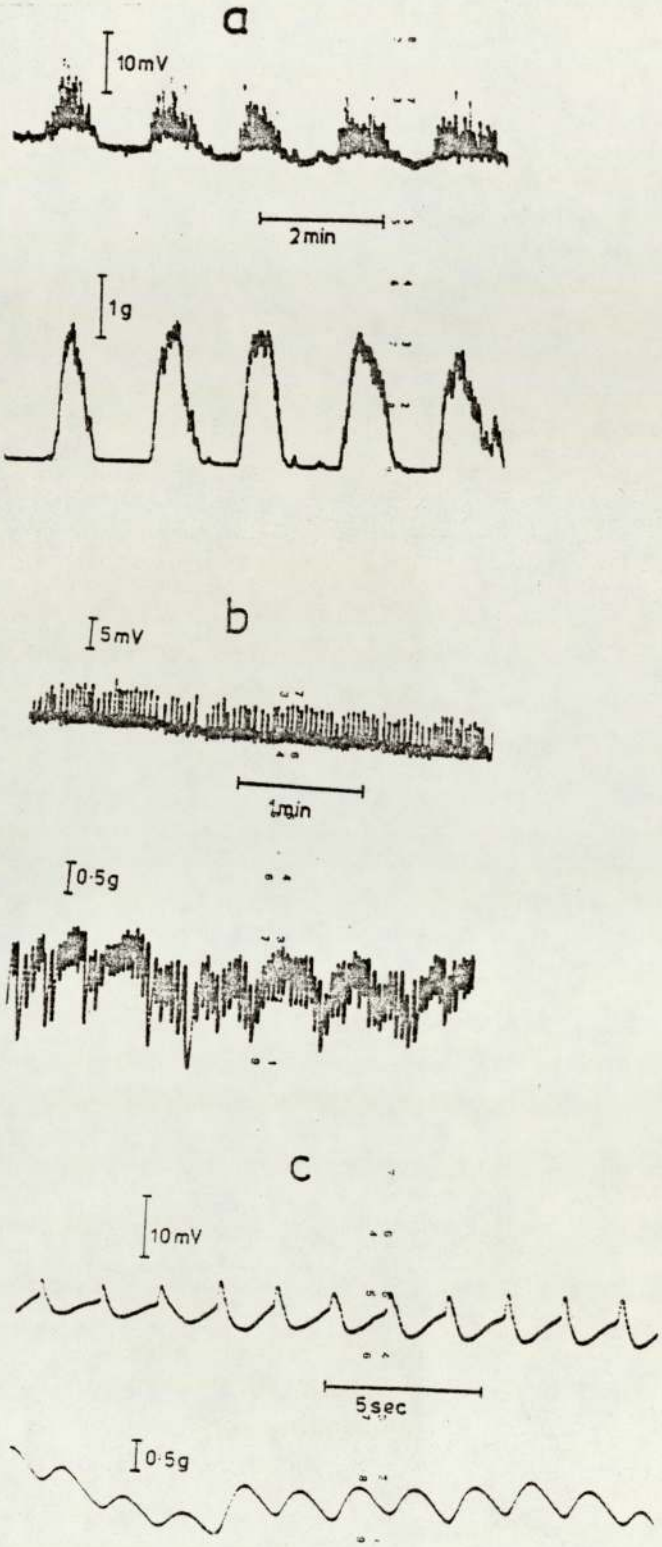
(i) Longitudinal Muscle of Rat Descending Colon

This muscle preparation was quiescent, and displayed neither spontaneous electrical activity nor contractions.

(ii) Guinea-pig Taenia Coli

As the resting membrane potential approached a stable value 20-30 minutes after the addition of isotonic potassium sulphate on the "inactive" side of the preparation the resting membrane potential became unstable and displayed

cyclic fluctuation (slow waves). These consisted of a depolarization at the test end of up to 5mV which was accompanied by a discharge of action potentials (spikes) at the crests of depolarization followed by a repolarization of the membrane potential and abolition of spikes (Fig 19a). These cyclic electrical changes varied in duration and frequency of occurrence in different preparations. Spontaneous mechanical activity also changed in harmony with the electrical fluctuation, hence membrane depolarization and spiking resulted in an increase in resting tension and phasic contractions which were followed by muscle relaxation during membrane repolarization (Fig 19a). The observed spike activity was irregular varying in amplitude (2-8mV) and frequency of occurrence. In some preparations the magnitude of the slow waves was small (1-2mV) with a short interval between them, in such preparations the spikes occurred more regularly and the preparation showed more spontaneous contractions (Fig 19b). The spontaneous activity of the preparation shown in Fig 19b was recorded at a higher chart speed (Fig 19c) for more detailed analysis, and it was observed that every spike was associated with a phasic contraction. The spikes were similar to those described by other investigators (Bulbring, 1957; Burnstock and Straub, 1958; Holman, 1957). They occurred at a frequency of 0.5 per second and showed an initial slow wave of depolarization followed by a rapid repolarization with an after-hyperpolarization. Their amplitude ranged from 5-7mV.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C. Effect of Angiotensin and Potassium Chloride on Longitudinal Muscle of the Rat Descending Colon and Guinea-pig Taenia Coli

(i) Longitudinal Muscle of Rat Descending Colon

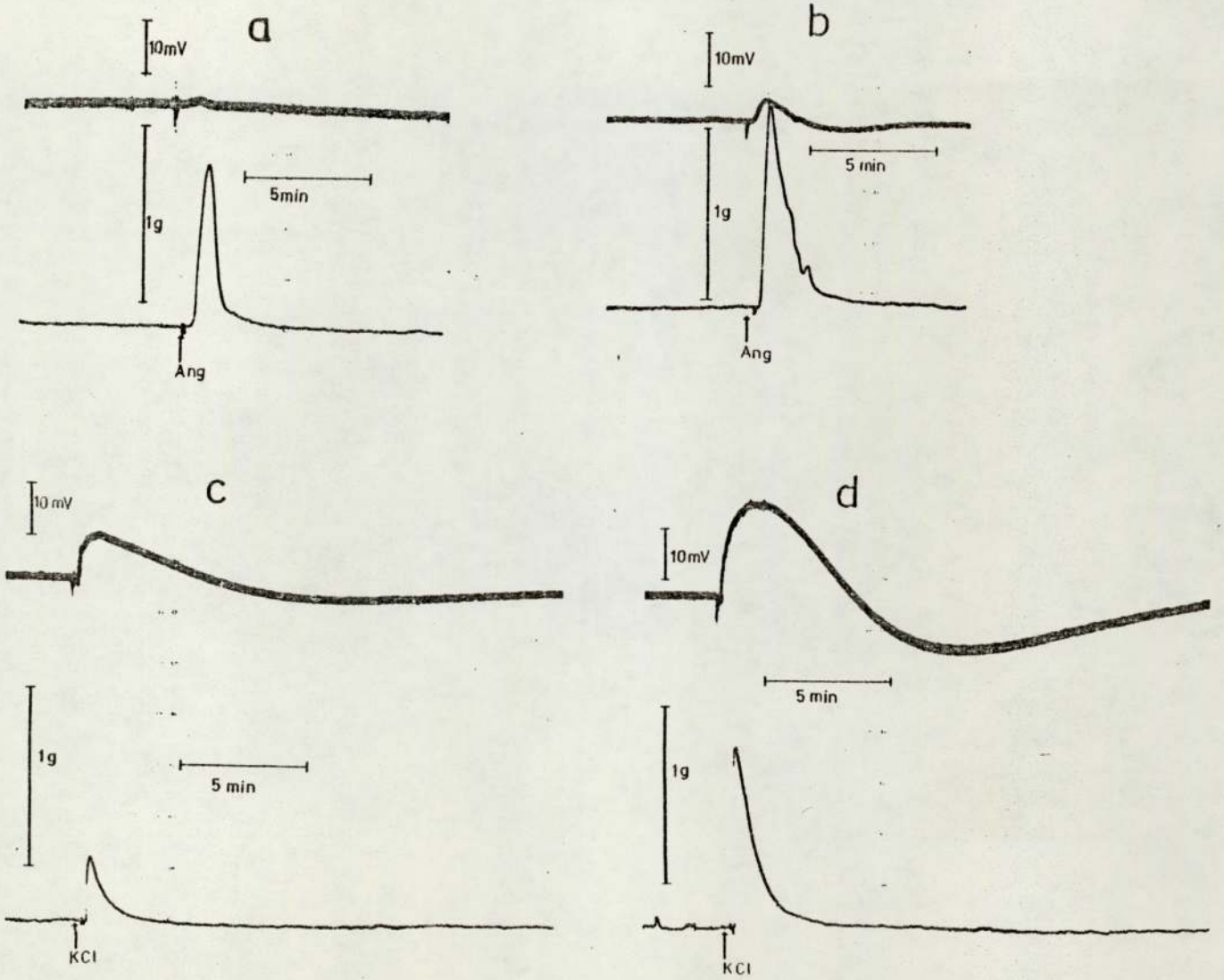
(a) Effect of Angiotensin on Longitudinal Muscle of Rat Descending Colon

Addition of a bolus of 20μl of angiotensin (2×10^{-7}M) to the longitudinal muscle of the rat descending colon increased the resting tension and caused a membrane depolarization of 1mV (Fig 20a). A similar addition of angiotensin (8×10^{-7}M), induced a greater isometric contraction and caused a greater membrane depolarization of 4mV (Fig 20b). However, in some preparations bolus injections of angiotensin that caused maximal contraction were not associated with a detectable membrane depolarization. Nonetheless in those cases where membrane depolarization was accompanied by contraction the membrane effects paralleled the mechanical events. Thus membrane depolarization nearly always preceded the initiation of contraction to angiotensin and the maximal depolarization occurred before maximal contraction. Maximum depolarization was followed by a slower repolarization and relaxation. Repolarization was followed by an afterpolarization of 1-5mV above the initial resting level.

(b) Effect of Potassium Chloride on Longitudinal Muscle of Rat Descending Colon

Addition of a 20μl bolus of 0.5 molar KCl to the longitudinal muscle of the rat descending colon caused a large membrane depolarization of 8mV (Fig 20c). A 20μl bolus injection of higher concentration of KCl (2 molar) caused an even larger membrane depolarization of 18mV (Fig20d) with a correspondingly larger isometric contraction. Membrane depolarization always preceded initiation of contraction and maximal contraction coincided with or occurred before maximal depolarization. In this case membrane depolarization lasted longer than the duration of contraction. Repolarization of the membrane was followed by an after hyperpolarization of 3-9mV which was dependent upon the concentration of the bolus of KCl used (Fig20c,d).

These results show that the angiotensin contractile responses of longitudinal muscle of rat colon were accompanied by a small membrane depolarization and in some cases the membrane depolarization was not detectable. Unlike angiotensin, contractile responses to potassium chloride were always accompanied by membrane depolarization, the degree of depolarization in any one preparation was related to the amount of KCl added.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(ii) Guinea-pig Taenia Coli

(a) Effect of Angiotensin on Guinea-pig Taenia Coli

Addition of 20μl bolus injection of angiotensin (2×10^{-7}M) to the guinea-pig's taenia coli caused an increase in spike frequency and a depolarization of the membrane potential of 2mV (Fig 21a). These changes in membrane electrical activity were associated with a sustained contraction. A 20μl bolus of higher concentration of angiotensin (4×10^{-7}M) caused a further increase in spike frequency and a depolarization of 7mV (Fig 21b). The corresponding sustained contraction was also slightly larger with this bolus injection of higher angiotensin concentration. A more detailed analysis of the effect of angiotensin upon spike activity revealed an initial increase in spike frequency without change in size that was followed by an increase in spike height at a lower frequency. Thus in a typical preparation which had a basal spike rate of 36 per minute with an average spike height of 7mV; on addition of angiotensin the spike frequency increased to 60 per minute in the first 25 seconds without increase in spike amplitude, which was followed by an increase in spike size to 9mV at 40 spikes per minute. In general the addition of angiotensin was followed by gradual depolarization to a steady level with increase in frequency and size of spikes. The maximum depolarization often coincided with the maximum increase in sustained tension but sometimes occurred a few

seconds before. After reaching the maximum tension and maximum depolarization the tension gradually declined to the original level. The membrane potential however, recovered more slowly and also returned to a level of 1 - 2mV higher than the resting value recorded initially. The addition of angiotensin always caused an increase in spike size and frequency, and membrane depolarization depending on the concentration of angiotensin in the bolus injection, it was however observed that in some preparations of guinea-pig taenia coli (2 in 6 experiments) the membrane depolarization was never more than 3mV irrespective of the concentration of angiotensin.

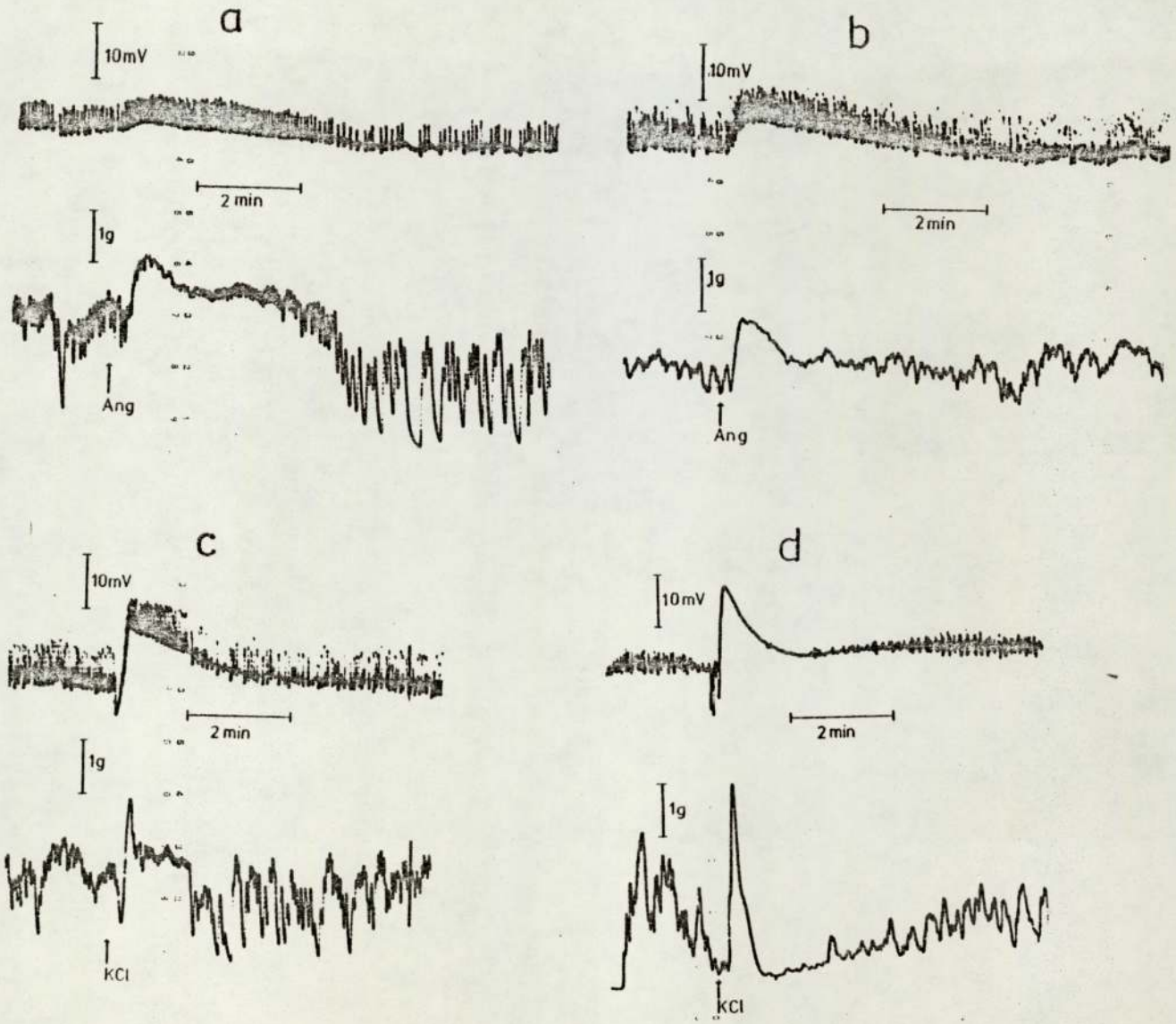
(b) Effect of Potassium Chloride on Guinea-pig Taenia Coli

When guinea-pig taenia coli was treated with a 20μl bolus injection of potassium chloride (0.5 Molar) there was a brief period of hyperpolarization which was accompanied by a relaxation (Fig 21c). The initial hyperpolarization was followed by membrane depolarization of 12mV with an increase in spike frequency. The initial hyperpolarization occurred in four out of six preparations studied and is similar to that reported by Burnstock and Prosser (1960a) when spontaneously active taenia coli was activated by quick stretch. It has been attributed to desynchronization or inhibition of spike activity. More recently, Gabella (1978) has suggested that the initial fall in tension is

caused by release of an inhibitory transmitter from non-adrenergic nerve endings. Treatment of the preparation with a 20µl bolus injection of KCl (1 molar) there was a membrane depolarization of 16mV (Fig 21d) but in this case the membrane depolarization did not trigger spikes. In one out of six preparations this spike free depolarization was observed even at 20µl bolus injections of KCl (0.5 molar). This spike inhibition by KCl is similar to that observed by Shimo and Holland (1966) who considered it to be a consequence of membrane depolarization.

In general, membrane depolarization preceded the initiation of contraction and maximum contraction occurred at the same time as maximum depolarization. The contraction was rapid and after reaching maximum, the tension fell rapidly although the recovery of the membrane potential to its original resting value was slow. For instance in Fig 21d the muscle contraction to KCl reached maximum within 0.07 minutes and fell to the baseline within 0.25 minutes. The corresponding membrane depolarization reached maximum within 0.09 minutes and decayed to baseline within 0.68 minutes.

The results in this study indicate that contractile responses of guinea-pig taenia coli to angiotensin and KCl are associated with membrane depolarization and an increase in spike size and frequency.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2. Role of Calcium and Sodium Ions in the Electrical and Mechanical Effects of Angiotensin and Potassium Chloride on Longitudinal Muscle of Rat Descending Colon and Guinea-pig Taenia Coli

At the beginning of each of the following experiments submaximal doses of angiotensin and KCl that produced equal sized isometric contractions in normal physiological solution were selected. The spasmogens were then added alternately to a preparation at 15 minute intervals throughout the experiment. In a preliminary study, it was found that 20 μl bolus of angiotensin (4×10^{-7}M) and KCl (1M) consistently gave equal isometric responses on longitudinal muscle of rat descending colon preparations. Similarly 20 μl bolus of angiotensin (4×10^{-7}M) and KCl (0.5M) gave reproducible responses in guinea-pig taenia coli. These spasmogen concentrations were therefore used in this study.

The results of each experiment have been presented in three parts:

a) Control responses to the spasmogens in normal Tyrode (with longitudinal muscle of rat descending colon) or normal Krebs (with taenia coli)

b) Responses during experimental treatment

c) Responses during recovery after reintroduction of normal Tyrode or Krebs as in (a).

Comparison of the action of the two spasmogens during the experiments has been based on consecutive responses to angiotensin and to KCl.

A. Action of Angiotensin and of KCl on Smooth Muscle in Ca^{2+}-free solution

(i) Longitudinal Muscle of Rat Descending Colon

The electrical and mechanical responses of the longitudinal muscle of the rat descending colon in normal Tyrode's solution during the addition of either angiotensin or KCl are shown in Figs 22a and 22e respectively. When the preparation was exposed to Ca^{2+}-free Tyrode which contained an osmotic equivalent of sucrose (1.8mM) there was a fall in the resting membrane potential of 2 - 3mV (Fig 22b) which lasted for 5 - 10 minutes before the membrane potential reverted to its original level.

Five minutes after exposure of the preparation to Ca^{2+}-free Tyrode's solution both the electrical and the mechanical response to angiotensin were markedly reduced to 30% (Fig 22b) of control and after 35 minutes were abolished (Fig 22c). Perfusion with Ca^{2+}-free solution also caused a progressive diminution of the contraction to potassium, thus 20 minutes (Fig 22f) after perfusion with this solution the contractile response to potassium was abolished but the membrane depolarization was still present.

Restoration of Ca^{2+} to the bathing solution resulted in a gradual hyperpolarization of the membrane by 3 - 8mV (Figs 22d & g) which took 20-30 minutes to reach a stable value. The electrical and mechanical responses to angiotensin (Fig 22d) and to KCl (Fig22g) recovered and were increased to a slightly greater value than obtained before removing extracellular Ca^{2+}. Since calcium is essential for membrane stability (Frankenhauser and Hodgkin, 1957) the slight increase in the mechanical responses to angiotensin and potassium during recovery may be due to increased membrane permeability to ions following the removal of extracellular Ca^{2+}.

These results suggest that extracellular Ca^{2+} was necessary for the contraction and membrane depolarization induced by angiotensin in longitudinal muscle of rat descending colon. Although contraction to KCl was dependent upon extracellular Ca^{2+}, the membrane depolarization was relatively independent of extracellular Ca^{2+}.

(ii) Guinea-pig Taenia Coli

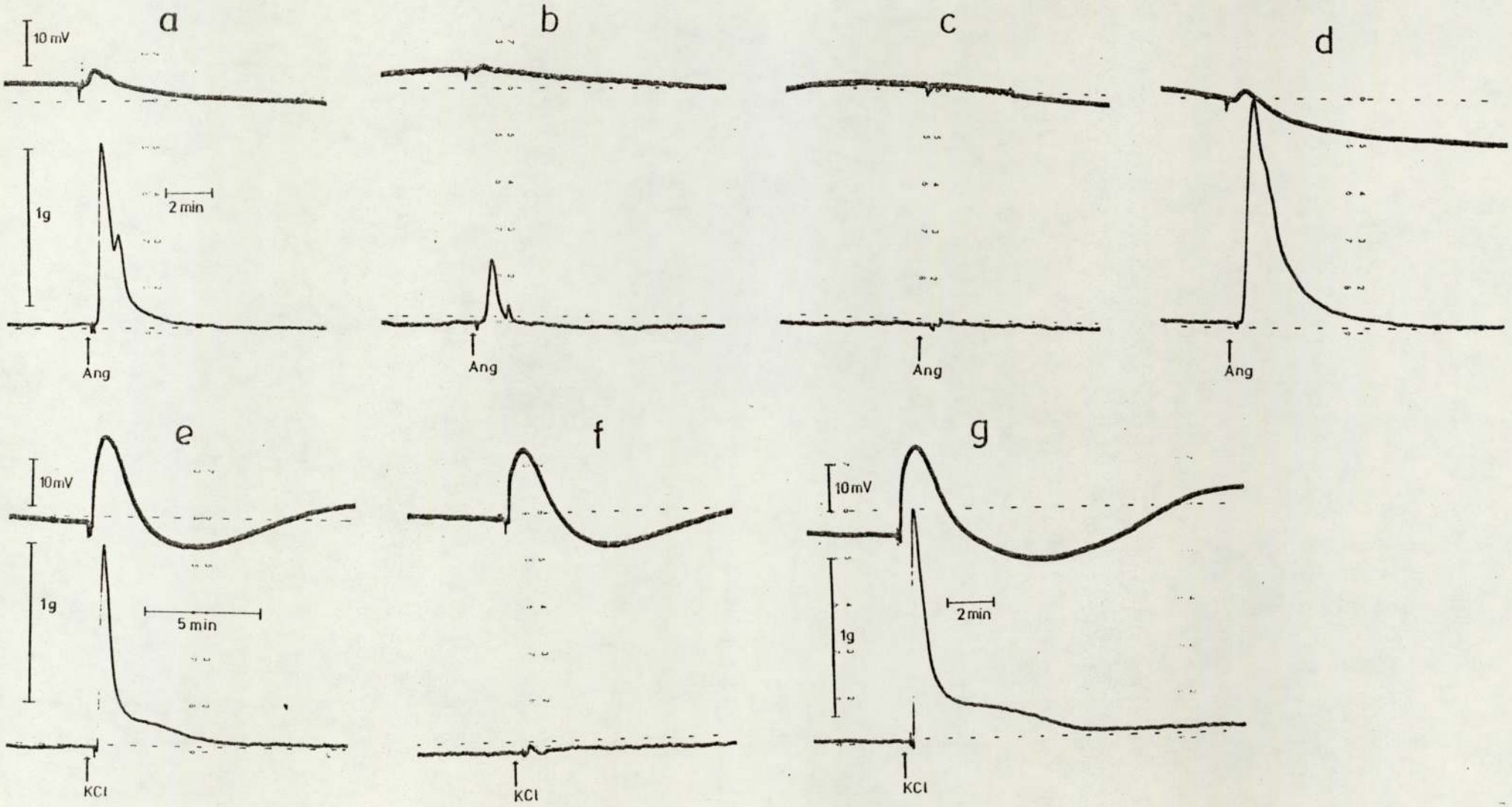
The electrical and mechanical responses of the guinea-pig taenia coli in normal Krebs' solution during addition of either angiotensin or KCl are shown in Figs 23a and 23d respectively. Exclusion of Ca^{2+} from the Krebs' solution bathing the guinea-pig taenia coli, resulted

in a fall of the resting membrane potential of 11.5 ± 1.2mV (n=4). This was followed by a disappearance of spontaneous spikes, abolition of spontaneous contractions and a 2.7g fall in resting tension (Fig 23b and 23e). The abolition of spontaneous spikes was normally rapid and occurred in about 10 minutes (mean 9.3 ± 2.2 min, n=4). Burnstock and Straub (1958) have reported a fall in resting membrane potential of 13mV during treatment of guinea-pig taenia coli with Ca^{2+}-free solution. A similar inhibition of spontaneous spikes and a fall in resting membrane potential has been reported by Holman (1958) who used microelectrode recording.

After exposure to Ca^{2+}-free solution for 50 minutes, the contractile response of the taenia coli to angiotensin (Fig 23b) was abolished. Similarly the contractile response to KCl (Fig 23e) obtained 35 minutes after exposure to the Ca^{2+}-free solution was also abolished. The membrane depolarization induced by either angiotensin (Fig 23b) or potassium (Fig 23e) persisted during perfusion with Ca^{2+}-free solution, the accompanying spikes remained abolished (Fig 23e) but were sometimes present (possibly due to incomplete removal of extracellular Ca^{2+} since EDTA was not used) although they were markedly diminished (Fig 23b). In one out of four preparations used in this study the membrane depolarization to the two spasmogens was slightly increased during perfusion with Ca^{2+}-free solution.

Following restoration of Ca^{2+} to the Krebs' solution the resting membrane potential reverted to its initial resting level in 10 - 20 minutes and the subsequent membrane and contractile responses to angiotensin (Fig 23c) and KCl (Fig 23f) recovered towards their control values obtained before removing extracellular Ca^{2+}.

These results showed that extracellular calcium ions are essential for the spikes and contractile responses of guinea-pig taenia coli to angiotensin and KCl. However the membrane depolarization to either angiotensin or potassium chloride was independent of these extracellular calcium ions.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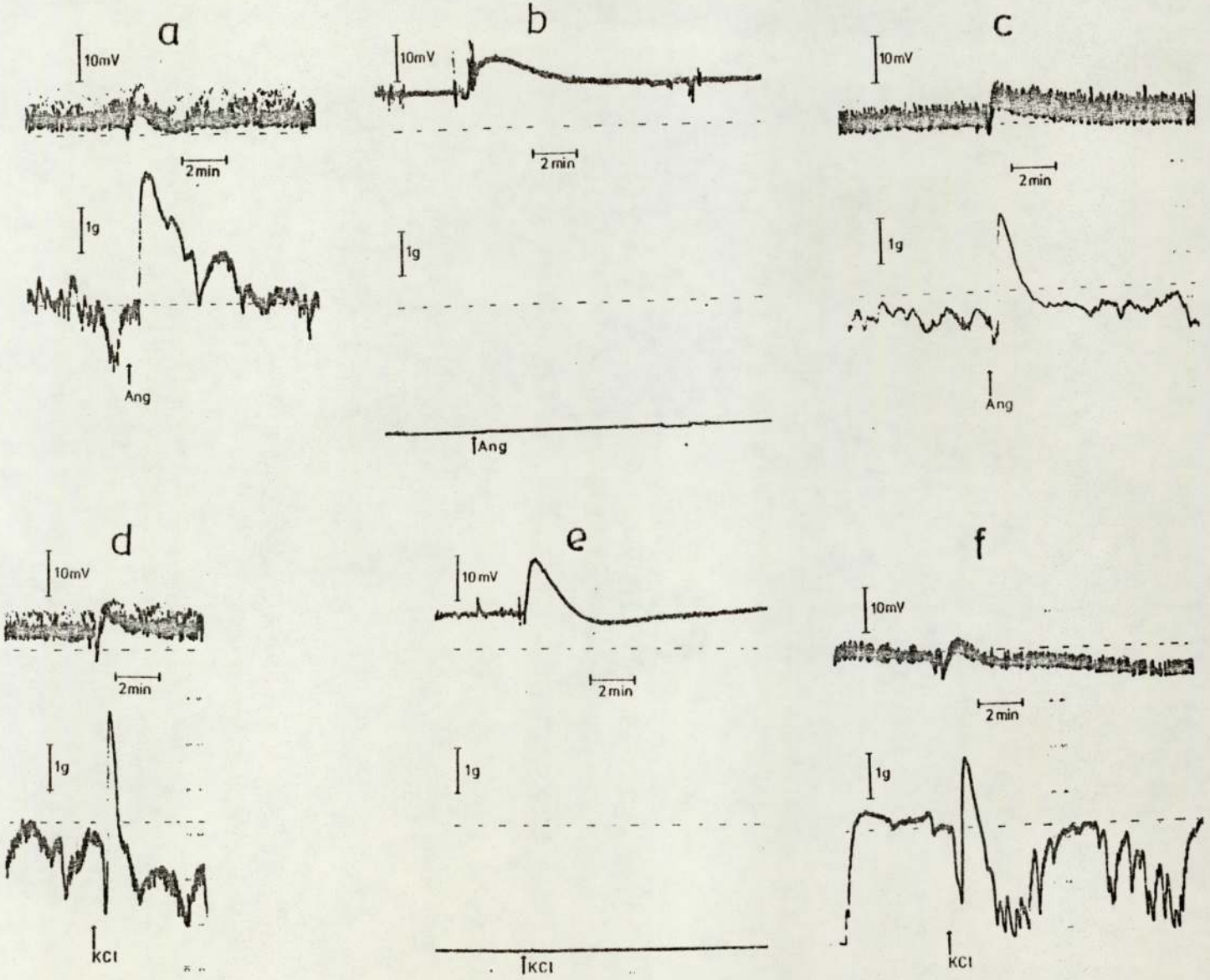
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B. Action of Angiotensin and Potassium Chloride on Smooth Muscle in High Ca^{2+} Solution

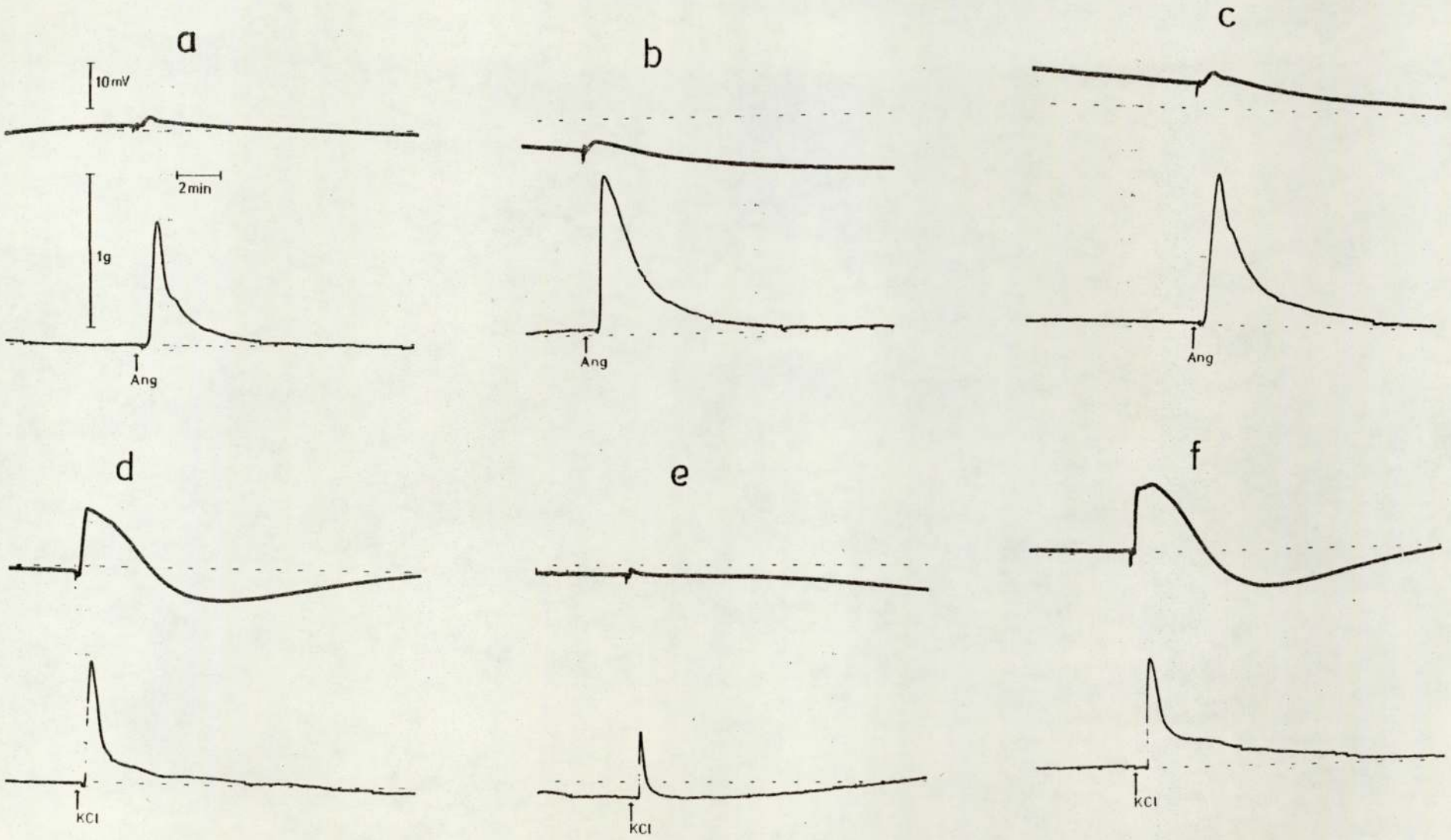
It has been reported that exposure of the guinea-pig taenia coli to a bathing solution containing excess Ca^{2+} resulted in a marked reduction of spontaneous spike frequency which was attributed to membrane stabilization (Holman, 1958; Bulbring and Kuriyama, 1963). In this experiment the effect of high $[Ca^{2+}]_e$ was studied upon the membrane depolarization and the contractile effect of angiotensin.

(i) Longitudinal Muscle of Rat Descending Colon

The electrical and mechanical responses of the longitudinal muscle of rat descending colon in normal Tyrode's solution during the addition of either angiotensin or KCl are shown in Figs 24a and 24d respectively. A six-fold increase in the calcium concentration of the Tyrode's solution from 1.8 to 10.8 millimolar resulted in a gradual increase of the resting membrane potential, and in 20 minutes of perfusion with this solution the membrane was hyperpolarized by 6.9 ± 1.3mV (n=4) (Fig24b). This hyper-polarization of the resting membrane decreased with con-tinued perfusion with the high Ca^{2+}-Tyrode. After 35 minutes of perfusion with this solution the resting membrane was hyperpolarized by 3.5 ± 1.3 (n=4) (Fig 24e). Both the

contractile response and membrane depolarization to angiotensin (Fig 24b) obtained after exposure of the preparation to the high Ca^{2+}-Tyrode were increased to 125% of control obtained in normal Tyrode. In contrast, 35 minutes after perfusion of the preparation with the high Ca^{2+}-Tyrode the membrane depolarization to KCl was reduced to 10% (Fig 24e) of control obtained in normal Tyrode, the corresponding contraction was reduced to 50% of control obtained in normal Tyrode.

When the Ca^{2+} concentration in the bathing solution was returned to normal the membrane potential showed a drop for 15 - 25 minutes (see Fig 24c) and then reverted to its original resting level (see Fig 24f). Twenty minutes after returning the preparation to normal Tyrode, the electrical and mechanical responses to angiotensin were still increased to 120% (Fig 24c) of control. The electrical and mechanical responses to KCl (Fig 24f) 35 minutes after returning the preparation to normal Tyrode recovered to 90% of control obtained before increasing Ca^{2+} in the Tyrode's solution.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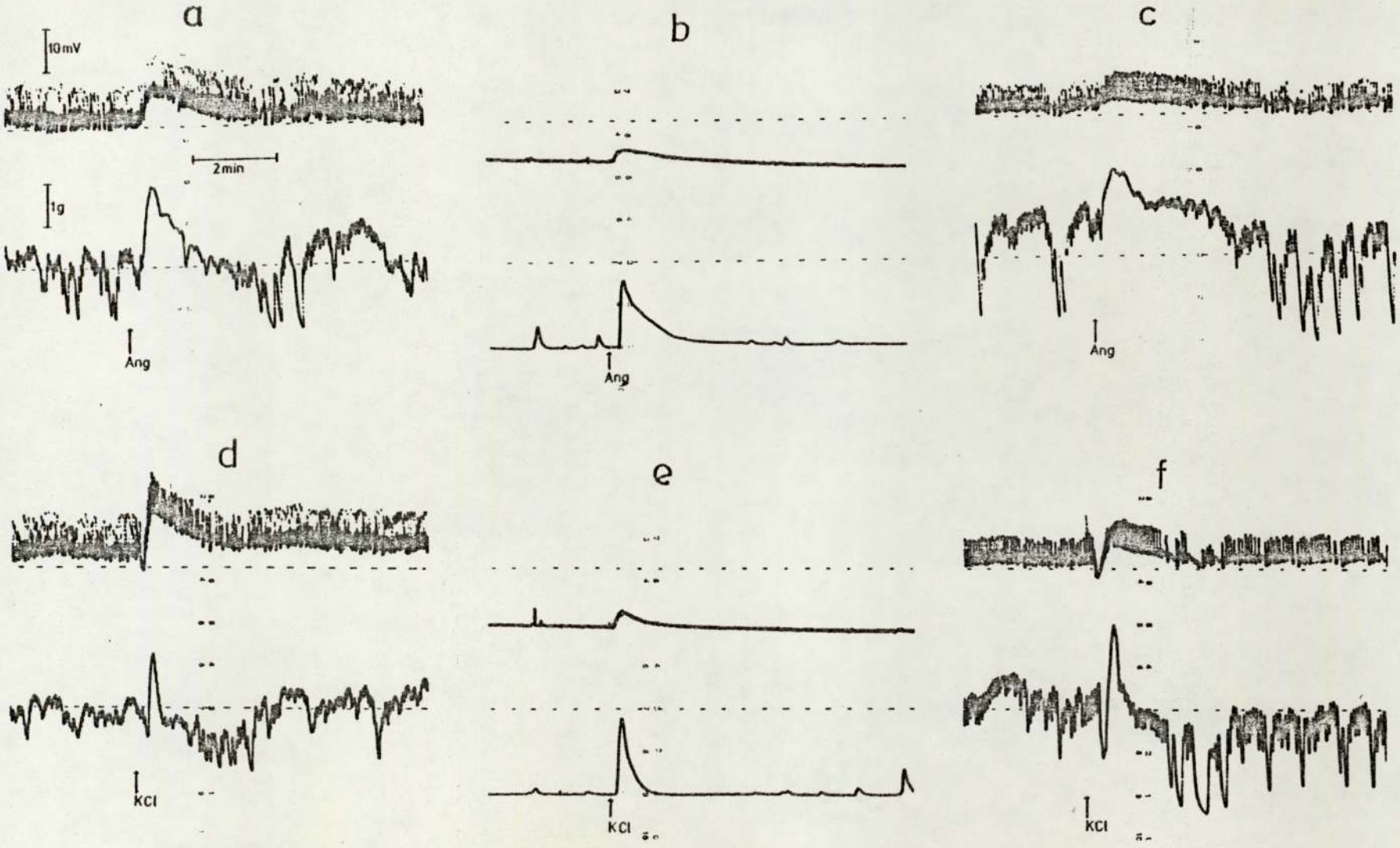
(ii) Guinea-pig Taenia Coli

The electrical and mechanical responses of the guinea-pig taenia coli in normal Krebs' solution during addition of either angiotensin or KCl are shown in Figs 25a and 25d respectively. A five-fold increase of the calcium concentration of the Krebs' solution from 2.4 to 12.0 millimolar resulted in an increase of the muscle resting membrane potential (9.2 ± 1.8mV, n=4). This was accompanied by diminution of spontaneous activity and a fall of resting tension of 2g (Fig 25b and e). Spontaneous spikes were diminished to a stable value in 5 - 10 minutes. During exposure to high calcium solution, after the resting membrane potential had reached a stable value, the membrane depolarization to both angiotensin and to potassium chloride were reduced to less than 50% of control (Fig25b & e), obtained in normal Krebs and the spikes which normally occur with the membrane depolarization were abolished. The effect of extracellular Ca^{2+} on the corresponding contractile effects of the two spasmogens was similar. There appeared to be a decrease in the contractile responses but this was difficult to assess owing to the fall in the resting tension. On returning the preparation to normal 2.4 millimolar Ca^{2+} Krebs the membrane potential fell to its original level. The resting tension, spontaneous mechanical and electrical activity returned towards the initial level in 5 - 10 minutes. The membrane depolarization and contractile effects to angiotensin (Fig25c) and to potassium chloride

(Fig25f) recovered to their respective control level. However, during recovery the spontaneous electrical and mechanical activity was frequently erratic.

In summary, these results show that high extracellular Ca^{2+} caused membrane hyperpolarization and a reduction of the membrane depolarization to potassium chloride in both longitudinal muscle of the rat descending colon and guinea-pig taenia coli preparations. The reduction of the membrane depolarization to potassium chloride in both preparations suggests a reduction of membrane permeability to potassium ions. This is consistent with muscle membrane stabilisation by a high concentration of extracellular Ca^{++} (Holman, 1958). However a high $[Ca^{2+}]_e$ caused a reduction of membrane depolarization to angiotensin in taenia coli but increased the membrane depolarization in the longitudinal muscle of the rat descending colon, this suggests that the mechanism involved in angiotensin induced depolarization may be different in the two preparations.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C. Action of Angiotensin and Potassium Chloride on Smooth Muscle in Na^+-deficient Solution

(i) Longitudinal Muscle of Rat Descending Colon in 34.5 mM Na^+-Tyrode

Fig 26a and 26e show the effect of angiotensin and of potassium chloride respectively upon the membrane potential and isometric tension of the longitudinal muscle of the rat descending colon perfused with normal Tyrode's solution containing 137mM Na^+. When the Na^+ concentration of the Tyrode's solution was reduced to 34.5mM with isotonic sucrose substitution, there was a fall in the resting membrane potential of 7.3 ± 2.8mV (n=5) which took 10 - 20 minutes to reach a stable value (Fig 26b & f). During perfusion with this Na^+-deficient solution the contractile response and membrane depolarization (1mV) to angiotensin were abolished (Fig 26b). In contrast, during this period the membrane depolarization and contractile response to potassium chloride was slightly increased (Fig26f) compared with the control responses obtained in normal Tyrode's solution (Fig26e). On reintroduction of normal Tyrode's solution the membrane potential gradually increased by 10 - 15 mV above the initial control level and took 20 to 30 minutes to reach a stable value (Fig 26c, d & g). The recovery of the mechanical response and membrane depolarization to angiotensin paralleled the recovery of the membrane potential (Fig 26c&d). When the membrane potential had

reached a stable value the membrane depolarization to both angiotensin (Fig 26d) and potassium chloride (Fig 26g) returned towards their control value, however the corresponding contractile responses were greater than the initial values obtained in normal Tyrode's solution. The angiotensin response was increased to 150% (Fig26d) and the response to KCl was 125% (Fig26g) of their respective controls obtained before reduction of extracellular Na^+.

(ii) Guinea-pig Taenia Coli

(a) Guinea-pig Taenia Coli in 48.5mM Na^+-Krebs

The electrical and mechanical responses of the guinea-pig taenia coli in normal Krebs' solution during addition of either angiotensin or KCl are shown in Figs 27a and 27d respectively. When the Na^+ concentration of the Krebs' solution was reduced to 48.5mM (sucrose substitution of sodium chloride) there was a depolarization of the membrane of 2 - 5mV, an increase in spike frequency and a sustained contraction which gradually declined as the spike frequency decreased. The membrane depolarization lasted for 10 - 15 minutes before the resting membrane potential reverted to its original level but the spontaneous spikes lasted for at least 30 minutes in three out of four preparations used in this study. The membrane effects to angiotensin during perfusion with this Na^+-deficient Krebs

were unaffected (Fig 27b). However the corresponding contractile response was slightly reduced to 90% of the control response in normal Krebs (Fig 27a). On the other hand both electrical and contractile effects to potassium chloride (Fig 27e) were slightly increased to 110% of control obtained in normal Krebs' solution (Fig 27d). On restoration of $[Na^+]_e$ there was a transient increase in the membrane potential of 2 - 5mV which lasted for 5 - 10 minutes and recovery of both electrical and mechanical spontaneous activity. The electrical effects to angiotensin (Fig 27c) and potassium chloride (Fig 27f) were restored towards their control level. The contractile response to angiotensin (Fig 27c) remained reduced to 90% of control and the contractile response to potassium chloride was reduced to 75% of control obtained before reduction of extracellular Na^+.

(ii) Guinea-pig Taenia Coli in 25mM Na^+-Krebs

In later experiments the Na^+ concentration of the Krebs' solution was reduced further to 25mM with the addition of either sucrose of tris-chloride as the substitute.

When sucrose was used as the Na^+ substitute, perfusion with the solution resulted in a fall of the resting membrane potential of 12.3 $\pm$ 2.9mV (n=4) (Fig 28b and e). The changes in spontaneous mechanical and electrical events were more pronounced than the ones described in the preceding section,

the membrane remained depolarized during perfusion of the preparation with this solution and there was a greater reduction of spontaneous electrical (spikes and slow waves) and mechanical events (Fig 28 b, e). Twenty-five minutes after perfusion of the preparation with this Na^+-deficient Krebs the mechanical and electrical response to angiotensin (Fig 28b) was reduced compared to the control in normal Krebs solution (Fig 28a). The contraction to angiotensin was smaller (less than 10% of control) and less sustained, the spikes which occurred during depolarization were present although markedly reduced (Fig 28b). In contrast perfusion with this solution increased the response to potassium chloride, thus after 40 minutes, the membrane depolarization and contraction to potassium chloride were increased to 120% (Fig 28e) of the control response in normal Krebs' solution (Fig 28d).

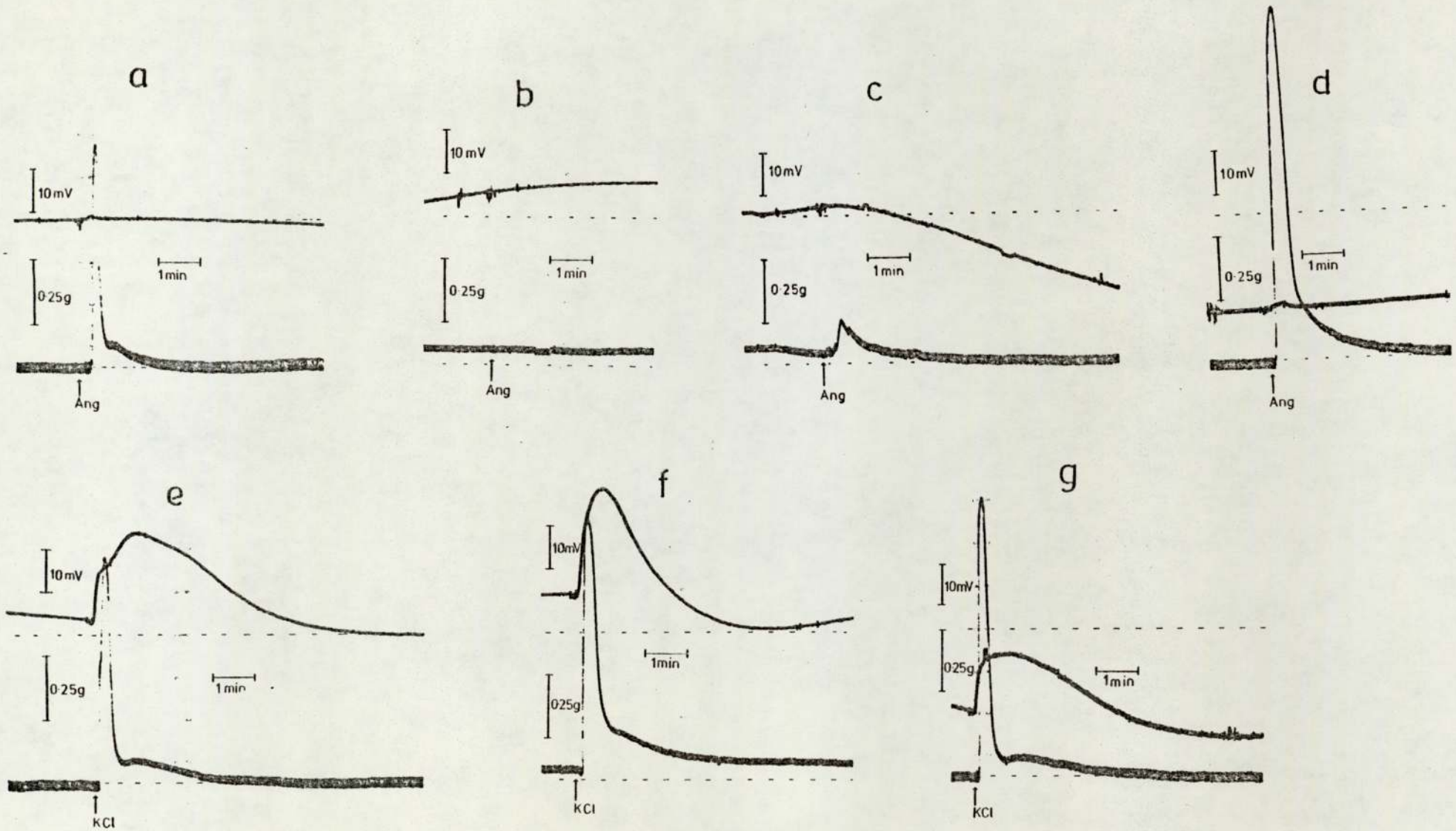
On restoration of $[Na^+]e$, the membrane potential gradually reverted to its initial level in 20 - 30 minutes and the mechanical and membrane response to angiotensin (Fig 28c) and to potassium chloride (Fig 28f) returned towards their initial level obtained in normal Krebs' solution.

When tris was used as a Na^+ substitute, perfusion with Krebs' solution containing 25mM Na^+ resulted in a hyperpolarization of 5.5 ± 1.5mV (n=5) (Fig 29b&e) which reached a stable value in 5 - 10 minutes. The local

potentials (slow waves) were markedly reduced and the spikes were more regular and persisted throughout the experimental time period although with decreased frequency. The resting tension was unchanged and the preparation displayed regular phasic contractions. Twenty-five minutes after exposure to Krebs' solution containing 25mM Na^+ the effects of angiotensin upon membrane depolarization and contraction (Fig 29b) were markedly diminished compared to control in normal Krebs (Fig 29a). The contractile response was reduced to less than 10% of control and the remaining electrical response showed only a slight increase in spike frequency. The contractile response to potassium chloride determined 40 minutes after perfusion of the preparation with this solution (Fig 29e) was increased in height to 150% of the control response in normal Krebs' solution (Fig 29d) but was not sustained and the corresponding membrane depolarization was markedly reduced in duration although the maximum depolarization was unaffected (Fig 29e). On readmission of normal Krebs' solution the resting membrane potential fell to its original level and reached a steady value in 5 - 10 minutes. The membrane depolarization and contractile effects to angiotensin (Fig 29c) and potassium chloride (Fig 29f) recovered towards their control value obtained in normal Krebs' solution.

In summary these results show that in both longitudinal muscle of rat descending colon and guinea-pig taenia coli extracellular Na^+ is essential for the membrane and

contractile effects to angiotensin but not to potassium chloride. It is also clear from these results that the different sodium substitutes used may affect the resulting level of the resting membrane potential. A fall of resting membrane potential was observed in both preparations when sucrose was used as a substitute, but the use of tris as a substitute for Na^+ caused a membrane hyperpolarization. These changes in resting membrane potential may indirectly influence the membrane response to angiotensin and hence emphasize the need to use a variety of substitutes in order to establish the role of sodium ions. Since the membrane depolarization of taenia coli to angiotensin persisted in Ca^{++}-free solutions (preceding studies) but diminished in this study during exposure of the preparation to Na^+-deficient solutions, the results suggest that the membrane depolarization to angiotensin of taenia coli may be due to an increase in membrane permeability to Na^+. Although the abolition of membrane depolarization of the longitudinal muscle of the rat descending colon to angiotensin may suggest a depolarization involving an increase in Na^+ permeability, this is complicated by the preceding observations that the membrane depolarization of this preparation to angiotensin was also dependent on the presence of extracellular Ca^{2+}. The significance of this will be discussed in detail later in the discussion chapter.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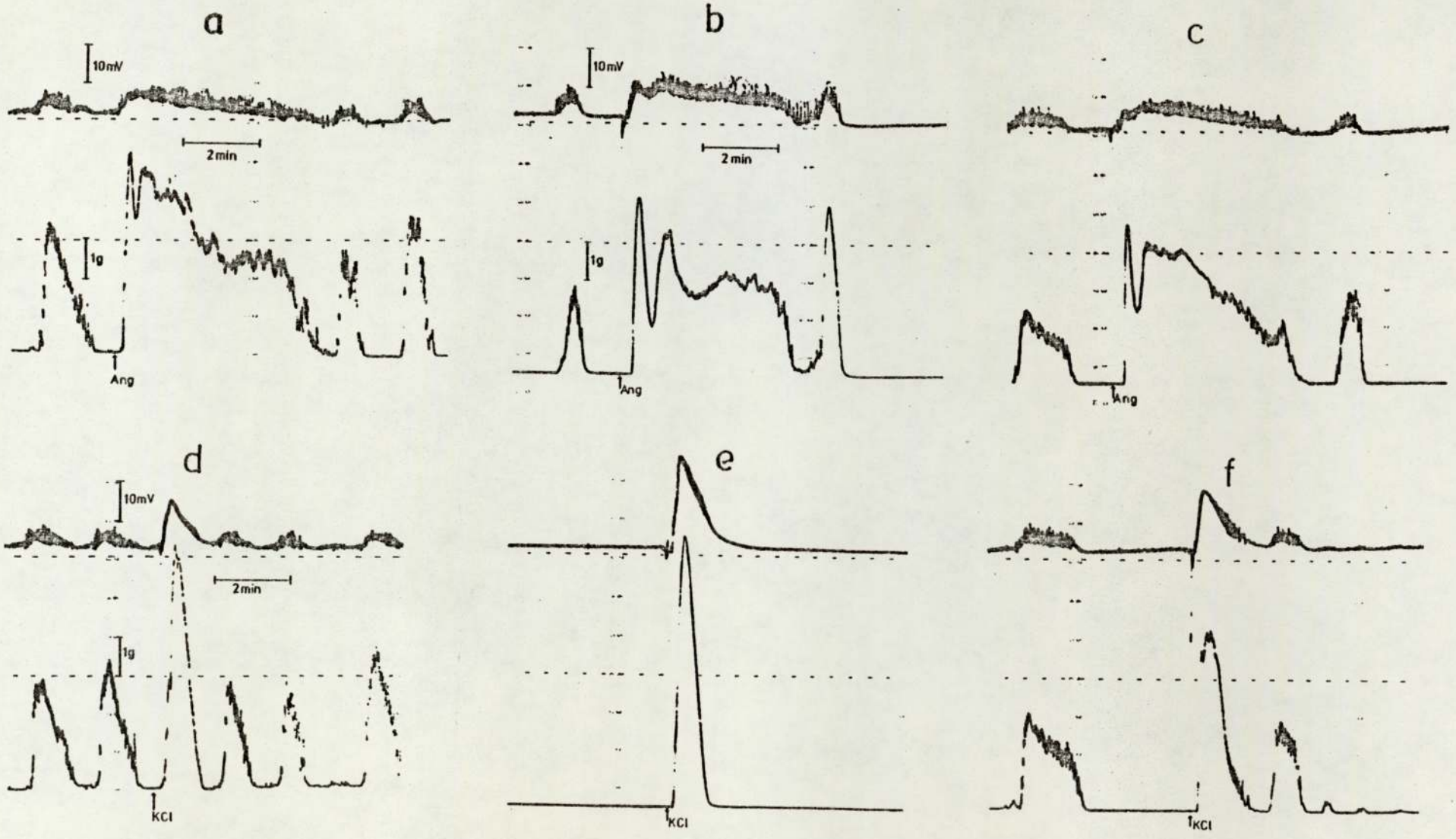
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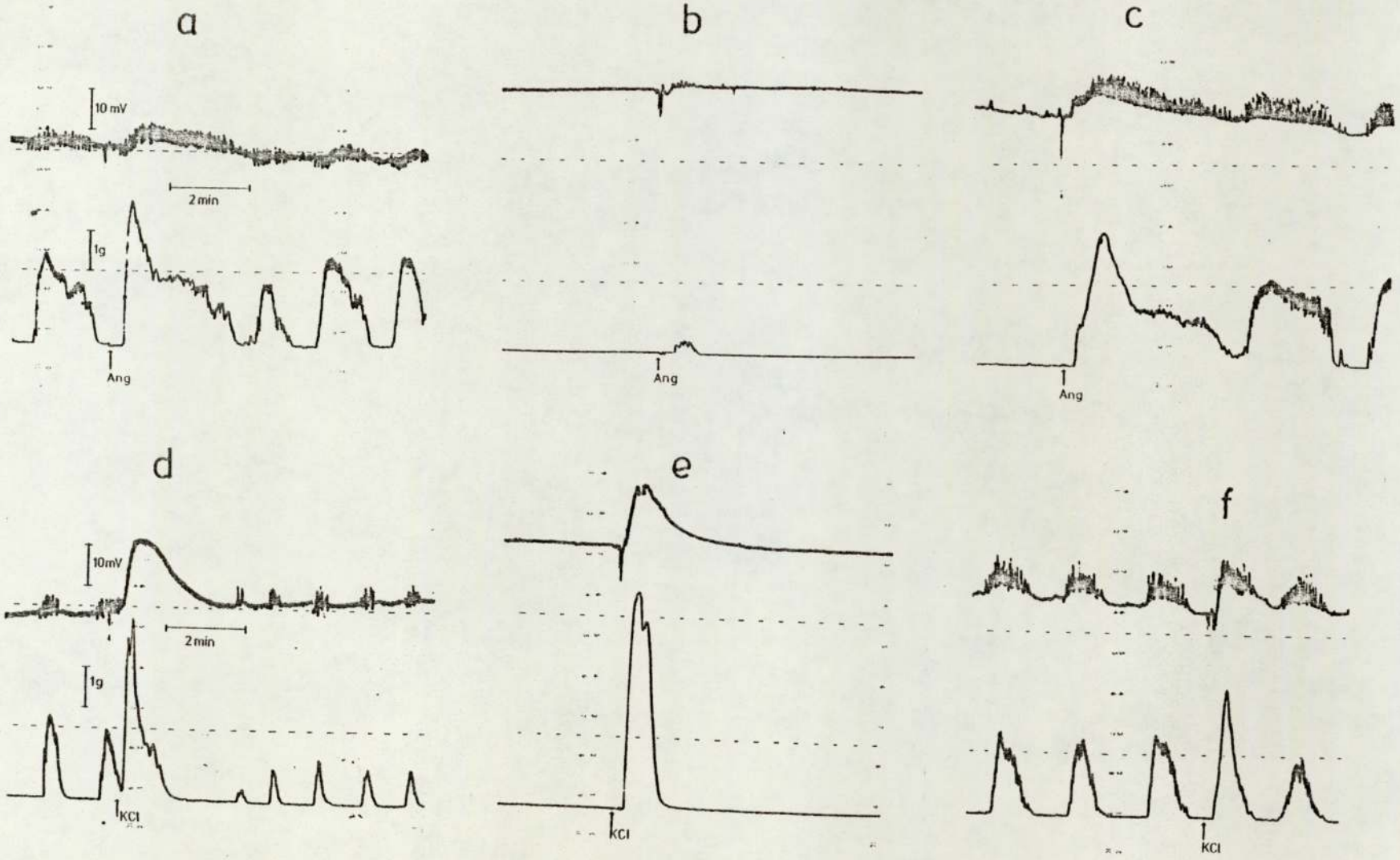
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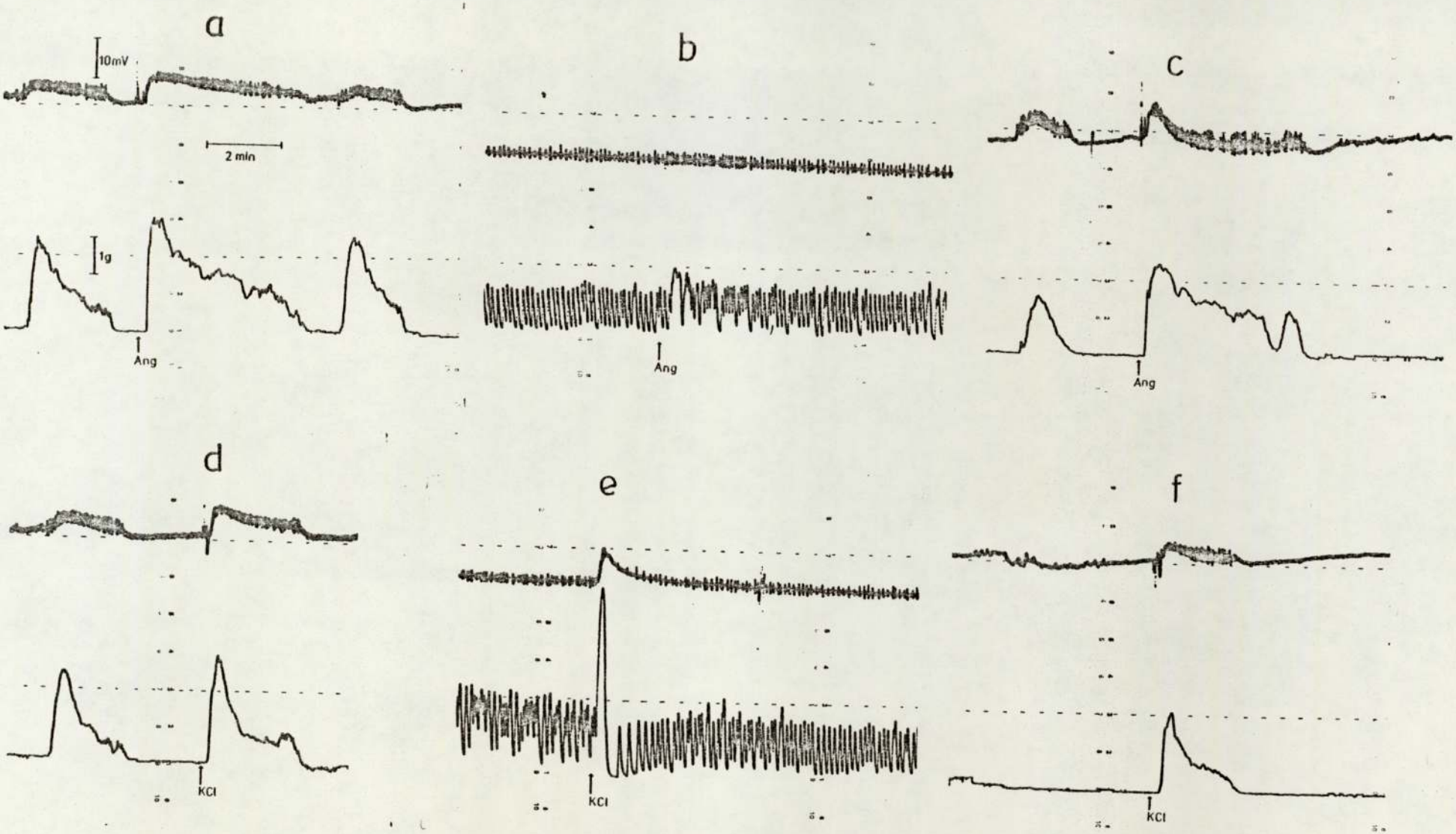
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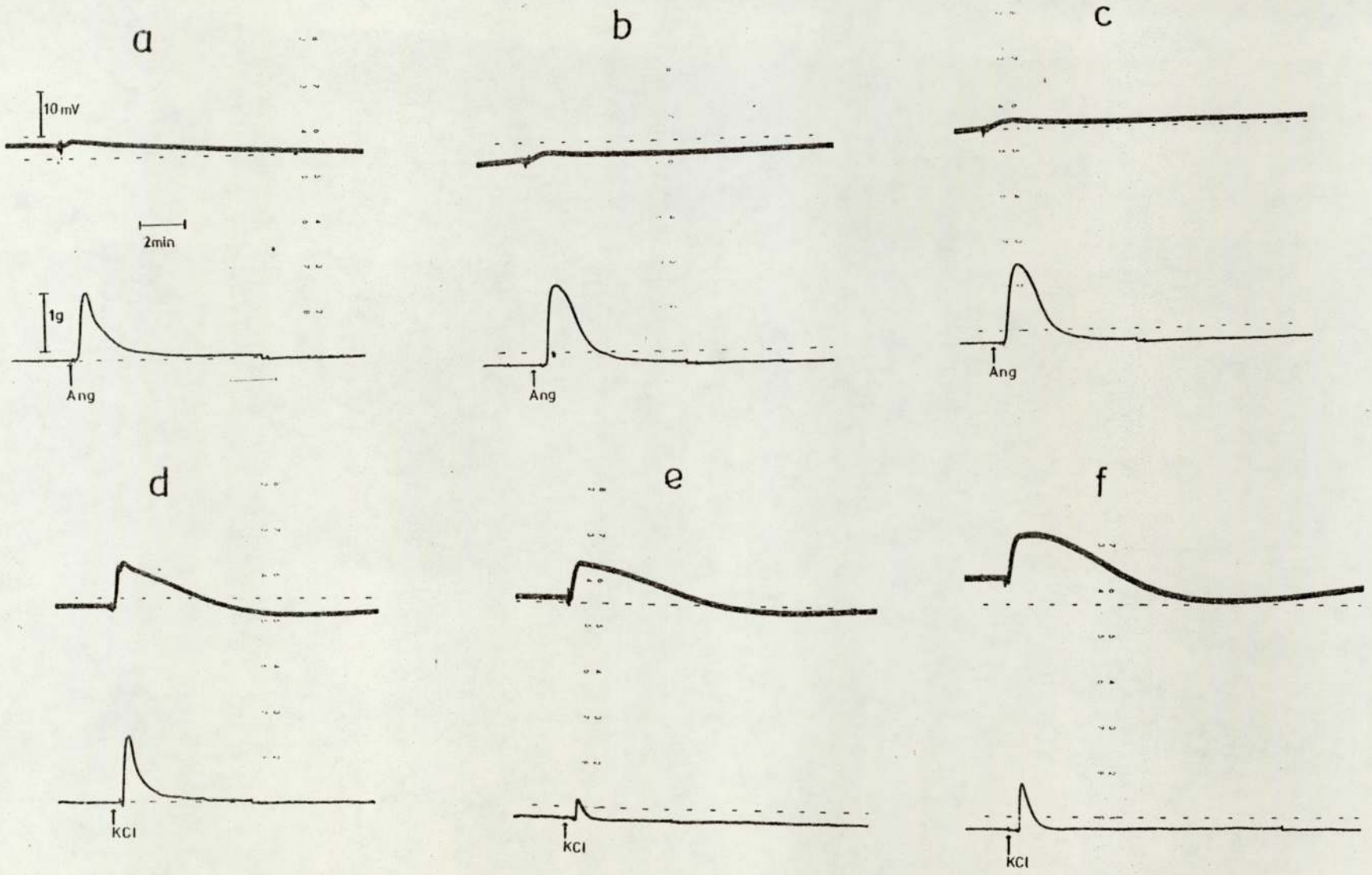
D. Action of Angiotensin and Potassium Chloride on Smooth Muscle in the Presence of Verapamil

(i) Longitudinal Muscle of Rat Descending Colon

Fig30 shows the effect upon the electrical and mechanical responses to angiotensin and potassium chloride of exposure of the longitudinal muscle of rat descending colon to Tyrode's solution containing verapamil (6×10^{-6}M). Perfusion of the preparation with this solution had no effect on the resting membrane potential and there was only a slight fall in the resting tension of the muscle (Fig 30b & e). In some preparations however, there was a small increase of resting potential (1 - 2mV). After 105 minutes of exposure to Tyrode containing verapamil the membrane depolarization and contractile response to angiotensin were generally unchanged (Fig30b) when compared to the control in normal Tyrode (Fig30a) although in some preparations there was a slight reduction of the response. The mechanical response to potassium chloride declined progressively and 90 minutes after exposure to a solution containing verapamil the contractile response was reduced (Fig30e) to less than a 25% of the control (Fig30d) obtained in normal Tyrode. The corresponding membrane depolarization was unchanged. Thus verapamil uncoupled excitation-contraction to potassium without any effect on the angiotensin response.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When the preparation was returned to normal Tyrode's solution the mechanical response to potassium chloride gradually recovered to 75% of control value within 75 minutes (Fig 30f). The membrane depolarization and contractile effects to angiotensin (Fig30c) during the recovery period were equivalent to the initial control effects in normal Tyrode.

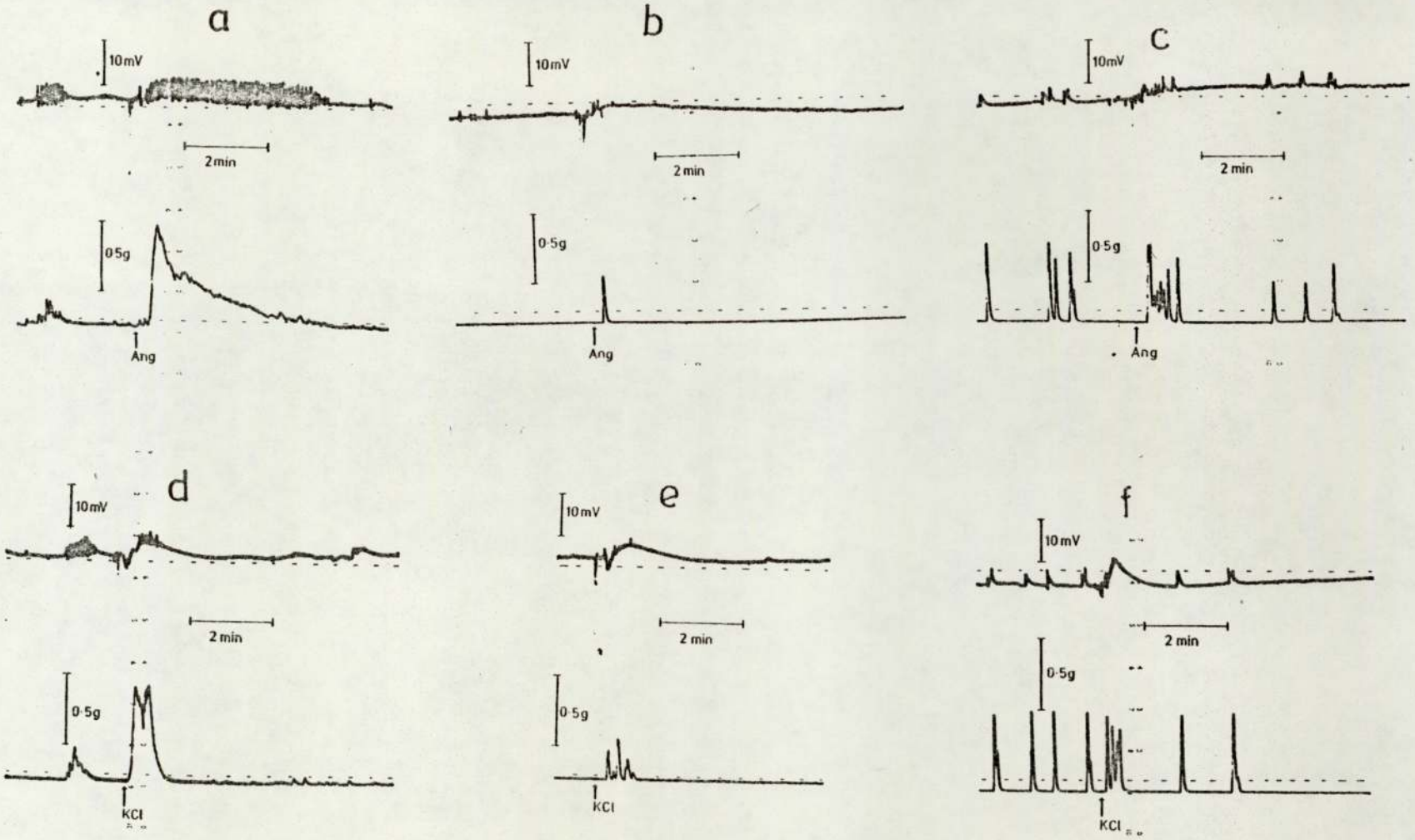
(ii) Guinea-pig Taenia Coli

When guinea-pig taenia coli was exposed to Krebs' solution containing verapamil (3.5×10^{-6}M) the resting membrane potential was generally unchanged (Fig 31b & d) although in some preparations there was a small increase of the resting membrane potential (1 - 3mV). The frequency of spontaneous spikes was progressively reduced as were the phasic contractions. This was accompanied by a fall in the resting tension, particularly in preparations with marked spontaneous activity, and a diminution of the slow waves. Seventy minutes after exposure to this solution (Fig 31b) the mechanical response to angiotensin was markedly reduced to a small phasic contraction which was associated with a few spikes although the corresponding membrane depolarization was not different from the control in normal Tyrode (Fig31a). The mechanical response to potassium chloride 55 minutes following the addition of verapamil (Fig31e) was also reduced to small phasic contractions each being

associated with a spike. The corresponding membrane depolarization was also not different from the control (Fig31d) obtained in normal Tyrode's solution. Removal of verapamil was followed by a slow progressive recovery of spontaneous spikes and phasic contractions. However both spontaneous electrical and mechanical activity failed to recover to their initial level. Thus 60 and 75 minutes after removal of verapamil, the mechanical responses to angiotensin (Fig 31c) and potassium chloride respectively (Fig 31f) were only partially sustained which indicated an incomplete recovery.

In summary, these results show that verapamil uncoupled excitation-contraction to potassium chloride without any effect on the angiotensin membrane depolarization and contractile response of the longitudinal muscle of rat descending colon. However in guinea-pig taenia coli the calcium antagonist verapamil, abolished the spikes and inhibited the contractile responses to both angiotensin and potassium chloride without affecting the membrane depolarization. This suggested that the mobilisation of calcium by angiotensin in longitudinal muscle of rat descending colon was resistant to the calcium antagonist verapamil whereas that in guinea-pig taenia coli was sensitive to this agent. The results also suggested that the membrane depolarization of taenia coli to angiotensin is not due to an increase membrane permeability to Ca^{2+}.

In a preliminary study the effect of higher concentration of verapamil (6×10^{-6}M) was investigated. However both the contractile activity and the membrane potential changes produced by angiotensin and potassium chloride were abolished which indicated a non-selective action. Therefore the use of higher concentrations of verapamil was abandoned.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E. Action of Angiotensin and Potassium Chloride on Smooth Muscle in the Presence of Sodium Nitroprusside

In 1976, Golenhofen proposed two calcium activation mechanisms in smooth muscle. The first was a phasic mechanism dependent upon spike discharge that was blocked by the calcium antagonists verapamil or D600. The second was a tonic or spike free mechanism which was sensitive to calcium depletion and yet was resistant to verapamil and D600. In some preparations this latter mechanism was said to be antagonized by sodium nitroprusside (Na-nitroprusside). In view of the above suggestion the effect of Na-nitroprusside was studied on the action of angiotensin upon the longitudinal muscle of the rat descending colon and the guinea-pig taenia coli.

(i) Longitudinal Muscle of Rat Descending Colon

Fig 32a and 32c show the effect of angiotensin and potassium chloride respectively upon the membrane potential and isometric tension of the longitudinal muscle of the rat descending colon perfused with normal Tyrode's solution. When the preparation was exposed to Tyrode's solution containing Na-nitroprusside (4×10^{-3}M) there was a small increase in the resting membrane potential of 2 - 3mV but no change in resting tension (Fig 32b&d). Thirty-five minutes after exposure to this solution the membrane depolarization, and contractile response to angiotensin

(Fig 32b) were not noticeably different from the control responses observed in normal Tyrode's solution (Fig32a). Similarly, the electrical and mechanical response to potassium chloride recorded after 50 minutes perfusion with solution containing Na-nitroprusside (Fig32d) were similar to those recorded in normal Tyrode's solution (Fig32c). Thus this concentration of Na-nitroprusside was without effect on the membrane depolarization and contractile effects to angiotensin and potassium chloride.

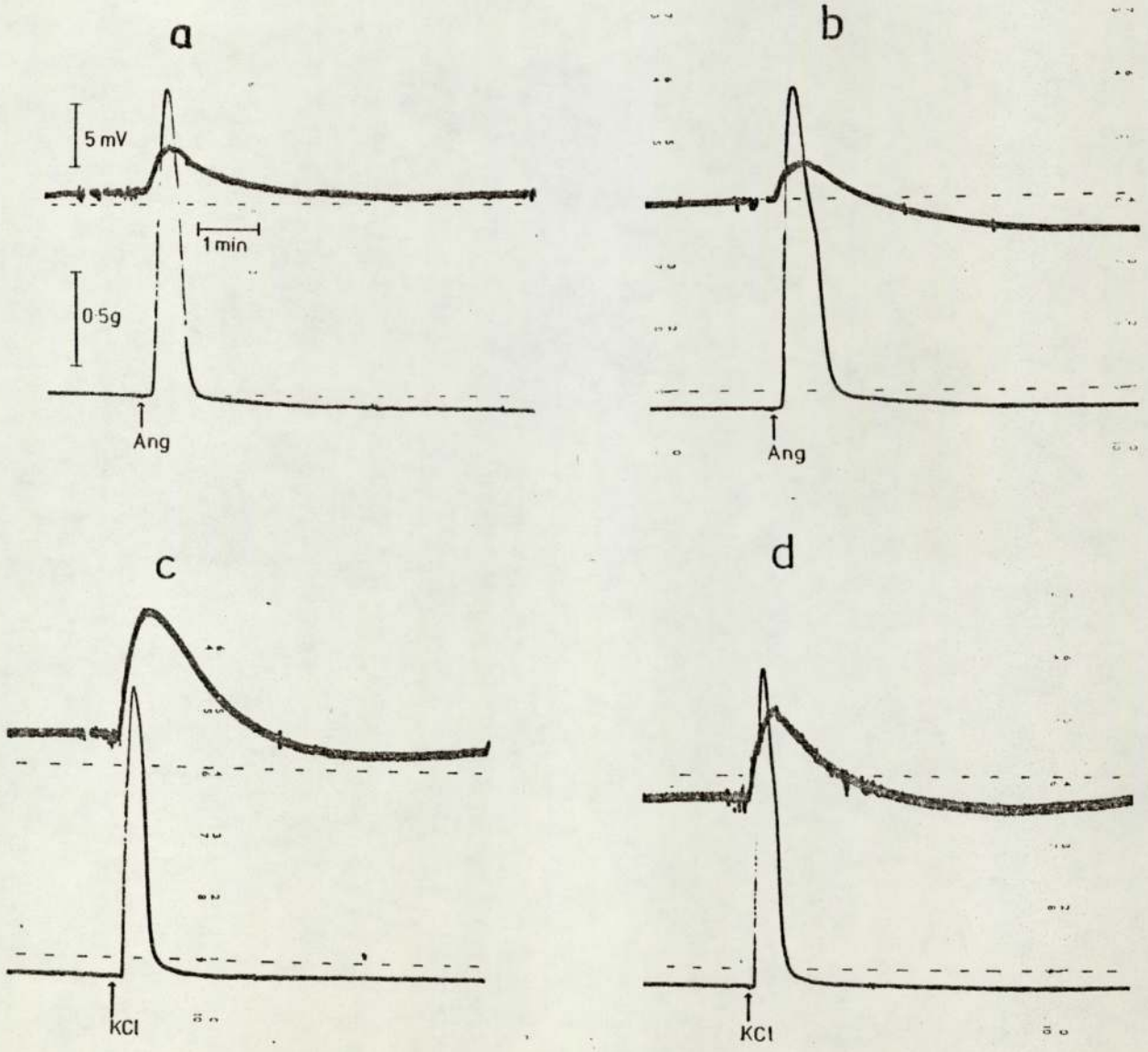
In a preliminary study the effect of a higher concentration of Na-nitroprusside (10^{-2}M) was investigated. This had no effect on the membrane and contractile effects of either angiotensin or potassium chloride on this preparation.

(ii) Guinea-pig Taenia Coli

Fig 33a and 33d show the effect of angiotensin and potassium chloride respectively upon the membrane potential and isometric tension of guinea-pig taenia coli perfused with normal Krebs' solution. Exposure of the preparation to Krebs' solution containing Na-nitroprusside (4×10^{-3}M) resulted in a fall in the resting membrane potential of 5.5 ± 1.5mV (n=5) (Fig 33b&e). There was a slight reduction in spontaneous spiking in two out of five preparations used in this study. Twenty minutes after exposure to this solution

(Fig33b) there was a reduction in the size and duration of the membrane depolarization and contractile responses to angiotensin. The membrane depolarization and contractile effects to potassium chloride recorded 35 minutes after addition of sodium nitroprusside (Fig33e) were also reduced in magnitude and duration. In both cases the size of the isometric contractile responses were reduced to approximately 50% of the control value while the corresponding membrane depolarization was always associated with an increase in spike frequency. On washing out with normal Krebs' solution the resting membrane potential reverted to its original level in 10 - 15 minutes. There was a recovery of the responses to angiotensin (Fig33c) and potassium chloride (Fig33f) towards control value obtained before addition of Na-nitroprusside.

These results show that sodium nitroprusside at the high concentration used in this study had no effect on the spike-free membrane depolarization and contraction of longitudinal muscle of rat descending colon to angiotensin and potassium chloride but exerted a non-selective inhibitory effect on the responses to these spasmogens in guinea-pig taenia coli.


a
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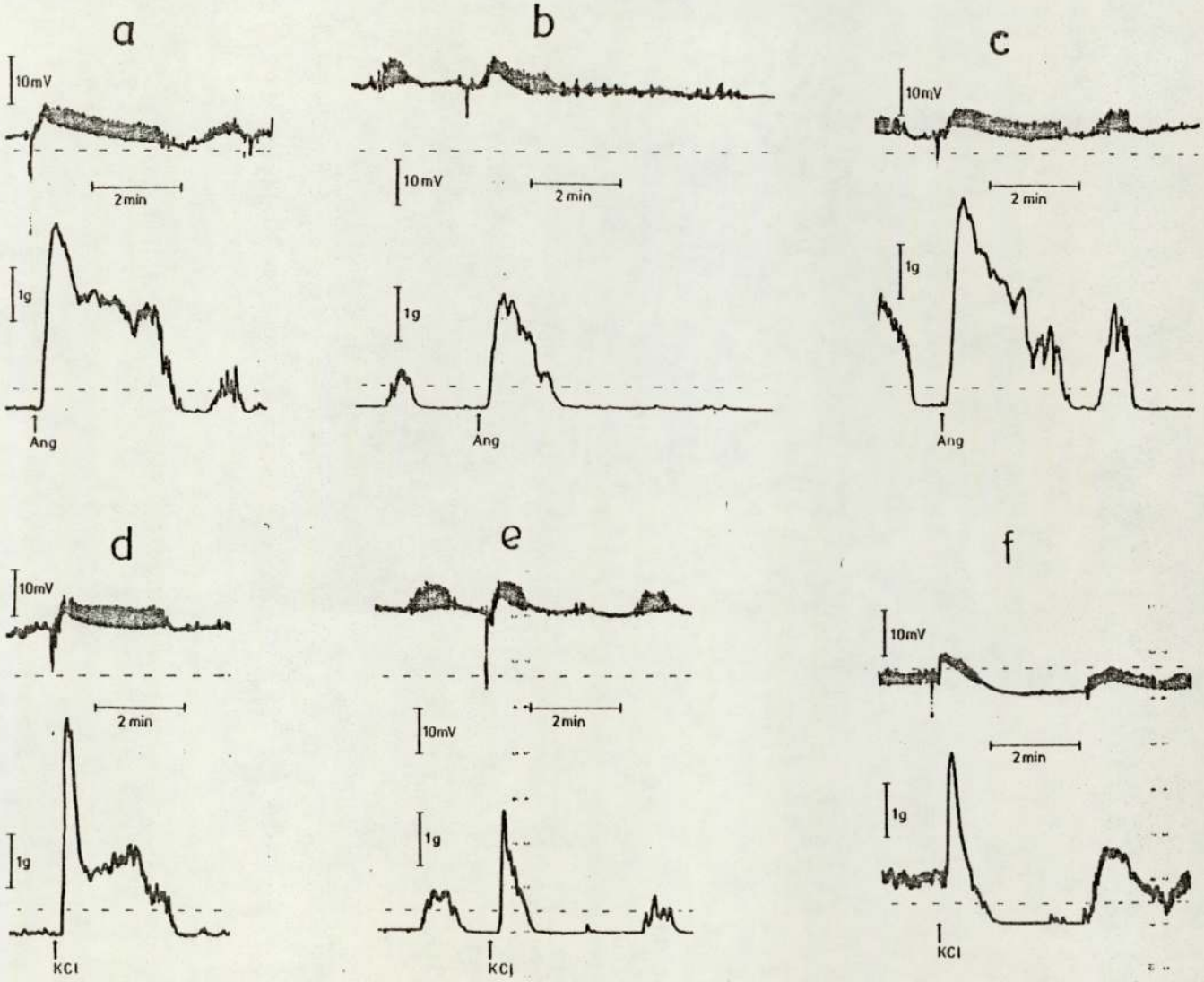
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SECTION III: ^{45}Ca Uptake in the Longitudinal Muscle of Rat Descending Colon

The previous experiments revealed that responses of the longitudinal muscle of the rat descending colon to angiotensin were abolished during exposure of the preparation to Ca^{2+}-free Tyrode's solution and yet persisted during exposure of this preparation to the calcium antagonist verapamil. However two mechanisms of mobilisation of external calcium for contraction have been reported for smooth muscle contraction; one sensitive and the other resistant to the verapamil-like calcium antagonists (Edney and Downes, 1976, Lamell, 1977). In order to locate the source of calcium in this contractile response it appeared appropriate to study the uptake of ^{45}Ca by this muscle during activation by angiotensin, both in the presence of and absence of verapamil. Potassium chloride was used as a control spasmogen.

A. Cellular and Total ^{45}Ca Uptake by Longitudinal Muscle of Rat Descending Colon during Incubation in ^{45}Ca Uptake Solution

Extracellular space is in the closest proximity to the muscle cells, it is therefore logical to assume that any ^{45}Ca taken up by the muscle cell will come from this compartment which in turn is in equilibrium with the bathing fluid. Thus the objective of this initial experiment was to establish the incubation time required for penetration of sufficient ^{45}Ca into the extracellular space to enable easy detection of cellular ^{45}Ca uptake on addition of a spasmogen mobilising extracellular calcium for contraction.

Paired pieces of longitudinal muscle of rat descending colon were incubated for 2½, 5, 10 and 20 minutes in ^{45}Ca uptake solution. After this time of incubation one group was analysed for cellular (after lanthanum treatment) and the other for total ^{45}Ca uptake (Fig34). In this and subsequent experiments the uptake of ^{45}Ca has been expressed in micromoles of calcium per gramme of wet tissue. Both cellular and total ^{45}Ca uptake by the muscle preparations were initially more rapid and declined progressively with prolonged time of incubation. After 5 minutes, the increase in ^{45}Ca cellular uptake was much slower indicating that the uptake was approaching equilibrium. However at the end of the 20 minute maximum time of incubation studied total ^{45}Ca uptake was still increasing, this suggests that ^{45}Ca

uptake in the extracellular space takes longer to reach total equilibrium with the ^{45}Ca uptake solution.

Throughout the 20 minute incubation period, total ^{45}Ca uptake by the muscle preparations far exceeded cellular ^{45}Ca uptake. Thus after 20 minutes incubation, the total ^{45}Ca uptake was 0.4168 ± 0.0333 (n=5), which was significantly greater than the corresponding intracellular ^{45}Ca uptake of 0.0805 ± 0.0136 ($p < 0.001$, n=5). This showed that most of the ^{45}Ca taken up by the muscle preparations is stored in the extracellular space, and may be bound to phospholipids on the outside of the cell membrane or to other negative sites within the extracellular space (Borle, 1968). The cell membrane therefore provides a barrier to the entry of ^{45}Ca into the cell.

In the following experiments the effects of spasmogens were investigated upon cellular ^{45}Ca uptake after preincubation of the preparations with ^{45}Ca uptake solution for 10 minutes when "passive" cellular ^{45}Ca uptake was slower (see Fig34) and was therefore unlikely to mask spasmogen induced cellular ^{45}Ca uptake.

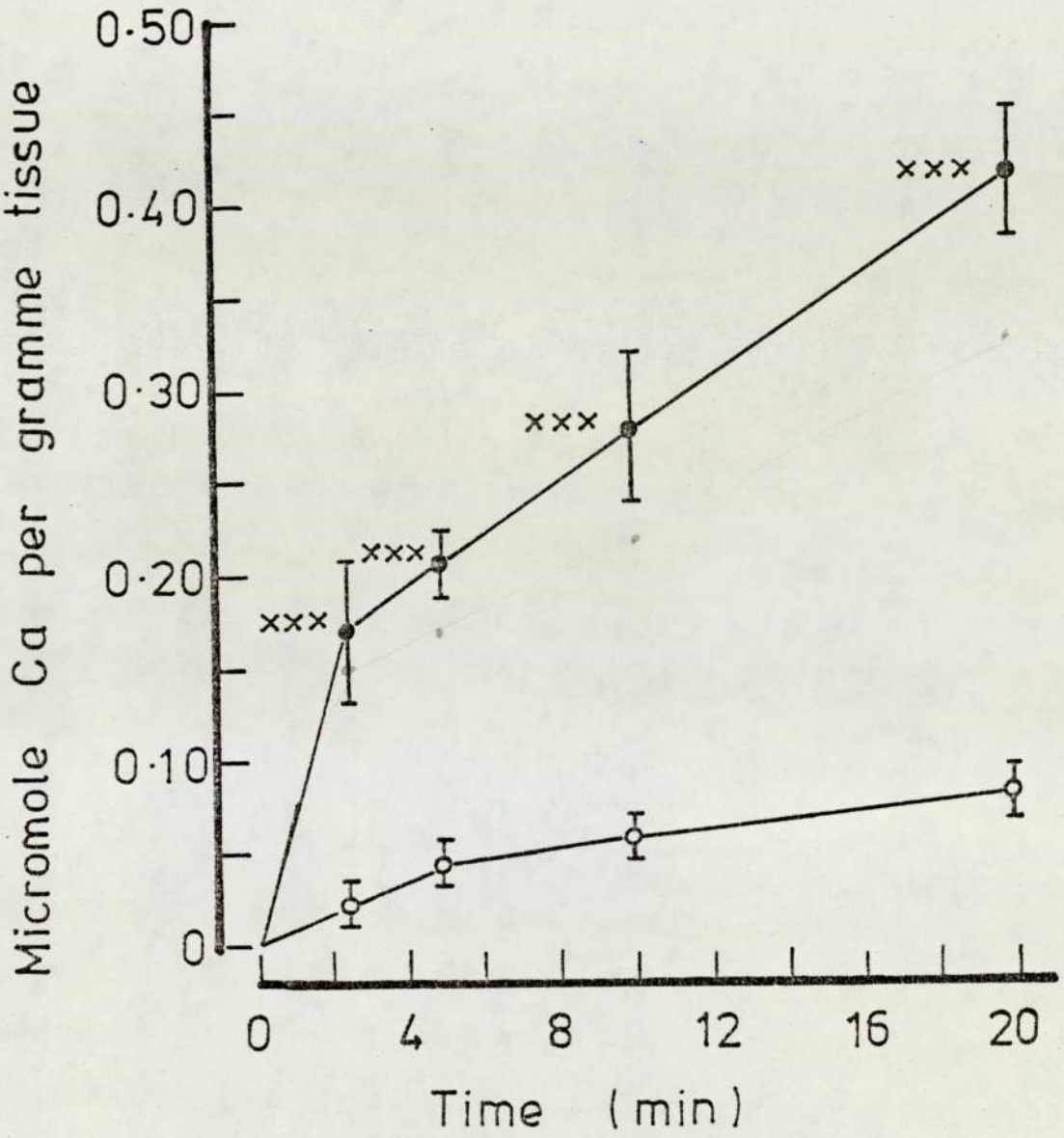

Fig. 34

Intracellular (O) and total (●) ^{45}Ca uptake by the longitudinal muscle of the rat descending colon after incubation in Tyrode's solution containing ^{45}Ca for 2.5 to 20 min. The points show the mean and the vertical bars denote the S.E.M. (n=5).

xxx $p < 0.001$ when comparing intracellular and total tissue ^{45}Ca uptake using a Student's t-test for paired data.

B. Effect of Verapamil on Cellular ^{45}Ca Uptake during Contraction of Rat Descending Colon to Angiotensin and to KCl

Muscle preparations were incubated for 10 minutes in Tyrode's solution containing ^{45}Ca prior to a 5 minute exposure to a supramaximal concentration of either angiotensin (5×10^{-8} molar) or potassium chloride (0.1 molar). These concentrations of spasmogens were selected because they gave approximately equal maximal contractile responses in this preparation. The cellular uptake of ^{45}Ca in muscles exposed to angiotensin was (0.1016 ± 0.0065, n=10) which was significantly greater than the uptake in control preparations (0.0575 ± 0.0045, n=10, $p < 0.001$) not exposed to the spasmogen (Fig35a). Similarly it can be seen in Fig35b that the uptake of ^{45}Ca (0.0963 ± 0.0066, n=8) in preparations exposed to potassium chloride was significantly greater than the uptake (0.0594 ± 0.0070, n=8, $p < 0.001$) in control preparations not exposed to the spasmogen (Fig35b). These results showed that both spasmogens caused an increase in calcium influx. Furthermore there was no significant difference between the ^{45}Ca uptake in preparations contracted by angiotensin compared with that in preparations activated by potassium chloride which suggested that both spasmogens caused a comparable influx of calcium.

When cellular ^{45}Ca uptake was determined in the presence of 10^{-5} molar verapamil the uptake during treatment

with angiotensin was 0.0931 ± 0.0097 (n=10). This was significantly greater than in control preparations not exposed to angiotensin ($p < 0.001$, n=10 in each case) but was similar in magnitude to the ^{45}Ca uptake observed in preparations exposed to angiotensin in the absence of the calcium antagonist ($p > 0.05$, n=10 in each case) (Fig35a). However, in the presence of verapamil, the uptake of ^{45}Ca by preparations exposed to potassium chloride was 0.0650 ± 0.0045 (n=8), which although not significantly different from the uptake by control preparations not exposed to the spasmogen (Fig35b), was much less than the uptake of 0.0963 ± 0.0066 (n=8) observed in preparations contracted by potassium chloride in the absence of verapamil ($p < 0.001$). These results showed that verapamil inhibited the ^{45}Ca uptake induced by potassium but was without effect on ^{45}Ca cellular uptake caused by the addition of angiotensin.

Simultaneous measurements of tension showed that angiotensin caused an increase in muscle tension of 0.59 ± 0.08g (n=10) which persisted in the presence of verapamil (0.51 ± 0.15g, n=10). On the other hand the increase in muscle tension produced by potassium chloride (0.56 ± 0.10g, n=8) was significantly reduced in the presence of verapamil to (0.08 ± 0.02g, n=8). The inhibitory effect of verapamil on muscle tension is in agreement with its effect on cellular ^{45}Ca uptake. Thus a reduced cellular ^{45}Ca uptake of preparations activated by potassium chloride in the presence

of verapamil was associated with reduced contractile response. The inability by verapamil to inhibit cellular ^{45}Ca uptake induced by angiotensin is consistent with the observed persistence of the contractile response to angiotensin in the presence of the antagonist. These results suggest that the angiotensin contractile action of longitudinal muscle of rat descending colon involves extracellular calcium influx resistant to inhibition by verapamil.

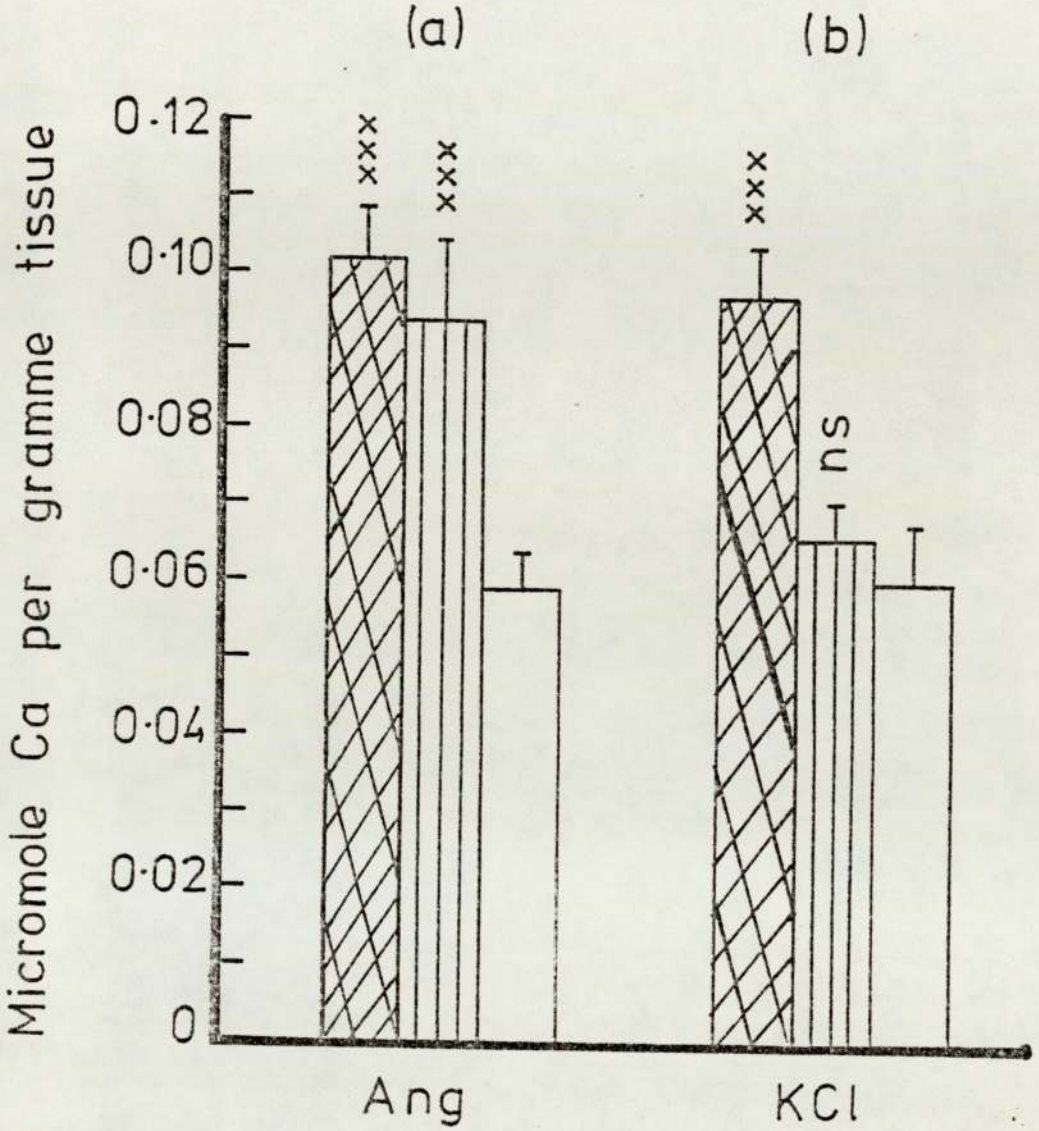

Fig. 35

Cellular ^{45}Ca tissue uptake by the longitudinal muscle of the rat descending colon during a 5 min exposure to equieffective concentration of

(a) angiotensin (Ang)(5×10^{-8}M, n=10 in each case)

(b) potassium chloride (KCl)(0.1M, n=8 in each case)

after a 10 min preincubation of tissue in the Tyrode containing ^{45}Ca.

Open columns: control uptake in normal Tyrode

Hatched columns: uptake in normal Tyrode in the presence of Ang or KCl

Vertical Columns: uptake in normal Tyrode in the presence of Ang or KCl with verapamil (10^{-5}M) added 2 min before addition of the spasmogen.

Vertical bars denote the S.E.M. xxx $p<0.001$; ns $p<0.05$ when comparing the measured uptake with that of control using a Student's t-test for paired data.

D I S C U S S I O N

SECTION I

CONTRACTION STUDIES: Angiotensin II Contractile Action of Rat Descending Colon

1. Introduction

It is generally accepted that regardless of the agent used the event that immediately precedes and is responsible for the mechanical end product in smooth muscle is always an increase in the concentration of ionized calcium (activator calcium) in the environment of the contractile protein (Bohr, 1964; Somlyo and Somlyo, 1968b; Ruegg, 1971; Hurwitz and Suria, 1971). The antepenultimate events leading to the increase in activator calcium, however, appear to depend on the particular agent used to initiate the sequence of events (refer to Introduction, pp 1). This study of the contractile action of angiotensin on smooth muscle was aimed at elucidating the mechanisms utilized by angiotensin to increase activator calcium. The effect of experimental treatments on the contractile responses to angiotensin has been compared with the effect upon potassium chloride responses since its mechanism of action is better understood. Reports in the literature have suggested a possible mediation of angiotensin responses by prostaglandins, thus PGE_2 was used as an additional control spasmogen in initial experiments in an attempt to study any correlation between its contractile activity and that of angiotensin.

2. Role of Calcium Ions in the Contractile Responses of Rat Descending Colon to Angiotensin

(a) Effect of Low $[Ca^{2+}]_e$

Calcium necessary for contraction of smooth muscle may originate from extracellular or intracellular sources (Somlyo and Somlyo, 1968b;Hurwitz and Suria, 1971; Devine et al, 1973). Exposure of rat descending colon to Ca^{2+}-free Tyrode resulted in a reduction of responses to the three spasmogens in the order angiotensin > KCl > PGE_2. The observed general decline of the responses to the spasmogens suggested that their contractile effect depends at least in part on external calcium ions. They further suggest that angiotensin responses are more dependent upon external calcium ions than KCl and PGE_2.

The reduction of responses to angiotensin during exposure of the preparations to Ca^{2+}-free solution are similar to the findings of other workers on guinea-pig ileum and rat uterus (Khairallah et al, 1965) and rat portal vein (Savino and Taquini, 1977). It could therefore be suggested that the reduction of angiotensin responses in Ca^{2+}-free media may be due to a dependence of angiotensin upon extracellular calcium ions (superficial or loosely bound calcium store) for its contractile action. However this hypothesis needs to be viewed with caution since it has been suggested that calcium ions are essential for

the interaction of angiotensin with its receptor (Freer, 1975a, 1975b; Paiva et al, 1976b)in tissues like rat uterus and guinea-pig ileum that have been found to require external Ca^{2+} for angiotensin to elicit contractions but not in rabbit aorta where angiotensin is thought to mobilise intracellular calcium for contraction (Freer 1975a, 1975b; Paiva, Mendes and Paiva, 1977). In contrast to the present findings on rat descending colon, the responses of dog mesenteric artery (Burks et al, 1967), rat tail artery (Hinke et al, 1964) and rabbit aorta (Kalsner et al, 1970) to angiotensin have been found to be less sensitive to tissue exposure to calcium free solution than responses to potassium chloride. This has been attributed to the ability of angiotensin to mobilise intracellular calcium for contraction in these tissues. In addition, angiotensin has been shown to displace calcium from isolated microsomes of rabbit aorta (Baudouin et al, 1972; Baudouin-Legros and Meyer, 1973) this has been suggested as additional evidence for the ability of angiotensin to mobilise intracellular calcium for contraction in the intact tissue. Evidence of intracellular mobilisation of calcium obtained from calcium binding studies on isolated microsomes should be interpreted cautiously since angiotensin is a big molecule and is therefore unlikely to enter the cell (see review, Regoli et al, 1974); although the possibility exists that intracellular calcium mobilisation may be a result of a secondary mediator which results from a drug receptor interaction.

The persistence of contractile responses of rat descending colon to potassium in Ca^{2+}-free solution is similar to the findings of other workers in a variety of smooth muscle preparations (Bozler, 1969; Andersson, 1972). However the contractions to potassium of rat uterus (Marshall and Kroeger, 1973), guinea-pig ileum (Goodman and Weiss, 1971) and rat aorta (Van Breemen et al, 1972) have been found to be dependent on extracellular calcium. Thus the observed persistence of contraction to potassium of rat descending colon in Ca^{2+}-free media may be due to an intracellular release of calcium resulting from an initial calcium influx linked to membrane depolarization. Such a process has been reported to occur in mouse rectal muscle (Cheng, 1976), rat ileum (Taniyama, 1974), rat portal vein (Sigurdsson et al, 1975) and in guinea-pig taenia coli (Imai and Takeda, 1957). This would only require a limited amount of extracellular Ca^{2+} and would therefore tend to persist in nominally Ca^{2+}-free solution or in a solution containing a concentration of Na_2EDTA as low as 25μM as used in these experiments.

The effects of prostaglandins on smooth muscle have been the subject of numerous reviews (Daniel, 1964; Bergström, Carlson and Weeks, 1968; Horton, 1969, 1979; Bennet and Flesher, 1970; Bolton, 1979) and these include both contraction and relaxation. Observations based on changes in external calcium ion concentration have established

that the contractile action of prostaglandins on smooth muscle is at least in part dependent on extracellular calcium (Bergström et al, 1968; Coceani and Wolfe,1966; Horton, 1969; Altura and Altura, 1976). However the persistence of PGE_2 responses during exposure of rat descending colon to Ca^{2+}-free Tyrode observed in this study suggests that part of the response is due to mobilisation of intracellular calcium (a tightly bound pool of calcium). Contractions of human myometrium (Bergström et al, 1968) to $PGF_{1\alpha}$ and of rat myometrium to PGE_1 (Grosset and Mironneau, 1977) have been found to persist in calcium-free solution. This has been taken as evidence that part of the contractile responses to prostaglandins are due to mobilisation of intracellular calcium (or tightly bound calcium) for contraction. Prostaglandins are able to be taken up by tissues (Bito, 1972; Greenberg, Kadowitz, Diecke and Long, 1974). Work with microsomal preparations from bovine or human myometrium has shown that PGE_2 or $PGF_{2\alpha}$, but not the physiologically inactive $PGF_{1\beta}$, inhibit calcium binding and increase calcium release (Carsten, 1973 a,b,c, 1974) which suggests that prostaglandin may increase the level of "activator calcium" by displacement from intracellular stores. However, other workers have found PGE_2 and $PGF_{2\alpha}$ to be without effect on calcium binding in rat aortic microsomal fractions (Webb and Bhalla, 1976). In any case prostaglandins do not appear to act directly on the contractile elements since glycerinated smooth muscles

from the rabbit intestine which contracted on addition of Ca^{2+} and ATP did not respond to PGE_1 (Miyazaki, Ishazawa, Sunano, Syuto and Sakagami, 1967).

(b) Effect of Dantrolene Sodium and Low $[Ca^{2+}]_e$

Dantrolene sodium is a skeletal muscle relaxant thought to act by inhibition of calcium release from the sarcoplasmic reticulum (Ellis and Bryant, 1972; Nott and Bowman, 1974; Putney and Bianchi, 1974; Brocklehurst, 1975). The addition of this agent to the tissue bathing solution failed to antagonize the component of the PGE_2 responses which persisted during treatment of the preparations with Ca^{2+}-free Tyrode. This observation would favour the view that responses to PGE_2 in Ca^{2+}-free solution are not due to mobilisation of intracellular calcium, but such evidence is weakened by the reported ineffectiveness of dantrolene sodium on cardiac and smooth muscle (Butterfield and Ellis, 1973; Ellis, Simpson, Tathan, Leighton and Williams, 1975). However more recently dantrolene sodium has been found to cause an immediate inhibition of contraction of smooth muscle (Bowman and Khan, 1977; Graves, Dretchen and Kruger, 1978) but this has been attributed to the action on superficial sites, possibly by blockade of calcium influx. The lack of effect of dantrolene sodium in smooth muscle, has sometimes been taken as evidence in favour of the opinion that unlike skeletal muscle, intracellular calcium stores

play a minor role in smooth muscle contraction (see Nott and Bowman, 1974).

(c) Effect of Increase in $[Ca^{2+}]_e$

An increase of Ca^{2+} in Tyrode's solution from 1.8 to 10.8mM markedly increased the responses to angiotensin thus providing further evidence that angiotensin responses are dependent on extracellular Ca^{2+}. Responses to PGE_2 were also increased but to a lesser extent. However the effect of an increase in extracellular Ca^{2+} concentration upon responses to potassium chloride was dependent upon the Ca^{2+} concentration. Thus an increase of external Ca^{2+} to 3.6mM potentiated the responses to potassium chloride, which may be attributed to a dependence upon extracellular Ca^{2+} for the induced contractions. A further increase of Ca^{2+} to 7.2mM and 10.8mM caused a decrease of the potassium chloride responses which is consistent with muscle membrane stabilisation that would occur with high calcium ion concentration (Holman, 1958). The fact that high concentrations of extracellular Ca^{2+} which inhibited the contractile responses to potassium (a depolarizing agent) also potentiated the responses to angiotensin, suggested that the responses to angiotensin are not dependent on extracellular Ca^{2+} influx associated with membrane depolarization. The significance of this observation will be discussed in detail later.

(d) Effect of SKF525A and Verapamil

To investigate further the sources of calcium for the contractile action of angiotensin preparations were exposed to either SKF525A or verapamil, agents which have been shown to block calcium influx linked to membrane depolarization (see Introduction pp 21).

The addition of either SKF525A or verapamil to Ca^{2+}-free solution resulted in a more rapid decline of the responses of rat descending colon to angiotensin, PGE_2 and potassium chloride than in Ca^{2+}-free solution alone, thus supporting the idea that the action of these spasmogens is mainly dependent on extracellular calcium for contraction. When Ca^{2+} was reintroduced to the Ca^{2+}-free bathing solution the responses of rat descending colon to potassium chloride (KCl) remained abolished in the continued presence of SKF525A and verapamil, which provided further evidence that these agents block calcium influx linked to membrane depolarization (Kalsner et al, 1970; Fleckenstein et al, 1971; Haeusler, 1972; Bilek et al, 1974). However, on readmission of Ca^{2+} to the Ca^{2+}-free bathing solution there was a partial recovery of responses to angiotensin and PGE_2 in the continued presence of SKF525A or verapamil. This suggested that mobilisation of extracellular calcium for contraction by angiotensin and PGE_2 is independent of membrane depolarization. These findings are in agreement

with those of Kalsner and co-workers (1970) who observed abolition of responses of rabbit aortic strip to potassium by concentrations of SKF525A that had little effect upon angiotensin and noradrenaline responses. Similarly, Freer (1975a) has observed a persistence of angiotensin responses of the rabbit aorta in the presence of verapamil. Kalsner and co-workers (1970) suggested that the persistence of responses of rabbit aorta to angiotensin and noradrenaline in the presence of SKF525A was due to the ability of these spasmogens to mobilise intracellular calcium for contraction. Since in this study the responses to angiotensin and PGE_2 recovered on restoration of extracellular calcium in the continued presence of SKF525A and verapamil, it is reasonable to suggest that the contractile response to these spasmogens involves an influx of extracellular calcium which is resistant to the calcium flux inhibitory effects of SKF525A and verapamil. A similar mechanism of calcium mobilisation for contraction has been proposed for the contractile action of the convulsant barbiturate (CHEB) on rabbit aorta (Edney and Downes, 1976) and of acetylcholine on guinea-pig fundus muscle (Lammel, 1977). It is possible that the observed persistence of angiotensin and PGE_2 in the presence of verapamil may represent extracellular calcium mobilisation by activation of a tonic mechanism as proposed by Golenhofen (1976).

Based on indirect evidence the findings in this

study favour the view that the angiotensin and PGE_2 contractile action of rat descending colon is independent of membrane depolarization. This is in agreement with the observation that angiotensin causes contraction of vascular (Keatinge, 1966; Shibata and Briggs, 1966) and uterine smooth muscle (Freer, 1975a) depolarized by exposure to high potassium solution. It is also supported by the observation that PGE_1 causes contraction of potassium depolarized uterus (Paton and Daniel, 1967). There is, however, a need for direct measurements of membrane potential since direct electrophysiological measurements have shown that angiotensin (Hamon and Worcel, 1977), PGE_1 (Grosset and Mironneau, 1977) and PGE_2 (Suzuki, Osa and Kuriyama, 1976) cause membrane depolarization of the rat myometrium even though they are able to contract the potassium depolarized muscle.

3. Role of Sodium Ions in the Contractile Responses of Rat Descending Colon to Angiotensin

Reports in the literature indicate that in smooth muscle the distribution of sodium ions is closely linked to that of calcium ions (see review Van Breemen, Aaronson and Loutzenhiser, 1979). Sodium ions contribute to the maintenance of the muscle membrane potential and interact with calcium ions in a variety of ways which include the competition by Na for Ca transporting sites at the membrane and the participation in Na-Ca exchange mechanisms (Goodford, 1970; Casteels, 1970; Reuter, 1973; Raeymaekers, Wuytack and Casteels, 1974). It is conceivable, therefore, that a spasmogen may cause calcium mobilisation in smooth muscle through a primary effect upon sodium distribution (see reviews Bohr, 1964; Bolton, 1979; Van Breemen et al, 1979). It was therefore pertinent to study the role of sodium ions in the contractile action of angiotensin.

(a) Effects of Reduction in $[Na^+]_e$

The effect of a reduction in the sodium concentration of the bathing medium on the responses to angiotensin was dependent upon both the degree of sodium reduction and the time of determination of the response following the reduction of external sodium. Thus the initial responses to angiotensin determined 5 minutes after reduction of extracellular Na^+ from 137mM (normal) to 68.5mM (sucrose

substitution) were potentiated whereas the subsequent responses were reduced. Further reduction of Na^+ to 34.3mM resulted in a greater potentiation of the initial angiotensin response and a further reduction of the subsequent responses.

An increase in muscle tone of the rat descending colon was observed following the reduction in external sodium concentration (Fig 12). Since the reduction of extracellular sodium concentration in smooth muscle has been shown to cause sodium efflux accompanied by calcium influx (Briggs and Melvin, 1961; Brading, 1973; Reuter et al, 1973), it is reasonable to assume that this mechanism may be responsible for the increase in tone in the rat descending colon. The sodium gradient is greatest soon after reduction of $[Na^+]e$, thus the observed potentiation of the initial angiotensin response could be explained if it is assumed that angiotensin causes calcium influx linked to sodium efflux as has been proposed for the action of the hormone on rat aortic muscle (Godfraind, 1973). The reduction in the angiotensin response due to prolonged periods of incubation in Na^+-deficient solution or prior exposure of the preparation to KCl or PGE_2, agents that may cause influx of extracellular calcium, is consistent with a reduction in the sodium concentration gradient that may occur during these treatments. Napodano and colleagues (1962) have also reported potentiation of responses to angiotensin following reduction of $[Na^+]e$ which they

attributed to an additive effect due to change in sodium gradient between the inside and the outside of the muscle cell with a resultant increase in muscle tone (Friedman et al, 1959). These workers also observed a decrease of angiotensin responses when the extracellular sodium concentration was increased. In contrast, a reduction of the external sodium ion concentration has been shown to cause a decrease in contractile responses to angiotensin of the rat uterus (Khairallah et al, 1965), guinea-pig ileum (Khairallah et al, 1965; Blair-West and McKenzie, 1966) and rat ventral artery (Hinke and Wilson, 1964) while the vasoconstrictor response of rabbit ear artery was reduced by either an increase or decrease in extracellular sodium concentration (Blair-West et al, 1968). More recently Freer and Smith (1976) have shown that reduction of extracellular sodium concentration causes reduction of angiotensin responses only when Li^{+} was the substitute but not when sucrose was used; they suggested that Li^{+} may either interfere with the angiotensin receptor or may compete with Ca^{++} for transport sites on the muscle membrane. It is possible that part of the reason for variation of the effects of reduction in extracellular sodium concentration presented in the literature is due to the different substitutes used and the time of determination of the angiotensin response after change in external sodium concentration. The significance of this will be considered in detail later. Because of the multifunctional role of sodium in the muscle cell,

it is therefore difficult to draw firm conclusions from experiments involving reduction in extracellular sodium concentration alone.

The effect of Na^+-deficient solution on the responses of rat descending colon to PGE_2 in this study was similar to that of angiotensin although the potentiation of the response determined 5 minutes after reduction of sodium in the bathing medium was less. The reduction in the responses to PGE_2 during exposure of the preparations to Na^+-deficient solution in this study is in agreement with the observed depression of contractions of the rabbit duodenum to prostaglandins when Li^+ was substituted for Na^+ (Miyazaki, Ishazawa, Sunano, Syuto and Sakagami, 1967). Unlike angiotensin and PGE_2 which showed an initial potentiation of the initial responses followed by a reduction of the subsequent responses during reduction of Na^+ in bathing solution, the responses to KCl were consistently potentiated during this period. A similar potentiation of responses to KCl has been observed on rat ventral artery by Hinke and co-workers (1964) and this was attributed to reduction in competition of Na^+ for Ca^{++} membrane diffusion sites or carriers as has been proposed in cardiac muscle (Niedergerke and Lüttgau, 1957).

In summary, the results in this section favour the view that angiotensin may exert its contractile action of

the rat descending colon by mobilising a Na-Ca mechanism as proposed by Godfraind (1973) for the action of the hormone in aortic muscle.

4. Role of Cyclic AMP in the Contractile Responses of Rat Descending Colon to Angiotensin

The smooth muscle relaxant effect of β-adrenoceptor stimulants (Marshall and Kroeger, 1973; Andersson, 1972) and of drugs that inhibit phosphodiesterase such as papaverine and theophylline (Butcher and Sutherland, 1962; Triner et al, 1970; Poch and Kukovetz, 1971) is mediated by an increase in intracellular cyclic AMP. The mode of action of cyclic AMP on the process of relaxation may depend at least in part on its ability to reduce free myoplasmic calcium (see Introduction pp 43). If some hormones and drugs are able to relax smooth muscles by an increase in the intracellular level of cyclic AMP it is logical to assume that a decreased level might be associated with contractile effect. This assumption was confirmed when imidazole, an agent that lowers intracellular concentration of cyclic AMP by activation of phosphodiesterase (Butcher and Sutherland, 1962) was found to antagonize the relaxant effects of β-stimulants but had no effects on the relaxant effect by α-stimulants (Wilkenfeld and Levy, 1969). Imidazole also causes a contraction of the rabbit colon and gall bladder muscles of the guinea-pig that is preceded by a stimulation of phosphodiesterase and a decrease of intracellular cyclic AMP level (Andersson, 1972). Attempts to relate changes in intracellular cyclic AMP to smooth muscle contraction induced by spasmogens has yielded conflicting results (see

reviews Andersson, 1972; Bar, 1974; Rasmussen and Goodman, 1977). Since many actions of hormones are mediated by cyclic AMP (see review Rasmussen and Goodman, 1977) the possible role of cyclic AMP in the contractile action of angiotensin upon rat descending colon was studied.

Both treatment of the muscle with isoprenaline, a β-adrenoceptor stimulant that has been reported to cause an increase in the intracellular level of cyclic AMP by activation of adenyl cyclase (Marshall and Kroeger, 1973) or theophylline an agent that increases intracellular cyclic AMP by inhibition of phosphodiesterase (Butcher and Sutherland 1962) inhibited the responses to angiotensin and PGE_2 more than those to potassium. Similarly, exogenously added dibutyryl cyclic AMP had a greater inhibitory effect on responses to angiotensin and PGE_2 than to potassium chloride. The dibutyryl derivative was used in this study since it is more stable to hydrolysis by phosphodiesterase than cyclic AMP (Robison, Butcher and Sutherland, 1971; Moore, Iorio and McManus, 1968). It penetrates the cells easily (Falbriard, Posternak and Sutherland, 1967) and acts by mimicking cyclic AMP at the site of action (Moore et al, 1968). The results suggest that changes in intracellular concentration of cyclic AMP may be involved in mediating the contractile responses of rat descending colon to angiotensin and PGE_2 but not with potassium chloride. These findings with angiotensin are in agreement with those of

Volicer and Hynie (1971) who on addition of angiotensin have observed a decrease of cyclic AMP levels in the rat tail artery and a reduction of theophylline induced increase of cyclic AMP in this tissue. These effects are consistent with the postulated role of the cyclic AMP system in smooth muscle, decreasing levels accompanying contraction and increasing levels accompanying relaxation. These workers suggested that a decrease in cyclic AMP may mediate angiotensin contraction although causal relationships of decrease in cyclic AMP and contraction were not established. In contrast to these findings Angles d'Auriac and Meyer (1972) found that angiotensin had no effect on the rate of cyclic AMP formation in rat uterus although it reduced the stimulatory effect of adrenaline on cyclic AMP formation. In addition the same workers have found that angiotensin does not affect the activity of phosphodiesterase nor the level of cyclic AMP or cyclic GMP in rat uterus (Angles d'Auriac and Meyer, 1973).

The reduction of the contractile responses of rat descending colon to PGE_2 by agents that have been reported to cause an increase in intracellular concentration of cyclic AMP is consistent with the findings of other workers who have reported that the contraction of the colon and mesenteric artery of the rabbit by PGE_2 was associated with decreases in intracellular cyclic AMP (Andersson, 1972).

Conversely, the relaxation induced by prostaglandin of tracheal and bronchial muscle was associated with an increase in intracellular cyclic AMP (Cuthbert, 1973) indicating that cyclic AMP may mediate prostaglandin action.

The significance of the observed equipotentiation of contractions of the rat descending colon to angiotensin, PGE_2 and to potassium by imidazole, an agent that decreases intracellular cyclic AMP by activation of phosphodiesterase (Butcher and Sutherland, 1962) is not clear. It indicates that imidazole acts non-specifically by affecting a mechanism common to all three spasmogens. It is unlikely that the potentiation by imidazole could arise from a direct effect on the contractile machinery per se, since it has been reported to have no effect on glycerol treated rabbit colon muscles contracted by direct addition of Ca^{2+} and ATP (Nilsson, Djarv and Andersson, 1976). It is possible that the non-specific potentiation may result from mobilisation of intracellular calcium since imidazole has been shown to release bound calcium from isolated microsomes, a phenomenon that could be due to reduction of cyclic AMP by imidazole (Nilsson et al, 1976). The slight increase in basal tone during exposure of rat descending colon to imidazole observed in this study could be interpreted in the light of the findings of Huang and Kemp (1971) who have reported imidazole to be effective in antagonizing increases in cyclic AMP while having little effect on basal levels of the nucleotide.

In preliminary experiments exogenous cyclic GMP had no effect on the response of rat descending colon to angiotensin, PGE_2 or to potassium. It is possible that the lack of effect may be due to the inability of the nucleotide to enter the cell as is the case with cyclic AMP (Falbriard et al, 1967; Moore et al, 1968). Nonetheless it is interesting to note that so far elevation of cyclic GMP during smooth muscle contraction has only been conclusively associated with cholinergic agonists through an atropine sensitive receptor (Murad and Kimura, 1974). These workers observed that incubation of tracheal rings with acetylcholine or carbachol increased cyclic GMP but angiotensin had no effect on the level of cyclic GMP or cyclic AMP.

5. Role of Prostaglandins in the Contractile Responses of Rat Descending Colon to Angiotensin

Angiotensin stimulates prostaglandin synthesis in a variety of tissues including the kidney (McGiff and Crowshaw, 1970; Aiken and Vane, 1973), spleen (Ferreira, Moncada and Vane, 1973; Douglas et al, 1973) and vascular endothelial cells (Gimrone and Alexander, 1975). Indomethacin an inhibitor of prostaglandin biosynthesis (Vane, 1971; Flower, 1974) has been reported to cause a selective inhibition of angiotensin contractile responses of the guinea-pig ileum that was reversed by addition of prostaglandin E_2, this has led to the proposition that prostaglandins may mediate contractions to angiotensin (Chong and Downing, 1973, 1974).

In concentrations of 3 - 6 μmol indomethacin inhibits prostaglandin synthesis in smooth muscle preparations (Ferreira, Moncada and Vane, 1972; Eckenfels and Vane, 1972; Flower, 1974). In this study, at a concentration of 5 μmol indomethacin had no effect on the responses of the rat descending colon to angiotensin, PGE_2 or potassium. This suggested that in this preparation prostaglandins are not involved in the contractile responses to any of the three spasmogens. However when the preparations were exposed to a higher concentration of indomethacin (50μmol) the responses to potassium chloride determined 5 minutes after treatment with indomethacin were reduced more than the

responses to angiotensin and PGE_2. The subsequent responses to all spasmogens were equally reduced. Northover (1971) has observed both inhibition of contraction and calcium uptake of electrically stimulated smooth muscle by the local anaesthetic cinchocaine and by indomethacin at high concentration of 0.3 - 0.6 mmol. It is likely that the marked reduction of responses to potassium observed in this study 5 minutes after exposure of the preparation to indomethacin (50μmol) is due to membrane effects of indomethacin exerting local anaesthetic like activity to inhibit calcium influx (Northover, 1971, 1972). This would be in agreement with the previous observations of inhibition of responses to potassium during treatment with calcium antagonists or exposure to high calcium concentration that may cause muscle membrane stabilisation. In further experiments Northover (1972 and 1973) has demonstrated that high concentrations of indomethacin caused a general inhibition of calcium uptake by smooth muscle and reduced calcium binding to isolated microsomes originating from the sarcoplasmic reticulum. The general inhibition of responses to angiotensin, PGE_2 and potassium on prolonged treatment of the preparations with 50μmol indomethacin in this study may therefore be a result of a non-specific effect of indomethacin upon calcium mobilisation at the membrane and at an intracellular site. It is unlikely to indicate a general involvement of prostaglandins in the contractile response to all three spasmogens. A similar non-selective inhibition

of noradrenaline and potassium responses by indomethacin (180μmol) has also been observed in rat mesenteric arteries (Manku and Horrobin, 1976; Kondo et al, 1977). The findings of this study do not support the hypothesis that angiotensin responses of the rat descending colon are mediated via prostaglandin synthesis and release. They are in agreement with those of Chong and Downing (1973) who found indomethacin (28 - 112μmol produced a selective inhibition of responses of guinea-pig ileum to angiotensin compared with responses to acetylcholine but was without effect on the responses of rat descending colon to angiotensin. They are also consistent with the report of Gagnon and Sirois (1972) who have observed contractions of angiotensin of rat descending colon in the presence of the prostaglandin blocker polyphloretin phosphate (PPP) (Eakins et al, 1970, 1971; Bennett and Posner, 1971). The results are further supported by a similar finding that angiotensin induced vasoconstriction in the hindlimb of the dog is not associated with prostaglandin release (Aiken and Vane, 1973). In contrast to the above findings there have been reports that prostaglandins serve as mediators of the contractile action of angiotensin in rat uterus (Boudouin-Legros, Meyer and Worcel, 1974) and guinea-pig ileum (Chong and Downing, 1973, 1974). In addition it has also been suggested that prostaglandins may serve to attenuate the vasoconstrictor response to angiotensin of rabbit coeliac and mesenteric arteries (Aiken, 1974). These reports lead to the conclusion that the involvement of prostaglandins in angiotensin induced contractions may vary in different smooth muscles.

6. Role of Metabolism in the Contractile Responses of Rat Descending Colon to Angiotensin

The energy requirements for smooth muscle contraction depend both upon the spasmogen and the type of smooth muscle preparation, (see Altura and Altura, 1970; Crocker and Wilson, 1974, 1975). 2,4-Dinitrophenol has been extensively used to investigate the metabolism of smooth muscle preparations and its effects have been shown to be consistent with its known mode of action as an uncoupler of oxidative phosphorylation (Born and Bulbring, 1955; Daniel, 1964; Rangachari, Paton and Daniel, 1972; Greenberg, Wilson and Long, 1973). In this study, 2,4-dinitrophenol was used in a Tyrode's solution containing glucose as an exogenous substrate for glycolysis so that, a limited energy production dependent upon anaerobic glycolysis would be present and the preparation would be capable of some degree of response to applied spasmogens (Daniel, 1964; Rangachari et al, 1972; Greenberg et al, 1973). Treatment with 2,4-dinitrophenol (0.05M) progressively reduced the responses of rat descending colon to angiotensin, PGE_2 and potassium but at all times the reduction of angiotensin and PGE_2 responses was significantly greater than of the corresponding responses to potassium. It appears from these results that angiotensin and PGE_2 responses are more dependent on oxidative energy than responses to potassium.

It has also been shown that angiotensin responses

of isolated rabbit aorta are more dependent on the presence of glucose than responses to catecholamines or potassium (Altura and Altura, 1970). Experiments by Crocker and Wilson with guinea-pig ileum (1974) and rat colon (1975) have also demonstrated that responses to angiotensin are more dependent upon the presence of glucose than the responses to acetylcholine. These observations were attributed to a difference in the ability by spasmogens to utilize endogenous glycogen and fats as a source of energy for contraction. However it has also been reported that anoxia inhibits the responses to angiotensin in guinea-pig ileum (Crocker and Wilson, 1974) and of prostaglandins on rat stomach strip and rat uterus (Coceani and Wolfe, 1966; Paton and Daniel, 1967) while having a relatively small effect on the responses to acetylcholine. This suggests that oxidative energy is essential for the contractile responses to both angiotensin and prostaglandins. Since the contractile action of potassium is due to membrane depolarization associated with calcium influx (Marshall and Kroeger, 1973) the only energy required by this spasmogen is likely to be the one required by the contractile machinery. Therefore the greater reduction of the responses to angiotensin and PGE_2 than potassium during treatment of rat descending colon with 2,4-dinitrophenol would suggest that extra ATP is required in the angiotensin and PGE_2 response at some stage prior to the contractile process. The nature of the ATP dependent mechanism is as yet unresolved.

SECTION III: Electrophysiological Studies of the Action of Angiotensin on Longitudinal Muscle of Rat Descending Colon and Guinea-pig Taenia Coli

Previous experiments with rat descending colon showed that the contractile action of angiotensin was greatly influenced by experimental treatments that affected the availability of external Ca and Na ions. Based upon this indirect evidence, it appeared that angiotensin was acting by affecting Ca and Na ion movements by a process independent of membrane depolarization. To investigate this possibility, the membrane effects of angiotensin on the longitudinal muscle of rat colon were studied using the sucrose gap technique. The method has the advantage that it allows simultaneous recording of electrical activity throughout the course of large contractions, which would preclude intracellular maintenance of microelectrodes (see Introduction pp 37). With this method it is possible to relate directly membrane effects to muscle contraction. The study was extended to include the action of the peptide on guinea-pig taenia coli as a control preparation.

1. Sucrose Gap Method and Normal Electrical Activity in Longitudinal Muscle of Rat Descending Colon and Guinea-Pig Taenia Coli

The modified sucrose-gap apparatus of Boev and Golenhofen (1974) was used in which junctional potentials were eliminated by use of rubber membranes. Resting membrane potential and electrical activity of guinea-pig taenia coli were recorded which consisted of slow waves and spikes similar to those reported by other workers (Burnstock and Straub, 1958; Shimo and Holland, 1966). This validated the use of this modified equipment in this study.

The origin of spontaneous activity of slow waves and spikes in taenia coli have been attributed to a periodically changing rate of active processes (affected by change in temperature and/or DNP) at the membrane which affect its stability and cause periodical shifts in the balance of ionic fluxes (Bulbring and Burnstock, 1960; Tomita and Watanabe, 1973). The main ionic current involved in the slow waves is that of sodium (Bülbring and Kuriyama, 1963; Bolton, 1971; Tomita and Watanabe, 1973). The spikes on the other hand are due to an inward flowing calcium current (Brading et al, 1969; Bülbring and Tomita, 1970). Hodgkin and Horowicz (1957) found that the part played by chloride ions can become important when the potassium concentration is suddenly changed and the chloride left unaltered. In this condition the chloride may temporarily dominate the

membrane potential until the chloride concentration inside the fibre has changed in accordance with the potassium potential. This may explain why a higher resting membrane potential was obtained when K_2SO_4 was used as a depolarizing agent than when KCl was used.

A higher resting membrane potential was also obtained with longitudinal muscle of rat colon when K_2SO_4 was used as a depolarizing agent compared with KCl. In this preparation however the resting membrane potential was stable. Similar electrical quiescence has been reported in the aorta and main pulmonary artery of both the rabbit and dog by Somlyo and Somlyo (1968a). Bozler (1948) has named smooth muscle tissues that display spontaneous spikes as "single-unit" tissues and those that show electrical quiescence as "multi unit". Presumably electrical quiescence in multi-unit systems may be due to either asynchronous electrical activity in muscle cells and hence an inability to conduct the action potentials or, possibly, to a lack of the membrane pump whose fluctuating activity affects ion permeability in single unit systems (Prosser et al, 1960; Burnstock and Prosser, 1960b; Burnstock et al, 1963).

2. Membrane Electrical Activity of Longitudinal Muscle of Rat Descending Colon and Guinea-pig Taenia Coli during Contraction to Angiotensin

The contractile responses of guinea-pig taenia coli to potassium were associated with membrane depolarization and an increase in spike size and frequency. These observations are similar to those reported by other workers (Burnstock and Straub, 1958; Shimo and Holland, 1966). Since the resting membrane is fully permeable to potassium ions in accordance with the Goldman equation (1943) the addition of external potassium ions would cause membrane depolarization by lowering the potassium equilibrium potential. Membrane depolarization by potassium in taenia coli has been reported to cause an increase in ^{45}Ca uptake and an increase in tension (Urakawa and Holland,1964). An increase in spike frequency may lead to an increase in extracellular calcium influx and in addition the spike calcium may trigger intracellular release of calcium (Mayer, Van Breemen and Casteels, 1972). It therefore appears that both membrane depolarization and increase in spike frequency function to increase intracellular calcium and hence increase muscle tension. The action of angiotensin on taenia coli was also associated with membrane depolarization and an increase in spike size and frequency although in some preparations the membrane depolarization was small ($\leq$ 3mV). Other workers have also found that the contractions to angiotensin in taenia coli (Ohashi et al, 1967),

rat uterus (Hamon and Worcel, 1977) and rabbit mesenteric veins (Cuthbert and Sutter, 1965; Somlyo and Somlyo, 1968a) are associated with membrane depolarization and an increase in spike size and frequency.

With longitudinal muscle of rat descending colon the membrane depolarization associated with contractions to angiotensin was small ($<$ 5mV) and in some cases undetectable. This would tend to suggest that the membrane depolarization is not the primary mechanism by which angiotensin exerts its contractile action. Since calcium contributes to the stability of the cell membrane (Frankenhauser and Hodgkin, 1957) it is likely that the membrane depolarization occurring with contractions to angiotensin is an epiphenomena resulting from changes in ion permeability, secondary to mobilisation of calcium. Since small depolarizations were associated with the action of angiotensin in this preparation, the failure to observe membrane potential changes in some preparations may be due to limitations in the sensitivity of the apparatus. Alternatively, the inability to detect membrane depolarization during contraction of longitudinal muscle of rat descending colon to angiotensin may further support the previous suggestion that angiotensin does not exert its contractile action in rat colon by membrane depolarization. Somlyo and Somlyo (1968a) have called the process by which some spasmogens may induce smooth muscle contraction without membrane depolarization as

"pharmacomechanical coupling".

It is interesting to note that using the sucrose gap technique, Somlyo and Somlyo (1968a) have also reported a small spike free depolarization of less than 8mV in rat pulmonary artery exposed to noradrenaline, to angiotensin, to 5-hydroxytryptamine or to histamine. Using the same technique Keatinge (1966) has also recorded a small ($\leq$ 9mV) spike free membrane depolarization in sheep carotid arteries exposed to angiotensin. Although the effect of any given drug in a tissue, as recorded by the sucrose-gap technique is the net statistical result of changes in the resting membrane potential or spike electrogenesis within a cell population, the findings obtained by the sucrose-gap technique often show close similarity to those obtained by microelectrodes. Thus by using intracellular microelectrodes Haeusler (1972) has recorded a maximum spike free depolarization of 6.3mV to noradrenaline in rabbit pulmonary artery which is in close agreement to the results obtained by Somlyo and Somlyo (1968a) with the sucrose gap technique.

3. Role of Calcium and Sodium Ions in the Electrical and Mechanical Effects of Angiotensin on Longitudinal Muscle of Rat Descending Colon and Guinea-pig Taenia Coli

Determination of the effect of changes in external potassium concentration on the resting membrane potential of guinea-pig taenia coli has revealed that the membrane potential decreases linearly with an increase in Log $[K^+]_e$ at concentrations above 30mM; and the change in potential per ten fold increase has been found to be between 26 and 51.5mV (Burnstock and Straub, 1958; Holman, 1958; Kuriyama, 1963; Riemer, Mayer, Ulbrecht, 1975). However the slope is not linear below 30mM $[K^+]_e$ and the change in potential is less than the 59mV per 10-fold change in $[K^+]_e$ predicted by the Nerst equation if K^+ were the sole determinant of membrane potential. Thus, Na^+ and Cl^- permeabilities contribute significantly to the maintenance of the resting membrane potential (Kuriyama, 1963). Given the electrochemical gradients estimated in guinea-pig taenia coli and taking into account the resting membrane potential in this tissue (Casteels and Kuriyama, 1966) depolarization can be induced by individual or simultaneous increases in Na^+, Ca^{2+} and Cl^- conductances. In order to identify the ions involved in the observed membrane effects to angiotensin in both guinea-pig taenia coli and longitudinal muscle of rat descending colon, the effect of changes in external Ca and Na ions was investigated.

(a) Role of Extracellular Calcium Ions

(i) Action of Angiotensin on Smooth Muscle in Ca^{2+}-free Solution

Perfusion with Ca^{2+}-free solution caused a fall in the resting membrane potential and markedly diminished the spontaneous spikes that are normally observed with taenia coli. It abolished contractile responses to both angiotensin and KCl but the corresponding membrane depolarization without the accompanying spikes remained. The diminution of spikes during perfusion of the preparation with Ca^{2+}-free solution is in agreement with the role of calcium as a carrier of the inward current responsible for the action potential in taenia coli (Brading, Bülbring and Tomita, 1969; Bulbring and Tomita, 1970; Kuriyama, Ito and Suzuki, 1977). Brading and colleagues (1969) have also observed a fall in the resting membrane potential during exposure of guinea-pig taenia coli to calcium free solution. Since the fall in resting membrane potential occurred only in the presence of sodium, they have attributed this phenomenon to an increase in sodium ion permeability as a result of reduced competition between Ca and Na ions for membrane transport sites (Goodford, 1967; Bülbring and Tomita, 1970; Goodford and Wolowyk, 1972). The abolition of the contractile responses of guinea-pig taenia coli to potassium is in agreement with reports by other workers of the dependence of the spasmogen on external calcium for contraction (Imai

and Takeda, 1957; Durbin and Jenkinson 1961b; Urakawa and Holland, 1964). In addition the persistence of membrane depolarization to potassium during perfusion of taenia coli with Ca^{2+}-free Krebs' solution is consistent with a mechanism of depolarization by a decrease of K^{+} equilibrium potential (see review Van Breemen et al, 1979). Since the contractile response of guinea-pig taenia coli to angiotensin was also abolished during exclusion of Ca^{2+} from the perfusing solution it is reasonable to assume that the response is dependent on extracellular Ca^{2+}. The persistence of membrane depolarization to angiotensin in the absence of external calcium in bathing solution suggested that angiotensin causes membrane depolarization by a mechanism unrelated to an increase in Ca^{2+} permeability. As in guinea-pig taenia coli, it has been reported that the addition of angiotensin on rat myometrium causes muscle contraction associated with membrane depolarization and an increase in spike frequency. Exposure of this preparation to Ca^{2+}-free solution also abolished spikes and muscle contraction, but did not affect the membrane depolarization to angiotensin (Hamon and Worcel, 1977).

With longitudinal muscle of rat colon, perfusion of preparation with Ca^{2+}-free Tyrode's solution caused a fall in the resting membrane potential similar to that observed with guinea-pig taenia coli. As in guinea-pig taenia coli the contractile response of longitudinal muscle

of rat descending colon to potassium was abolished during perfusion with Ca^{2+}-free solution without affecting the corresponding membrane potential. In contrast both the membrane depolarization and contractile response of longitudinal muscle of rat descending colon to angiotensin were abolished when the preparation was exposed to Ca^{2+}-free solution. This suggested that the observed membrane depolarization to angiotensin in longitudinal muscle of rat descending colon was either due to an increase in membrane permeability to calcium ions or was an epiphenomena which occurs with contraction but does not trigger contraction. This epiphenomena may have occurred due to a secondary change in permeability to other ions as a result of calcium mobilisation by angiotensin.

(ii) Action of Angiotensin on Smooth Muscle in High Ca^{2+}-solution

Calcium is necessary for the activation of the contractile apparatus in smooth muscle (Sparrow et al, 1970; Ruegg, 1971). It is also essential for cell membrane stability (Frankenhauser and Hodgkin, 1957). Exposure of guinea-pig taenia coli to a solution containing 10.8mM Ca^{++} caused a hyperpolarization of the membrane, an inhibition of spontaneous spikes and a fall in resting tension. These findings are in agreement with those of other workers (Holman, 1958; Bülbring and Kuriyama, 1963) who have attributed

the reduction in spontaneous spike frequency observed when taenia coli was exposed to high calcium solution to be due to muscle membrane stabilisation. Since Bülbring (1955) has found a correlation between spike frequency and muscle tension in taenia coli, it is logical to assume that the observed fall in resting tension was a result of inhibition of the spontaneous spiking. Exposure of taenia coli to the high calcium solution also caused a reduction in membrane depolarization, and contractile responses to angiotensin and potassium. The spikes which normally occur during membrane depolarization to these spasmogens were also abolished A reduction of membrane depolarization to potassium in taenia coli has also been reported by Kuriyama (1963) when the preparation was exposed to an $[Ca^{++}]_e$ of 7.5mM. The observed reduction of membrane depolarization of guinea-pig taenia coli to potassium in this study suggests a decrease of membrane permeability to K^+ and is consistent with membrane stabilization due to elevated extracellular calcium concentration (Holman, 1958). Since Ca and Na ions compete for transport sites (Bülbring and Tomita, 1970) it is reasonable to assume that a marked increase in extracellular calcium concentration would reduce the membrane permeability to sodium ions. These results therefore suggest that an increase in sodium ion permeability may participate in the membrane depolarization of guinea-pig taenia coli to angiotensin. They further suggest that in addition to an increase in spike frequency angiotensin may exert its contractile action

by causing membrane depolarization.

As in taenia coli, exposure of longitudinal muscle of rat descending colon to a high calcium solution caused hyperpolarization of the membrane and a decrease of membrane depolarization and contractile response to potassium. The reduction of membrane depolarization to potassium was consistent with muscle membrane stabilization by high calcium. It is reasonable to assume that a spasmogen eliciting contraction primarily through membrane depolarization would have a diminished activity in this condition. Since both the membrane depolarization and the contraction to angiotensin on longitudinal muscle of rat descending colon were increased these results therefore suggest that membrane depolarization was not a primary step in the contraCtile action of angiotensin in this tissue. The present results confirm the previous observation (in organ bath experiments) of the dependence of the angiotensin response on extracellular calcium for contraction. The results add further evidence to suggest that the observed membrane depolarization to angiotensin in this tissue is either due to increased permeability to calcium ions or is an epiphenomena occurring during contraction.

(b) Role of Extracellular Sodium Ions

(i) Action of Angiotensin on Smooth Muscle in Na^+-deficient Solution

The possibility that the observed membrane depolarization of guinea-pig taenia coli and longitudinal muscle of rat descending colon during exposure to angiotensin may be due to an increase in permeability to sodium ions was studied by reduction in external sodium of perfusing solution with the addition of sucrose or tris as an osmotic substitute.

With guinea-pig taenia coli reduction of external Na^+ from 119mM (normal) to 25mM with isomolar sucrose substitution resulted in a fall in resting membrane potential, a transient increase of both spike frequency and tension followed by decline in tension and a reduction (but not abolition) of spontaneous spikes. However when tris was used as a substitute for Na^+ the membrane was hyperpolarized and spontaneous spikes persisted throughout the experimental time period. The reasons for these differences are not clear. However the reduction of one ion does affect the permeability to another (Casteels, 1970) and so results in ionic redistribution. The differences in the resulting membrane potential could be due to the effect of replacing chloride by a non-permeant substance, sucrose, since this has been demonstrated to reduce the potassium permeability; whereas replacing Na^+ by tris or choline reduced K^+ uptake and increased passive

loss of K+ from cells (see Casteels, 1970). The persistence of spontaneous spikes during perfusion of taenia coli with Na^+-deficient solution indicated that sodium is not necessary for spikes, although it probably exerts a modulatory role perhaps via competition for or near the calcium influx site (Brading et al, 1969; Bülbring and Tomita, 1970). The changes in spontaneous electrical activity and resting membrane potential observed in this study when taenia coli was exposed to Na^+-deficient solutions are similar to those reported by other workers (Bülbring and Kuriyama, 1963; Kuriyama, 1963). Casteels and Van Breemen (1975) have studied the effect of a reduction in external Na^+ on the intracellular Ca content of taenia coli. They found that replacement of all but 7mM Na^+ with choline caused a small plateau type of increase in ^{45}Ca uptake over that seen with normal Na^+. The observed increase in ^{45}Ca content by these workers may represent the transient increase in spike frequency and tension observed in this study.

In this study when sucrose was used as a substitute for sodium chloride the membrane depolarization and contractile response of taenia coli to potassium were increased. The increase in contractile response could be due to an increase in calcium influx due to reduced competition by sodium for calcium transporting sites as in cardiac muscle (Lüttgau and Niedergerke, 1958). It is reasonable to assume that replacement of sodium chloride by sucrose leads to depletion

of tissue sodium and chloride, in which case K^+ would predominantly contribute to the maintenance of membrane potential. Since depolarization to potassium is by a decrease in K^+ equilibrium potential, the addition of potassium in such circumstances would therefore cause greater membrane depolarization, as observed in this study. However, when tris was used as a substitute for sodium the membrane depolarization to potassium of guinea-pig taenia coli was slightly reduced. This suggested that in the hyperpolarized state the membrane displayed reduced permeability to potassium ions.

The observed reduction of membrane depolarization to angiotensin in guinea-pig taenia coli whether tris or sucrose was used as a substitute for Na^+ in guinea-pig taenia coli suggests that the membrane depolarization to angiotensin may be due to an increase in permeability to sodium ions (PNa). Since the reduction in membrane depolarization was accompanied by a corresponding reduction in contraction this suggested that in addition to increased spikes angiotensin exerts its contractile action through membrane depolarization. Because these experiments were not designed to study the role of chloride ions in membrane depolarization in taenia coli, one cannot reject a priori a possible involvement of chloride ions. This would therefore be interesting to study in future work.

The findings in this study of the action of angiotensin on guinea-pig taenia coli are similar to those of Hamon and Worcel (1973) who have reported that angiotensin increased ^{24}Na influx in both polarized and depolarized uterine muscle. These workers also observed an increase in ^{42}K and ^{36}Cl efflux in polarized muscles but this was absent in potassium depolarized tissues. Hence the effect of angiotensin on these two latter ions was regarded as a consequence of the depolarization induced by angiotensin through a primary increase in sodium influx. In electrophysiological experiments using the double-sucrose gap method, Hamon and Worcel (1977) have found that in the same tissue angiotensin causes membrane depolarization and an increase in spike frequency. In experiments which involved reduction of $[Na^+]e$ and voltage clamp recording they established conclusively that the membrane depolarization to angiotensin was mainly due to an increase in sodium conductance. They observed a tonic muscle contraction associated with an increase in membrane conductance when sodium was present even when the membrane potential was held at a baseline level. Since, in the present study, angiotensin also caused membrane depolarization and an increase in spike frequency in guinea-pig taenia coli it is possible that the membrane depolarization is also due to an increase in Na^+ conductance. It is therefore necessary to confirm the findings of the present study both by voltage clamp, by the double-sucrose gap method (Bülbring and Tomita, 1969a)

and ^{24}Na ion flux measurements.

Like taenia coli, substitution of sodium chloride in the bathing solution by sucrose caused a fall in resting membrane potential and increased the membrane depolarization and contractile response of longitudinal muscle of rat descending colon to potassium. Friedman and co-workers (1959) have also reported that equilibration of rat colon strip in a solution in which some of the sodium was substituted with sucrose increased the sensitivity of the tissue to a threshold dose of carbachol, while equilibration in a high Na^+ medium had the opposite effect. This they attributed to a change in sodium concentration gradient across the plasmalemma. Although tris chloride was not used as a substitute for sodium chloride in experiments on the longitudinal muscle of rat descending colon, it remains to be seen if this would have similar effect upon the membrane depolarization and contractile responses to potassium to those observed in taenia coli.

As with taenia coli, the membrane depolarization and contractile response to angiotensin on longitudinal muscle of the rat descending colon were also diminished when external sodium was reduced. Since it was observed previously that the membrane depolarization to angiotensin was abolished when the preparation was exposed to calcium-free medium in which the external sodium concentration was unchanged, these

results do not support a primary role of sodium ions in membrane depolarization of longitudinal muscle of rat descending colon to angiotensin. The observed inhibition of membrane depolarization could be a consequence of abolition of contraction to angiotensin. A possible mechanism of abolition of contraction during reduction of $[Na^+]e$ could be impairment of Na-Ca exchange which may be involved in the contractile action of angiotensin as has been suggested in this study by previous findings in organ bath experiments. It would be interesting in future experiments to see if increases in $[Na^+]e$ have the opposite effect to low $[Na^+]e$ on the responses to angiotensin.

4. Action of Angiotensin on Longitudinal Muscle of Rat Descending Colon and Guinea-pig Taenia Coli in the Presence of Verampamil

It has been observed that the verapamil-like calcium antagonists selectively abolish potassium induced and spike activated contractions in smooth muscle (P system) without affecting contractions elicited by spike-free activation mechanism (T system) (see Introduction pp 27). In the present study, the contractile responses of guinea-pig taenia coli were associated with membrane depolarization and an increase in spike frequency. Perfusion of this preparation with a solution which contained verapamil (3.5×10^{-6}M) inhibited spontaneous electrical and mechanical activity. The spikes were inhibited without markedly affecting the resting membrane potential. The contractile responses to angiotensin and potassium were diminished but the membrane depolarization remained although the accompanying spikes were markedly reduced. These results suggest that an increase in spike frequency is essential for the contractile action of angiotensin in taenia coli. It has been reported that D600 (methoxy-verapamil) inhibits spike induced ^{45}Ca uptake in taenia coli (Mayer et al, 1972) and voltage clamp studies have also revealed that D600 inhibits the inward calcium current in uterine smooth muscle (Anderson et al, 1971; Mironneau, 1973). In addition it has been observed that verapamil blocks the spike activity and the contractile responses of rat uterus to oxytocin and potassium chloride without affecting the membrane depolarization (Fleckenstein

et al, 1971). The inhibitory activity of verapamil was antagonized by an increase in extracellular calcium thus furnishing further evidence that verapamil exerts its action by inhibition of calcium influx (Fleckenstein et al, 1971). In the present study the observed blockade of spikes in taenia coli provides support for the role of calcium as the carrier of the inward current in the spike. Since the membrane depolarization to angiotensin was unaffected by verapamil, the results favour the view that the membrane depolarization is not due to an increase in conductance to calcium ions. This is in agreement with the results obtained with low $[Na^+]_e$ which suggested that the membrane depolarization to angiotensin is a result of an increase in permeability to sodium ions.

In addition to inhibition of calcium fluxes, at high concentrations verapamil acts as a local anaesthetic to block sodium and potassium ion permeability (Singh and Vaughan-Williams, 1972; Van der Kloot and Kita, 1975). This may explain the observed abolition of membrane depolarization to both angiotensin and potassium by a high concentration of verapamil (6×10^{-6}M). This also indicates that in taenia coli, a similarity exists in the Ca^{2+} and Na^+ channels.

It has been reported that the addition of noradrenaline (NA) on guinea-pig portal vein (Golenhofen, Hermestein and Lammel, 1973) and of acetylcholine on guinea-pig circular

stomach muscle (Golenhofen and Wegner, 1975) resulted in muscle contraction associated with membrane depolarization and an increase in spike frequency. In the presence of verapamil the spikes and phasic contractions were abolished leaving a tonic contraction accompanied by spike free membrane depolarization. This indicated that in these tissues contraction involved activation of both "P" and "T" systems as defined by Golenhofen (1976). However, in this study treatment of guinea-pig taenia coli with verapamil did not reveal any spike free contraction to angiotensin suggesting that the contraction in this tissue is wholly through activation of the "P" system. A similar abolition by verapamil of the contractile responses of guinea-pig taenia coli to acetylcholine has been reported by Golenhofen and Wegner (1975). Since Freer (1975a) has observed an abolition of the contractile response to angiotensin of rat uterus by SKF525A and verapamil, it is possible that the contractile action of angiotensin in rat uterine muscle and guinea-pig taenia coli may involve a similar mechanism.

On longitudinal muscle of rat descending colon verapamil (6×10^{-6}M) selectively blocked contraction to potassium without affecting the membrane depolarization. At this concentration verapamil had no effect on the membrane depolarization and contractile responses to angiotensin. This suggested the angiotensin contractile effect in this tissue involves a verapamil resistant spike free activation ("T"

mechanism) as has been reported for the contractile response of rat aorta to NA and of rat fundus to cholecytokinin (Golenhofen, 1976). At high concentration, verapamil-like calcium antagonists may also affect the "T" mechanism (Golenhofen and Hermstein, 1975; Golenhofen, 1976), this may explain the reduction in the angiotensin electrical and mechanical responses observed in some tissues in this study.

5. Action of Angiotensin on Longitudinal Muscle of Rat Descending Colon and Guinea-pig Taenia Coli in the Presence of Sodium Nitroprusside

It has been reported that the contractile responses of rat aorta to NA and of stomach fundus muscle to acetylcholine which are resistant to verapamil and D600 can be selectively inhibited by sodium nitroprusside (Golenhofen, 1976; Boev, Golenhofen and Lukanow, 1976). In addition, Na-nitroprusside has been shown to be less effective in antagonizing smooth muscle contractions to potassium and other agonist induced contractions in smooth muscles displaying phasic activity (Haeusler and Thorens, 1976; Kreye et al, 1975; Kreye and Lüth, 1976). It has become evident from these observations that sodium nitroprusside may serve to identify agonist induced contractions which involve a tonic mechanism.

In smooth muscle preparations where sodium nitroprusside shows a specific activity, inhibition of smooth muscle contraction is generally observed at very low concentrations. Thus the threshold concentration of sodium nitroprusside for relaxation of helical strips from rat aorta contracted by 10^{-8}M noradrenaline is 10^{-10}M (see Kreye and Gross, 1977). Low concentrations of Na-nitroprusside showed no effect on the contractile responses to angiotensin and potassium in both guinea-pig taenia coli and longitudinal muscle of rat colon. This led to the use

of a higher concentration of Na-nitroprusside (4×10^{-3}M) in this study.

Perfusion of guinea-pig taenia coli with a solution containing Na-nitroprusside (4×10^{-3}M) caused a fall in the resting membrane potential and a reduction of electrical and mechanical responses to potassium and angiotensin. Previous results with verapamil in this study had revealed that the angiotensin response of taenia coli does not display a spike free tonic contractile component. It was therefore assumed that at the high concentration used, sodium nitroprusside was exerting a non-specific effect in taenia coli. It has been proposed that part of the action of sodium nitroprusside may be due to a de novo release of cyanide which impairs the formation of ATP (Kreye et al, 1975; Kreye and Luth, 1976). It is therefore possible that this may interfere with the energy dependent Na-K pump which is very active in guinea-pig taenia coli (Casteels, 1966) which would explain the observed fall in resting membrane potential.

Sodium nitroprusside (4×10^{-3}M) had no effect upon the membrane depolarization and contractile responses of longitudinal muscle of rat colon to angiotensin and potassium. The lack of effect of sodium nitroprusside on the membrane and contractile response of longitudinal muscle of rat descending colon to angiotensin does not constitute prima facia evidence against the involvement of a tonic

mechanism in the contractile responses to the peptide. Rather, it may suggest that the angiotensin contraction in this preparation involves a tonic mechanism which is resistant to verapamil but which is not sensitive to inhibition by Na-nitroprusside. Na-nitroprusside has already been shown to be without effect on the D600 resistant tonic contractions of tissues like vas deferens and rat uterus (Golenhofen, 1976; Kreye and Gross, 1977) which indicates that Na-nitroprusside lacks universal specificity in its ability to block the "T" system.

SECTION III: ^{45}Ca Uptake Studies in Longitudinal Muscle of Rat Descending Colon

Calcium required for contraction of smooth muscle may originate from extracellular or intracellular sources (Somlyo and Somlyo, 1968b; Devine et al, 1973; Deth and Van Breemen, 1974). Previous experiments in this study showed that the contractile responses of rat descending colon to angiotensin were greatly influenced by changes in external calcium concentration. The results suggested that angiotensin may exert its contractile action by causing influx of extracellular calcium. To investigate this possibility, the effect of angiotensin on ^{45}Ca cellular uptake of the longitudinal muscle of rat descending colon was studied using the lanthanum technique (see Methods pp 83). The method has the advantage that it enables measurement of intracellular calcium which is more directly related to smooth muscle contraction than total tissue calcium (Van Breemen and McNaughton, 1970; Van Breemen et al, 1972). The prediction by Lettvin and co-workers (1964) that by virtue of an ionic radius similar to that of calcium and higher valency than calcium (i.e. La^{3+} has a higher charge density than Ca^{2+}), lanthanum will bind at superficially located calcium sites in less reversible manner than does calcium may be regarded as the idea that led to the development of the lanthanum method. The lanthanum method developed by Van Breemen and co-workers (1970, 1972) is based upon

the assumptions that in sufficiently high extracellular concentration lanthanum will (a) displace and replace extracellular calcium (b) block both calcium uptake and efflux, and (c) not enter the cell in appreciable quantities to displace or alter cellular calcium distribution. Some investigators have expressed considerable doubt whether lanthanum would displace all the membrane bound calcium and would remain entirely outside the cell (Hodgson, Kidwai and Daniel, 1972; Weiss, 1974; Widdicombe, 1975). They have therefore expressed the opinion that the lanthanum-resistant calcium fraction may represent only one of several such fractions (Freeman and Daniel, 1973) nonetheless its measurement provides an estimate of transmembrane calcium flux associated with the excitatory action of spasmogens like potassium (Van Breemen et al, 1972; Mayer et al, 1972) and noradrenaline (Godfraind, 1976). Thus, with the advent of the lanthanum method it has been demonstrated that the contractile response to potassium of a variety of smooth muscles including rabbit aorta (Van Breemen et al, 1972; Deth and Van Breemen, 1974), rat uterus (Marshall and Kroeger, 1973) and guinea-pig taenia coli (Mayer et al, 1972) was accompanied by an increase in ^{45}Ca cellular uptake that was inhibited by SKF525A, verapamil or D600. This formed a reasonable basis for the use of potassium chloride as a control spasmogen in this study.

When longitudinal muscle of rat descending colon

was exposed to a solution containing ^{45}Ca, total tissue ^{45}Ca uptake far exceeded cellular ^{45}Ca uptake. This indicated that the bulk of the tissue ^{45}Ca is stored in the extracellular space. Since it is the intracellular calcium that is related to muscle contraction, the large ^{45}Ca uptake in the extracellular space may mask any subsequent changes that may occur during muscle contraction to a spasmogen and hence emphasises the need for the lanthanum method (Van Breemen and McNaughton, 1970). In this study the cellular ^{45}Ca uptake during incubation showed an initial rapid uptake that slowed down with prolonged time of incubation. Calcium entry into the cell is by multiple processes, and thus the net cellular calcium uptake is a balance of mechanisms that favour calcium entry versus those factors that operate to lower intracellular calcium concentration (see Introduction pp 14). When the tissue is first exposed to ^{45}Ca containing solution the uptake is fast possibly because of diffusion of calcium down an electrical and concentration gradient. Subsequently the rate of uptake decreases presumably due to increased extrusion. Similarly total tissue ^{45}Ca uptake was rapid initially but slowed down with time of incubation. Since most of the total tissue ^{45}Ca uptake is in the extracellular space, one would expect the uptake curve to level off when the extracellular space reaches total equilibrium with the uptake solution. It would therefore be valuable in future work to determine the total tissue ^{45}Ca uptake at longer periods of incubation

than the maximum of 20 minutes used in this study.

It was observed in this study that the contractile responses of longitudinal muscle of rat descending colon to angiotensin and potassium was associated with an increase in cellular ^{45}Ca uptake. This suggested that the contractile responses to potassium and angiotensin involves an influx of extracellular calcium although an additional mechanism involving a decrease of ^{45}Ca efflux could not be ruled out by the present experiments. The cellular ^{45}Ca uptake as well as the contractile response to potassium were inhibited by verapamil. This finding is in agreement with the inhibition by verapamil-like calcium antagonists of both contraction and cellular ^{45}Ca uptake to potassium reported by other workers in a variety of smooth muscles (Mayer et al, 1972; Marshall and Kroeger, 1973; Lammel, 1977). In contrast, both the cellular ^{45}Ca uptake and contractile response to angiotensin were resistant to verapamil. It was concluded from these observations that the angiotensin contractile action of longitudinal muscle of rat descending colon involves an influx of extracellular calcium resistant to inhibition by verapamil. It also suggests that the contractile action of angiotensin in this muscle may involve activation of a "T" system. A similar resistance of both contraction and ^{45}Ca cellular uptake to nifedipine in rat fundus muscle contracted by acetylcholine has been reported by Lammel (1977). The observed increase in cellular ^{45}Ca when

longitudinal muscle of rat descending colon was exposed to angiotensin confirms the previous observation that angiotensin contractions of rat descending colon are dependent upon the presence of calcium ions in the bathing medium.

SECTION IV: Conclusions

Calcium necessary for contraction of smooth muscle may originate from extracellular or intracellular sources (Hurwitz and Suria, 1971; Devine et al, 1973; Deth and Van Breemen, 1974). Changes in external calcium concentrations showed that angiotensin contractile responses of rat descending colon are more dependent on extracellular Ca^{2+} than potassium chloride or PGE_2 responses. This suggested that angiotensin exerts its contractile action by mobilisation of extracellular Ca^{2+}. Treatment of rat colon with either SKF525A or verapamil in Ca^{2+}-free solution completely abolished the responses to angiotensin, PGE_2 and potassium. On readmission of Ca^{2+} the responses to angiotensin and PGE_2 partially recovered while the responses to potassium remained abolished. Since SKF525A and verapamil have been shown to inhibit contractile responses due to extracellular calcium influx associated with membrane depolarization (Kalsner et al, 1970; Fleckeinstein et al, 1971) these results suggest that the responses to angiotensin and PGE_2 are independent of calcium influx linked to membrane depolarization. The observed partial recovery of responses to angiotensin and PGE_2 on readmission of Ca^{2+} in the continued presence of SKF525A and verapamil in preparations which at the end of the initial preincubation were unresponsive to the spasmogens led to the suggestion that the responses to these spasmogens involve extracellular calcium influx resistant to

calcium antagonists as suggested by Lammel (1977) for the contractile action of acetylcholine on rat fundic strip.

The distribution of cellular Na^+ is closely linked to that of Ca^{2+} (see Van Breemen et al, 1979). Thus a spasmogen may cause calcium mobilisation secondary to its effect on sodium distribution. There have been reports that angiotensin may exert its contractile action by activation of Na-K pump (Turker et al, 1967), stimulation of Na-Ca exchange (Godfraind, 1973) and by membrane depolarization due to an increase in membrane permeability to Na^+ (Hamon and Worcel, 1977). In this study reduction of $[Na^+]_e$ caused an increase of the initial angiotensin response followed by a decline of the subsequent responses. The potentiation of the initial angiotensin response declined with an increase in time of preparation incubation in Na^+-deficient solution which was attributed to depletion of an intracellular sodium pool by exchange with extracellular calcium. It was therefore suggested that angiotensin exerted its contractile action by mobilising extracellular calcium by a Na-Ca exchange mechanism. Treatment of the preparation with 2,4-dinitrophenol caused a greater inhibition of angiotensin and PGE_2 responses than of responses to potassium; which suggested that the contractile responses of the rat descending colon to angiotensin required additional energy for some step prior to the contractile machinery. The nature of the energy dependent step is not clear, but one possible candidate

would be an activation mechanism which is dependent upon an inwardly directed calcium pump coupled to outward movement of sodium ions (Bohr et al, 1969; Brading, 1973; Reuter et al, 1973) against the sodium electrochemical gradient.

The action of many peptide hormones in a variety of tissues is mediated by cyclic AMP (see Rasmussen and Goodman, 1977). Evidence also suggests that spasmogen contractile action may be mediated by changes in cyclic AMP (Bär, 1974). Treatment of rat descending colon with either isoprenaline, theophylline or dibutyryl cyclic AMP; agents which are expected to cause an increase in cyclic AMP caused a greater reduction of responses to angiotensin and PGE_2 than potassium chloride responses, this suggested that the contractile action of angiotensin may be mediated by a decrease in cyclic AMP. Imidazole an agent that causes a decrease in intracellular cyclic AMP by activation of phosphodiesterase (Butcher and Sutherland, 1962) did not show any selective potentiation of angiotensin and PGE_2 responses, this may be due to lack of specificity of action of imidazole. These findings need to be substantiated by direct measurements of changes in cyclic AMP during contraction to angiotensin.

In addition it has been proposed that angiotensin contractile action may be mediated by release of prostaglandins

(Chong and Downing, 1973, 1974). Indomethacin is a potent inhibitor of prostaglandin biosynthesis (see Flower, 1974). Treatment of rat descending colon with indomethacin (5μmol) had no effect on the contractile responses to the three spasmogens, but at higher concentration (50μmol) it caused a non selective inhibition of the responses to angiotensin, PGE_2 and potassium possibly by exerting a non specific membrane effect on calcium fluxes (Northover, 1972, 1973). This suggested that prostaglandins are not involved in mediating the angiotensin contractile action of rat descending colon.

Based on indirect evidence the results obtained from the preceding experiments suggested that angiotensin exerts a direct contractile action of the rat descending colon possibly by affecting calcium and sodium ion movement by a process independent of membrane depolarization. To confirm this possibility an electrophysiological study of the effect of angiotensin on longitudinal muscle of rat descending colon and of guinea-pig taenia coli was carried out using the sucrose gap technique.

These electrophysiological studies revealed that the contractile response of longitudinal smooth muscle of rat descending colon to angiotensin was associated with a small membrane depolarization ($<$ 5mV) which was dependent on extracellular Ca^{2+}. In some preparations this was not

detectable possibly due to insensitivity of the method, which could be resolved in future by use of intracellular microelectrodes. The membrane and contractile response to angiotensin were increased during exposure to a high extracellular Ca^{2+} concentration that inhibited contraction and membrane depolarization to potassium probably by muscle membrane stabilization (Holman, 1958). In addition the contractile response to potassium was inhibited by verapamil without affecting the membrane depolarization while both membrane depolarization and contraction to angiotensin were unaffected. It was concluded from these observations that angiotensin contraction does not involve extracellular Ca^{2+} influx linked to membrane depolarization. It was also suggested that although the observed membrane depolarization to angiotensin occurred with contraction it did not trigger contraction and was therefore an epiphenomena of contraction. The possibility remained, however, that the membrane depolarization to angiotensin could be a spike free depolarization consistent with smooth muscle tonic activation mechanism as proposed by Golenhofen (1976). However, sodium nitroprusside, an agent proposed to be a blocker of the "T" system (Golenhofen, 1976) did not affect the membrane and contractile response to angiotensin. This was attributed to lack of activity of sodium nitroprusside in this tissue.

On the other hand the contractile response of the guinea-pig taenia coli to angiotensin was associated with

membrane depolarization and an increase in spike size and frequency. The spikes were dependent on extracellular Ca^{2+} and were therefore abolished by either removal of external Ca^{2+} or treatment of the preparation with verapamil. The membrane depolarization to angiotensin was unaffected by treatment of the preparation with Ca^{2+}-free solution and addition of verapamil but was abolished by reduction of external Na^{+} and reduced by a high increase in external Ca^{2+} that also reduced the membrane depolarization to potassium. It was concluded that the membrane depolarization to angiotensin was due to an increase in membrane permeability to extracellular Na^{+}. The changes in external Ca^{2+} and Na^{+} concentration were accompanied by secondary changes in the resting membrane potential. The resulting resting membrane potential after changes in external Na^{+} concentration dependent on the substitute used. This in turn influenced the membrane response to the spasmogens. This problem resulting from secondary changes in resting membrane potential may be resolved in future experiments with voltage clamp, using the double sucrose gap technique. In addition, verapamil caused abolition of both contractile responses to angiotensin and potassium, suggesting that in taenia coli the contractile response to angiotensin involves extracellular calcium influx linked to membrane depolarization and does not involve a spike-free component resistant to verapamil.

In summary, different mechanisms are utilized by angiotensin in eliciting contractions of longitudinal muscle of rat descending colon and guinea-pig taenia coli. In the former preparation responses are independent of membrane depolarization whereas in the latter they are dependent both upon membrane depolarization and on increase in spike frequency. This idea of different mechanisms, is consistent with work with angiotensin derivatives and analogues which has shown that the receptors to angiotensin differ in different smooth muscles (Meyer, Papadimitriou and Worcel, 1970; Peach, 1972; Park, Regoli and Rioux, 1973).

Determination of cellular ^{45}Ca uptake using the lanthanum method showed that the angiotensin contractile responses of longitudinal muscle of rat descending colon was associated with an increase in ^{45}Ca uptake which was resistant to verapamil. These results supported the previous observations of dependence on extracellular Ca^{2+} of contractile responses of rat descending colon to angiotensin, they are in agreement with the hypothesis that the contractile responses of rat descending colon to angiotensin are due to influx of extracellular Ca^{2+} which is resistant to calcium antagonists.

REFERENCES

1. Aboulafia, J., Mendes, G.B., Miyamoto, M.E., Paiva, A.C.M. & Paiva, T.B. (1976). Effect of indomethacin and prostaglandin on the smooth muscle contracting activity of angiotensin and other agonists. Br. J. Pharmac., 58, 223-228.

2. Aiken, J.W. & Vane, J.R. (1973). Intrarenal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. J. Pharmac. exp. Ther., 194, 678-687.

3. Aiken, J.W. (1974). Effects of prostaglandin synthesis inhibitors on angiotensin tachyphylaxis in the isolated coeliac and mesenteric arteries of the rabbit. Pol. J. Pharmac. Pharm., 26, 217-227.

4. Altura, B.M. & Altura, B.T. (1970). Differential effects of substrate depletion on drug-induced contractions of rabbit aorta. Am. J. Physiol., 219, 1698-1705.

5. Altura, B.M. & Altura, B.T. (1976). Vascular smooth muscle and prostaglandins. Fedn Proc., 35, 2360-2366.

6. Ambache, N. (1954). Separation of the longitudinal muscle of the rabbit's ileum as a broad sheet. J. Physiol., Lond., 125, 53-54p.

7. Andersson, R.G.G. (1972). Cyclic AMP and calcium ions in mechanical and metabolic responses of smooth muscle; influence of hormones and drugs. Acta physiol. scand. suppl., 382, 1-59.

8. Andersson, R. & Nilsson, K. (1972). Cyclic AMP and calcium in relaxation in intestinal smooth muscle. Nature, New Biol., 238, 119-120.

9. Anderson, N.C., Ramon, F. & Synder, A. (1971). Studies on calcium and sodium in uterine smooth muscle excitation under current-clamp and voltage-clamp conditions. J. gen. Physiol., 58, 322-339.

10. Andersson, R., Nilsson, K., Wikberg, J., Johansson, S. & Lundholm, L. (1975). Cyclic nucleotides and the contraction of smooth muscle. In Advances in Cyclic Nucleotide Research, Vol.5, ed. Drummond, G.I., Greengard, P. & Robison, G.A. pp491-518. New York: Rowen Press.

11. Angles d'Auriac, G., Baudouin, M. & Meyer, P., (1972). Mechanism of action of angiotensin in smooth muscle cell: Biochemical changes following interaction of the hormone with its membrane receptors. Circulation Res. Suppl. 30-31, Part II, 151-160.

12. Angles d'Auriac, G. & Meyer, P. (1972). Effects of angiotensin II on cyclic 3', 5'-AMP in rat uterus. Life Sci., 11, part 1, 631-641.

13. Angles d'Auriac, G. & Meyer, P. (1973). 3',5'-cyclic AMP phosphodiesterases in cytol of uterine smooth muscle effects of angiotensin. Life Sci., 12, Part II, 233-239.

14. Axelrod, J. (1976). Catecholamines and hypertension. Clin. Sci. molec. Med. Suppl., 51, 415s-421s.

15. Baker, P.F. (1972). Transport and metabolism of calcium ions in nerve. Prog. Biophys. Molec. Biol., 24, 177-223.

16. Baker, P.F., Meves, H. & Ridgway, E.B. (1973). Effects of manganese and other agents on the calcium uptake that follows depolarization of squid axons. J. Physiol., Lond., 231, 511-526.

17. Bakhle, Y.S., Reynard, A.M. & Vane, J.R. (1969). Metabolism of angiotensins in isolated perfused tissues. Nature, 222, 956-959.

18. Bär, H.P. (1974). Cyclic nucleotides and smooth muscle. In: Advances in Cyclic Nucleotide Research, Vol. 4, ed. Greengard, P. & Robison, G.A., pp195-237. New York: Raven Press.

19. Barabé, J., Park, W.K. & Regoli, D. (1975). Application of drug receptor theories to the analysis of the myotropic effects of bradykinin. Can. J. Physiol. Pharmac., 53, 345-353.

20. Baraclough, M.A., Jones, N.F., Marsden, C.D. & Bradford, B.C. (1967). Renal and pressor actions of angiotensin in salt loaded and depleted rabbits. Experimentia, 23, 553-555.

21. Barajas, L. & Muller, J. (1973). The innervation of the juxtaglomerular apparatus and surrounding tissues: A quantitative analysis by serial section microscopy. J. Ultrastruct. Res., 43, 107-132.

22. Batra, S. (1973). The role of mitochondrial calcium uptake in contraction and relaxation of the human myometrium. Biochem. Biophys. Acta, 305, 428-432.

23. Batra, S. (1975). The role of mitochondria in the regulation of myoplasmic calcium concentration in smooth muscle. In: Calcium Transport in Contraction and Secretion, ed. Carafoli, E., Clementi, F., Drabikowski, W. & Margareth, A., pp. 87-94. Amsterdam: North Holland Publishing Company.

24. Batra, S.C. & Daniel, E.E. (1971). ATP dependent calcium uptake by subcellular fractions of uterine smooth muscle. Comp. Biochem. Physiol., 38A, 369-385.

25. Battachrya, B.K., Dadkar, N.K. & Dohadwala, A.N. (1977) Vascular reactivity of perfused vascular bed in spontaneously hypertensive and normotensive rats. Br. J. Pharmac., 59, 243-246.

26. Baudouin, M., Meyer, P., Fermandjian, S. & Morgat, J.L. (1972). Calcium release induced by interaction of angiotensin with its receptors in smooth muscle cell microsomes. Nature, Lond., 235, 336-338.

27. Baudouin-Legros, M. & Meyer, P. (1973). Effects of angiotensin, catecholamines and cyclic AMP on calcium storage in aortic microsomes. Br. J. Pharmac., 47, 377-385.

28. Baudouin-Legros, M.Y., Meyer, P. & Worcel, M. (1974). Influence of prostaglandins and oestrous cycle on the spasmodic action of angiotensin II and oxytocin on rat uterus. Br. J. Pharmac., 51, 121-122p.

29. Bayer, R., Hennekes, R., Kaufmann, R. & Mannhold, R. (1975a). Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. I. Pattern of inotropic effects of the racemic compounds. Naunyn-Schmiedebergs Arch. Pharmac., 290, 49-68.

30. Bayer, R., Kaufmann, R. & Mannhold, R. (1975b). Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. II. Pattern of inotropic effects of the optical isomers. Naunyn-Schmiedebergs Arch. Pharmac., 290, 69-80.

31. Bazler, H. (1972). The effect of quinidine and drugs with quinidine-like action (propranolol, verapamil, and tetracaine) on the calcium transport system in isolated sarcoplasmic reticulum vesicles of rabbit skeletal muscle. Naunyn Schmiedebergs Arch. Pharmac., 274, 256-272.

32. Bell, C. (1972). Mechanism of enhancement by angiotensin II of sympathetic adrenergic transmission in the guinea-pig. Circulation Res., 31, 348-355.

33. Bennett, A. & Flesher, B. (1970). Prostaglandins and the gastrointestinal tract. Gastroenterology, 59, 790-800.

34. Bennett, A. & Posner, J. (1971). Studies on prostaglandin antagonists. Br. J. Pharmac., 42, 584-594.

35. Berger, W. (1963). Die Doppelsaccharosetrennwandtechnik: Eine Methode zur Untersuchung des Membranpotentials und der Membraneigenschaften glatter Muskelzellen. Pflugers Arch. ges. Physiol., 277, 570-576.

36. Berger, W. & Barr L. (1969). Use of rubber membranes to improve sucrose-gap and other electrical recording techniques. J. appl. Physiol., 26, 378-382.

37. Bergofsky, E.H. & Holtzman, S. (1967). A Study of the mechanisms involved in the pulmonary arterial pressor response to hypoxia. Circulation Res., 20, 506-519.

38. Bergstrom, S., Carlson, L.A. & Weeks, J.R. (1968). The prostaglandins: A family of biologically active lipids. Pharmac. Rev., 20, 1-48.

39. Bernstein, J. (1912). Electrobiologie. Braunschweig: Vieweg.

40. Bevan, J.A., Osher, J.V. & Su, C. (1963). Response of vascular smooth muscle to potassium and its antagonism by phenoxybenzamine. J. Pharmac. exp. Ther., 139, 216-221.

41. Bilek, I., Laven, R., Peiper, U. & Regnat, K. (1974). The effect of verapamil on the response to noradrenaline or to potassium-depolarization in isolated vascular strips. Microvasc. Res., 7, 181-189.

42. Bisset, G.W. & Lewis, G.P. (1962). A spectrum of pharmacological activity in some biologically active peptides. Br. J. Pharmac. Chemother., 19, 168-182.

43. Bito, L.Z. (1972). Accumulation and apparent active transport of prostaglandins by some rabbit tissues in vitro. J. Physiol., Lond., 221, 371-387.

44. Blair-West, J.R., Coghlan, J.P., Denton, D.A., Goding, J.R., Munro, J.A. & Wright, R.D. (1963). The reduction of the pressor action of angiotensin II in sodium deficient conscious sheep. Aust. . J. exp. Biol. med. Sci., 41, 369-376.

45. Blair-West, J.R., Harding, R. & McKenzie, J.S. (1967) The action of angiotensin II on guinea-pig ileum and its modification by changes in sodium concentration. Br. J. Pharmac. Chemother., 31, 229-243.

46. Blair-West, J.R., Harding, R. & McKenzie, J.S. (1968) Effect of sodium concentration on the vasoconstrictor action of angiotensin in the rabbit ear. Eur. J. Pharmac. 4, 77-82.

47. Blair-West, J.R. & McKenzie, J.S. (1966). Sodium concentration and the effect of angiotensin II on ileal smooth muscle. Experimentia, 22, 291-292.

48. Blaustein, M.P. (1977). The role of Na-Ca exchange in the regulation of tone in vascular smooth muscle. In: Excitation-Contraction Coupling in Smooth Muscle, ed. Casteels, R., Godfraind, T. & Ruegg, J.C. pp 101-107. Amsterdam: Elsevier.

49. Blaustein, M.P. & Goldman, D.E. (1966) Origin of axon membrane hyperpolarization under sucrose-gap. Biophys. J., 6, 453-470.

50. Boev, K.K. (1971). Method of simultaneous stimulation and recording of the electrical and contractile activity of smooth muscle. C.R. Acad. Bulg. Sci., 24, 933-936.

51. Boev, K. & Golenhofen, K. (1974). Sucrose-gap technique with pressed-rubber membranes. Pflugers Arch. ges. Physiol., 349, 277-283.

52. Boev, K., Golenhofen, K. & Lukanow, J. (1976). Selective suppression of phasic and tonic activation mechanisms in stomach smooth muscle. In: Physiology of Smooth Muscle, ed. Bulbring, E. & Shuba, M.F. pp. 203-208. New York: Raven Press.

53. Bohr, D.F. (1963). Vascular smooth muscle: Dual effect of calcium. Science, 139, 597-599.

54. Bohr, D.F. (1964). Electrolytes and smooth muscle contraction. Pharmac. Rev., 16, 85-111.

55. Bohr, D.F. (1973). Vascular smooth muscle updated. Circulation Res., 32, 665-672.

56. Bohr, D.F. (1974). Angiotensin on vascular smooth muscle. In 'Angiotensin', Handbook of Experimental Pharmacology, Vol XXXVII, ed. Page, I.H. & Bumpus, F.M. pp 425-440. Berlin-Heideberg-New York: Springer-Verlag.

57. Bohr, D.F., Seidel, C. & Sabieski, J. (1969). The role of sodium-calcium pumps in tension development of vascular smooth muscle. Microvasc. Res., 1, 335-343.

58. Bohr, D.F. & Uchida, E. (1967). Individualities of vascular smooth muscles in response to angiotensin. Circulation Res. 21, Suppl. 2, 135-143.

59. Bolton, T.B. (1971). On the nature of the oscillations of the membrane potential (slow waves) produced by acetylcholine or carbachol in intestinal smooth muscle. J. Physiol., Lond., 216, 403-418.

60. Bolton, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev., 59, 606-718.

61. Bondi, A.Y. (1976). Effects of verapamil on excitation contraction coupling in frog sartorius muscle. Ph.D. Thesis, Columbia University, New York.

62. Borle, A.B. (1968). Calcium metabolism in Hela cells and the effects of parathyroid hormone. J. Cell Biol., 36, 567-582.

63. Born, G.V.R. & Bulbring, E. (1955). The effect of 2,4-dinitrophenol (DNP) on the smooth muscle of the guinea-pig's taenia coli. J. Physiol., Lond., 127, 626-635.

64. Bowman, W.C. & Khan, H.H. (1977). Effects of dantrolene sodium on isolated skeletal, smooth and cardiac muscle of the guinea pig. J. Pharm. Pharmac., 29, 628-630.

65. Boyle, P.J. & Conway, E.J. (1941). Potassium accumulation in muscle and associated changes. J. Physiol. Lond., 100, 1-63.

66. Bozler, E. (1948). Conduction, automaticity and tonus of visceral muscle. Experimentai, 4, 213-218.

67. Bozler, E. (1969). Role of calcium in initiation of activity of smooth muscle. Am. J. Physiol., 216, 671-674.

68. Brading, A.F. (1973). Ion distribution and the role of calcium in cellular function. Phil. Trans. R. Soc. Lond. B., 265, 35-46.

69. Brading, A., Bulbring, E. & Tomita, T. (1969). The effect of sodium and calcium on the action potential of the smooth muscle of the guinea-pig taenia coli. J. Physiol., Lond., 200, 637-654.

70. Braun-Menendez, E. & Page, I.H. (1958). Suggested revision of nomenclature. Angiotensin. Science, 127, 242.

71. Briggs, A.H. & Melvin, S. (1961). Ion movements in isolated rabbit aortic strips. Am. J. Physiol., 201, 365-368.

72. Brocklehurst, L. (1975). Dantrolene sodium and 'skinned' muscle fibres. Nature, 254, 364.

73. Brunner, H.R., Chang, P., Wallach, R., Sealey, J.E. & Laragh, J.H. (1972). Angiotensin II vascular receptors. Their avidity in relationship to sodium balance, the autonomic nervous system and hypertension. J. Clin. Invest., 51, 58-67.

74. Bueding, E., Butcher, R.W., Hawkins, J., Timms, A.R. & Sutherland, E.W. Jr. (1966). Effect of epinephrine on cyclic adenosine 3',5'-phosphate and hexose phosphates in intestinal smooth muscle. Biochim. Biophys. Acta, 115, 173-178.

75. Bülbring, E. (1954). Membrane potential of smooth muscle fibres of the taenia coli of the guinea pig. J. Physiol., Lond., 125, 302-305.

76. Bülbring, E. (1955). Correlation between membrane potential, spike discharge and tension in smooth muscle. J. Physiol., Lond., 128, 200-221.

77. Bülbring, E. (1957). Changes in configuration of spontaneously discharged spike potentials from smooth muscle of the guinea-pig's taenia coli. The effect of electrotonic currents and of adrenaline, acetylcholine and histamine. J. Physiol, Lond., 135, 412-425.

78. Bülbring, E., Brading, A.F., Jones, A.W. & Tomita, T. (1970). Smooth Muscle. London: Edward Arnold.

79. Bülbring, E. & Burnstock, G. (1960). Membrane potential changes associated with tachyphylaxis and potentiation of the stimulating drugs in smooth muscle. Br. J. Pharmac., 15, 611-624.

80. Bülbring, E. & Kuriyama, H. (1963). Effects of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea-pig taenia coli. J. Physiol., Lond., 166, 29-58.

81. Bülbring, E. & Tomita, T. (1969a). Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. Proc. Roy. Soc. B., 172, 89-102.

82. Bülbring, E. & Tomita, T. (1969b). Effect of calcium, barium and manganese on the action of adrenaline in the smooth muscle of the guinea pig taenia coli. Proc. Roy. Soc. B., 172, 121-136.

83. Bülbring, E. & Tomita, T. (1970). Effects of Ca removal on the smooth muscle of the guinea pig taenia coli. J. Physiol., Lond., 210, 217-232.

84. Burnstock, G., Holman, M.E. & Prosser, C.L. (1963). Electrophysiology of smooth muscle. Physiol. Rev., 43, 482-527.

85. Burnstock, G. & Straub, R.W. (1958). A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. J. Physiol., Lond., 140, 156-167.

86. Burnstock, G. & Prosser, C.L. (1960a). Responses of smooth muscles to quick stretch; relation of stretch to conduction. Am. J. Physiol., 198, 921-925.

87. Burnstock, G. & Prosser, C.L. (1960b). Conduction in smooth muscles: Comparative electrical properties. Am. J. Physiol. 199, 553-559.

88. Burks, T.F., Whitacre, T.S. & Long, J.P. (1967). Effects of calcium-deficient perfusion on isolated mesenteric arteries. Arch. int. Pharmacodyn., 168, 304-311.

89. Butcher, R.W. & Sutherland, E.W. (1962). Adenosine 3',5'-phosphate in biological materials. 1. Purification and properties of cyclic 3', 5'-nucleotide phosphodiesterase and use of this enzyme to charactize adenosine 3', 5'-phosphate in human urine. J. Biol. Chem., 237, 1244-1250.

90. Butterfield, J.L. & Ellis, K.O. (1973). Effects of dantrolene sodium, a skeletal muscle relaxant, on the contractility of cardiac and smooth muscle. Fedn. Proc. Am. Soc. exp. Biol., 32, 772.

91. Caldwell, P.C. (1968). Factors governing movement and distribution of inorganic ions in nerve and muscle. Physiol. Rev., 48, 1-64.

92. Callingham, B.A. (1976). Current aspects of pharmacology. Angiotensin. Pharm. J., 217, 410-412.

93. Carruba, M., Mandelli, V. & Mantegazza, P. (1973). The effect of angiotensin II and other vasoactive drugs on isolated portal vein preparations. Arch. int. Pharmacodyn., 201, 224-233.

94. Carsten, M.E. (1969). Role of calcium binding by sarcoplasmic reticulum in the contraction and relaxation of uterine smooth muscle. J. gen. Physiol., 53, 414-426.

95. Carsten, M.E. (1973a). Sarcoplasmic reticulum from pregnant bovine uterus. Calcium binding. Gynecol. Invest., 4, 84-94.

96. Carsten, M.E. (1973b). Sarcoplasmic reticulum from bovine uterus. Prostaglandins and calcium. Gynecol. Invest., 4, 95-105.

97. Carsten, M.E. (1973c). Prostaglandins and cellular calcium transport in the pregnant human uterus. Am. J. Obstet. Gynecol., 117, 824-832.

98. Carsten, M.E. (1974). Prostaglandins and oxytocin. Their effects on uterine smooth muscle. Prostaglandins, 5, 33-40.

99. Casteels, R. (1966). The action of oubain on the smooth muscle cells of the guinea pig's taenia coli. J. Physiol., Lond., 184, 131-142.

100. Casteels, R. (1970). The relationship between the membrane potential and the ion distribution in smooth muscle cells. In: Smooth Muscle, ed. Bulbring, E., Brading, A.F., Jones, A.W. and Tomita, T. pp 70-99. London: Arnold.

101. Casteels, R., Droogmans, G. & Hendrickx, H. (1973). Active ion transport and resting potential in smooth muscle cells. Phil. Trans. R. Soc. Lond. B., 265, 47-56.

102. Casteels, R. & Kuriyama (1966). Membrane potential and ion content in smooth muscle of the guinea pig's taenia coli at different external potassium concentrations. J. Physiol., Lond., 184, 120-130.

103. Casteels, R. & Van Breemen, C. (1975). Active and passive Ca^{2+} fluxes across cell membranes of the guinea-pig taenia coli. Pflugers Arch. ges. Physiol. 359, 197-207.

104. Cheng, J. (1976). "Calcium-induced release of calcium" in rectal smooth muscle of mice. Jap. J. Pharmac., 26, 73-78.

105. Chong, E.K.S. & Downing, O.A. (1973). Selective inhibition of angiotensin-induced contractions of smooth muscle by indomethacin. J. Pharm. Pharmac., 25, 170-171.

106. Chong, E.K.S. & Downing, O.A. (1974). Reversal by prostaglandin E_2 of the inhibitory effect of indomethacin on contractions of guinea-pig ileum induced by angiotensin. J. Pharm. Pharmac., 26, 729-730.

107. Coceani, F. & Wolfe, L.S. (1966). On the action of prostaglandin E_1 and prostaglandins from brain on the isolated rat stomach. Can. J. Physiol. Pharmac., 44, 933-950.

108. Collins, M.G. & Alps, B.J. (1975). Vascular reactivity to noradrenaline, potassium chloride, and angiotensin II in the rat perfused mesenteric vasculature preparation, during the development of renal hypertension. Cardiovasc. Res., 9, 118-126.

109. Constantin, L.L. (1975). Electrical properties of the transverse tubular system. Fedn. Proc., 34, 1390-1394.

110. Cowley, A.W. Jr. & McCaa, R.E. (1976). Acute and chronic dose-response relationships for angiotensin aldosterone and arterial pressure at varying levels of sodium intake. Circulation Res., 39, 788-797.

111. Cranefield, P.F., Aronson, R.S. & Wit, A.L. (1974). Effect of verapamil on the normal action potential and on a calcium-dependent slow response of canine cardiac purkinje fibres. Circulation Res., 34, 204-213.

112. Crankshaw, D.J., Janis, R.A. & Daniel, E.E. (1977). The effects of Ca^{2+} antagonists on Ca^{2+} accumulation by subcellular fractions of the rat myometrium. Can. J. Physiol. Pharmac., 55, 1028-1032.

113. Crocker, A.D. & Wilson, K.A. (1974). A study of the metabolic requirements for the contractile action of angiotensin upon guinea-pig ileum. Br. J. Pharmac., 51, 73-79.

114. Crocker, A.D. & Wilson, K.A. (1975). A further investigation into the energy dependence of angiotensin II-induced contractions of isolated smooth muscle preparations. Br. J. Pharmac., 53, 59-60.

115. Cuthbert, M.F. (1973). Prostaglandins and respiratory smooth muscle. In: The Prostaglandins, Pharmacological and Therapeutic Advances, ed. Cuthbert, M.F. pp 253-285. London: William Heinemann.

116. Cuthbert, A.W. & Sutter, M.C. (1965). The effects of drugs on the relation between the action potential discharge and tension in the mammalian vein. Br. J. Pharmac., 25, 592-601.

117. Daniel, E.E. (1964). The interconnection between active transport and contracture in uterine tissue. Can. J. Physiol. Pharmac., 42, 453-495.

118. Davis, J.O. (1971). The renin-angiotensin system in the control of aldosterone secretion. In: Kidney Hormones, ed. Fisher, J.W. pp. 173-205. London - New York: Academic Press.

119. Davis, J.O. (1974). The renin-angiotensin system in the control of aldosterone secretion. In: 'Angiotensin', Handbook of Experimental Pharmacology, Vol. XXXVii, ed. Page, I.H. & Bumpus, F.M. pp 323-336. Berlin-Heidelberg-New York: Springer-Verlag.

120. Davis, J.O. & Freeman, R.H. (1976). Mechanisms regulating renin release. Physiol. Rev., 56, 1-56.

121. Davis, J.O., Hartroft, P.M., Titus, E.O., Carpenter, C.C.J., Ayers, C.R. & Spiegel, H.E. (1962). Role of the renin-angiotensin system in the control of aldosterone secretion. J. clin. Invest., 41, 378-389.

122. Detar, R. & Gellai, M. (1971). Oxygen and isolated vascular smooth muscle from the main pulmonary artery of the rabbit. Am. J. Physiol., 221, 1791-1794.

123. Deth, R. & Van Breemen, C. (1974). Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release during drug-induced activation of the rabbit aorta. Pflugers Arch. ges. Physiol., 348, 13-22.

124. Devine, C.E., Somlyo, A.V. & Somlyo, A.P. (1972). Sarcoplasmic reticulum and excitation-contraction coupling in mammalian smooth muscles. J. Cell Biol., 52, 690-718.

125. Devine, C.E., Somlyo, A.V. & Somlyo, A.P. (1973). Sarcoplasmic reticulum and mitochondria as cation accumulation sites in smooth muscle. Phil. Trans. R. Soc. Lond. B., 265, 17-23.

126. Douglas, J.R., Johnson, E.M., Marshall, G.R., Jaffe, B.M. & Needleman, P. (1973). Stimulation of splenic prostaglandin release by angiotensin and specific inhibition by cysteine-8-angiotensin II. Prostaglandins, 3, 67-73.

127. Durbin, R.P. & Jenkinson, D.H. (1961a). The effect of carbachol on the permeability of depolarized smooth muscle to inorganic ions. J. Physiol., Lond., 157, 74-89.

128. Durbin, R.P. & Jenkinson, D.H. (1961b). The calcium dependence of tension development in depolarized smooth muscle. J. Physiol., Lond., 90-96.

129. Eakins, K.E., Karim, S.M.M. & Miller, J.D. (1970). Antagonism of some smooth muscle actions of prostaglandins by polyphloretin phosphate. Br. J. Pharmac., 39, 556-563.

130. Eakins, K.E., Miller, J.D. & Karim, S.M.M. (1971). The nature of the prostaglandin-blocking activity of polyphloretin phosphate. J. Pharmac exp. Ther., 176, 441-447.

131. Ebashi, S. (1976). Excitation-contraction coupling. Ann. Rev. Physiol., 38, 293-313.

132. Ebashi, S. & Endo, M. (1968). Calcium ion and muscle contraction. Progr. Biophys. Mol. Biol. 18, 123-183.

133. Eckenfels, A. & Vane, J.R. (1972). Prostaglandins, oxygen tension and smooth muscle tone. Br. J. Pharmac., 45, 451-462.

134. Edman, K.A.P. & Schild, H.O. (1962). The need for calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus. J. Physiol., Lond., 161, 424-441.

135. Edney, S.M. & Downes, H. (1976). Calcium-dependent, iproveratril-resistant contractions of aortic strips induced by a convulsant barbiturate. Arch. int. Pharmacodyn, 224, 77-90.

136. Ellis, K.O. & Bryant, S.H. (1972). Excitation-contraction uncoupling in skeletal muscle by dantrolene sodium. Naunyn-Schmiedebergs Arch. Pharmac., 274, 107-109.

137. Ellis, R.H., Simpson, P., Tathan, P., Leighton, M. & Williams, J. (1975). The cardiovascular effects of dantrolene sodium in dogs. Anaesthesia, 30, 318-322.

138. Endo, M. (1977). Calcium release from the sarcoplasmic reticulum. Physiol. Rev., 57, 71-108.

139. Entman, M.L., Allen, J.C., Bornet, E.P., Gillette, P.C. Wallick, E.T. & Schwart, A. (1972). Mechanisms of calcium accumulation and transport in cardiac relaxing system (sarcoplasmic reticulum membranes) : Effects of verapamil, D600, X537A and A23187. J. Mol. Cell. Cardiol. 4, 681-687.

140. Evans, D.H.L. & Schild, H.O. (1953). The reactions of plexus-free circular muscle of rat jejunum to drugs. J. Physiol., Lond., 119, 376-399.

141. Evans D.H.L. & Schild, H.O. (1956). Reactions of chick amnion to stretch and electrical stimulation. J. Physiol., Lond., 132, 31p.

142. Evans, D.H.L., Schild, H.O. & Thesleff, S. (1958). Effects of drugs on depolarized plain muscle. J. Physiol., Lond., 143, 474-485.

143. Falbriard, J.G., Posternak, T.H. & Sutherland, E.W. (1967). Preparation of derivatives of adenosine 3',5'-phosphate. Biochim. Biophys. Acta, 148, 99-105.

144. Feinstein, M.B. (1966). Inhibition of contraction and calcium exchangeability in rat uterus by local anaesthetics. J. Pharmac. exp. Ther., 152, 516-524.

145. Feinstein, M.B., Paimre, M. & Lee, M. (1968). Effect of local anaesthetics on excitation-coupling mechanisms. Trans. N.Y. Acad. Sci., 30, 1073-1081.

146. Ferreira, S.H., Moncada, S. & Vane, J.R. (1972). Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum. Br. J. Pharmac., 44, 328p.

147. Ferreira, S.H., Moncada, S. & Vane, J.R. (1973). Some effects of inhibiting endogenous prostaglandin formation on the responses of the cat spleen. Br. J. Pharmac., 47, 48-58.

148. Fleckenstein, A. (1971). Specific inhibitors and promoters of calcium action in the excitation-contraction coupling of heart muscle and their role in the prevention or production of myocardial lesions. In: Calcium and the Heart, ed. Harris, P. & Opie, L.H. pp 135-188. London-New York: Academic Press.

149. Fleckenstein, A. (1972). Physiologie and Pharmakologie der transmembraren natrium, Kalium und calcium bewegunen. Arz-Forsch., 22, 2019-2028.

150. Fleckenstein, A. (1976). Adalat, a new powerful Ca-antagonistic drug. In: New Therapy of Ischemic Heart Disease, ed. Lochner, W. pp 56-65. Berlin: Springer.

151. Fleckenstein, A. (1977). Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. Ann. Rev. Pharmac. Tox. 17, 149-166.

152. Fleckenstein, A., Doring, H.J. & Kammermeier, H. (1968) Einfluss von Beta-Receptorenblockern und verwandten Substanzen auf Erregung, Kontraktion und Energiestoff-wechsel der Myokardfaser. Klin. Wschr., 46, 343-351.

153. Fleckenstein, A., Grün, G., Tritthart, H. & Byon, K. (1971). Uterus-Relaxation durch hockaktive Ca^{++} antago-nistiche Hemmstoffe der electro-mechanischen Koppelung wie Isoptin (verapamil, iproveratril), Substanz D600 und Segontin (prenylamin). Klin. Wschr., 49, 32-41.

154. Fleckenstein, A., Nakaya, K., Fleckenstein-Grun, G. & Byon, T.K. (1975). Interactions of vasoactive ions and drugs with Ca-dependent excitation-contraction coupling of vascular smooth muscle. In: Calcium Transport in Contraction and Secretion, ed. Carafoli, E., Clementi, F., Drabikowski, W. & Margareth, A. pp 555-566. Amsterdam: North Holland Publishing Company.

155. Fleckenstein, A., Tritthart, H., Döring, H.J. & Byon, K.Y. (1972) BAY-a 1040-ein hochaktiver Ca^{++}-antagonisticher inhibitor der electro-mechanischen Koppelungsprozesse im Warmblutter-Myocard. Arz. Forsch. 22, 22-33.

156. Flower, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis. Pharmac. Rev., 26, 33-61.

157. Frankenhauser, B. & Hodgkin, A.L. (1957). The action of calcium on the electrical properties of squid axons. J. Physiol., Lond., 137, 218-244.

158. Freeman, D.J. & Daniel, E.E. (1973). Calcium movement in vascular smooth muscle and its detection using lanthanum as a tool. Can. J. Physiol. Pharmac., 51, 900-913.

159. Freeman, R.H., Davis, J.O., Gotshall, R.W., Johnson, J.A. & Spielman, W.S. (1974). The signal perceived by the macula densa during changes in renin release. Circulation Res., 35, 307-315.

160. Freer, R.J. (1975a). Calcium and angiotensin tachyphylaxis in rat uterine smooth muscle. Am. J. Physiol., 228, 1423-1430.

161. Freer, R.J. (1975b). Angiotensin as a tool to study excitation contraction coupling in smooth muscle. In: Peptides: Chemistry, Structure and Biology. Proceedings of the Fourth American Peptide Symposium, ed. Walter, R. & Meinhofer, J. pp559-564. Michigan: Ann Arbor.

162. Freer, R.J. & Smith, A.B. (1976). Effect of lithium on responsiveness of rat uterine smooth muscle to angiotensin. Am. J. Physiol., 230, 1132-1137.

163. Friedman, S.M. & Friedman, C.L. (1964). Ionic basis of vascular response to vasoactive substances. Can. Med. Ass. J., 90, 167-173.

164. Friedman, S.M. & Friedman, C.L. (1965). The emerging role of sodium and potassium in the regulation of vascular muscle tension. In: Electrolytes and Cardiovascular diseases, ed. Bajusz, E. pp. 323-341. Basel: Karger.

165. Friedman, S.M., Jamieson, J.D. & Friedman, C.L. (1959). Sodium gradient, smooth muscle tone and blood pressure regulation. Circulation Res., 7, 44-53.

166. Furchgott, R.F. & Bhadrakom, S. (1953). Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J. Pharmac. exp. Ther., 108, 129-143.

167. Gabella, G. (1971). Caveolae intracellulares and sarcoplasmic reticulum in smooth muscle. J. Cell Sci., 8, 601-609.

168. Gabella, G. (1973). Cellular structures and electrophysiological behaviour: Fine structure of smooth muscle. Phil. Trans. R. Soc. Lond. B., 265, 7 - 16.

169. Gabella, G. (1978). Effect of potassium on the mechanical activity of taenia coli, uterus, and portal vein of the guinea pig. Q.J. exp. Physiol, 63, 125-146.

170. Gaddum, J.H., Hameed, K.A., Hathway, D.E. & Stephens, F.F. (1955). Quantitative studies of antagonists for 5-hydroxytryptamine. Q.J. exp. Physiol., 40, 49-74.

171. Gagnon, D.J., Park, W.K. & Regoli, D. (1971). Specific antagonists for the myotropic action of angiotensin II and angiotensin I on the isolated rat colon. Br. J. Pharmac., 43, 409-410.

172. Gagnon, D.J. & Sirois, P. (1972). The rat isolated colon as a specific assay organ for angiotensin. Br. J. Pharmac. 46, 89-93.

173. Genest, J., Boucher, R., Nowaczynski & Kuchel, O. (1978). The role of the adrenal cortex in the pathogenesis of essential hypertension. In: The Endocrine Function of the Human Adrenal Cortex. Proceedings of the Serono Symposia, Vol. 18, ed. James, V.H.T., Serio, M., Guisti, G. & Martini, L. pp. 389-412. London: Academic Press.

174. Gimrone, M.A. Jr. & Alexander, R.W. (1975). Angiotensin II stimulation of prostaglandin production in cultured human vascular endothelium. Science, 189, 219-220.

175. Godfraind, T. (1968). Réponse contractile de muscles lisses isoles des stimulations répétees par l'angiotensine. Arch. int. Pharmacodyn., 176, 464-467.

176. Godfraind, T. (1970). Angiotensin auto-potentiation. Br. J. Pharmac., 40, 542p - 543p.

178. Godfraind, T., Kaba, A. (1972). The role of calcium in the action of drugs on vascular smooth muscle. Arch. int. Pharmacodyn. Suppl., 196, 35-49.

179. Godfraind, T. (1973). Na-Ca interaction and the mode of drug action. In: Pharmacology and the Future of Man. Proc. 5th Int. Congr. Pharmac. San Francisco; Vol. 4, pp. 344-358. Basel: Karger.

180. Godfraind, T. (1976). Calcium exchange in vascular smooth muscle, action of noradrenaline and lanthanum. J. Physiol., Lond., 260, 21-35.

181. Godfraind, T., Kaba, A. & Polster, P. (1966a). Dissociation in two contractile components on the isolated guinea-pig ileum response to angiotensin. Arch. int. Pharmacodyn., 163, 227-229.

182. Godfraind, T., Kaba, A. & Polster, P. (1966b). Specific antagonism to the direct and indirect action of angiotensin on isolated guinea-pig ileum. Br. J. Pharmac., 28, 93-104.

183. Goldberg, N.D., Haddox, M.K., Nicol, S.E., Glass, D.B., Sanford, C.H., Kuehl, F.A. Jr. & Estensen, R. (1975). Biological regulation through opposing influences of cyclic AMP and cyclic GMP. The Yin Yang hypothesis. In: Advances in Cyclic Nucleotide Research, Vol. 5, ed. Drummond, G.I., Greengard, P. & Robison, G.A. pp. 307-338. New York: Raven Press.

184. Goldblatt, H., Lynch, J., Hanzal, R.F. & Summerville, W. (1934). Studies on experimental hypertension. 1. The production of persistent elevation of systolic blood pressure by means of renal ischemia. J. exp. Med., 59, 347-379.

185. Goldman, D.E. (1943). Potential, impedance and rectification in membrane. J. gen. Physiol, 27, 37-60.

186. Golenhofen, K. (1976). The theory of P and T systems for calcium activation in smooth muscle. In: Physiology of Smooth Muscle, ed. Bulbring, E. and Shuba, M.F. pp. 197-202. New York: Raven Press.

187. Golenhofen, K., Hermstein, N. & Lammel, E. (1973). Membrane potential and contraction of vascular smooth muscle (portal vein) during application of noradrenaline and high potassium, and selective inhibitory effects of iproveratril (verapamil). Microvasc. Res., 5, 73-80.

188. Golenhofen, K. & Hermstein, N. (1975). Differentiation of calcium activation mechanisms in vascular smooth muscle by selective suppression with verapamil and D600 Blood Vessels, 12, 21-37.

189. Golenhofen, K. & Lammel, E. (1972). Selective suppression of some components of spontaneous activity in various types of smooth muscle by iproveratril (verapamil). Pflugers Arch. ges. Physiol., 331, 233-243.

190. Golenhofen, K., Wagner, B. & Weston, A.H. (1977). Calcium systems of smooth muscle and their selective inhibition. In: Excitation-Contraction Coupling in Smooth Muscle, ed. Casteels, R., Godfraind, T. & Ruegg, J.C. pp. 131-136. Amsterdam: Elsevier.

191. Golenhofen, K. & Wegner, H. (1975). Spike-free activation mechanisms in smooth muscle of guinea-pig stomach. Pflugers Arch. ges. Physiol., 354, 29-37.

192. Goodford, P.J. (1967). The calcium content of the smooth muscle of the guinea-pig taenia coli. J. Physiol., Lond., 192, 145-157.

193. Goodford, P.J. (1970). Ionic interactions in smooth muscle. In: Smooth Muscle, ed. Bulbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 100-121. London: Arnold.

194. Goodford, P.J. & Wolowyk, M.W. (1972). Localization of cation interactions in the smooth muscle of the guinea pig taenia coli. J. Physiol., Lond., 229, 521-535.

195. Goodfriend, T.L., Fyhrquist, F. & Allman, D. (1974). Biochemical effects of angiotensin. In: 'Angiotensin', Handbook of Experimental Pharmacology, Vol. XXXVII, ed. Page, I.H. & Bumpus, F.M. pp 511-517. Berlin-Heidelberg-New York: Springer-Verlag.

196. Goodman, F.R. & Weiss, B.G. (1971). Dissociation by lanthanum of smooth muscle responses to potassium and ACH. Am. J. Physiol., 220, 759-766.

197. Goto, M. & Woodbury, J.W. (1958). Effects of stretch and NaCl on transmembrane potentials and tension of pregnant rat uterus. Fedn. Proc. 17, suppl. II, 58.

198. Graves, S., Dretchen, K.L. & Kruger, G.D. (1978). Dantrolene sodium: effects on smooth muscle. Eur. J. Pharmac., 47, 29-35.

199. Greenberg, S., Kadowitz, P.J., Diecke, F.P. & Long, J.P. (1974). Effect of prostaglandin F2α (PGF2α) on venous contractility and ^{45}Ca uptake. Proc. Soc. exp. Biol. Med., 145, 80-84.

200. Greenberg, S., Wilson, W.R. & Long, J.P. (1973). Effect of metabolic inhibitors on mesenteric arterial smooth muscle. Arch. int. Pharmacodyn., 206, 213-228.

201. Gross, L. & Clark, A.J. (1923). The influence of oxygen supply on the response of the isolated intestine to drugs. J. Physiol., Lond., 57, 457-460.

202. Gross, F. (1971). Angiotensin. Pharmacology of naturally occurring polypeptides and lipid-soluble acids. In: International Encyclopaedia of Pharmacology and Therapeutics, Vol. 1, Section 72, pp 73-286. Oxford: Pergamon.

203. Gross, F., Brunner, H. & Ziegler, M. (1965). Renin-angiotensin system, aldosterone, and sodium balance. Recent Progr. Hormone Res., 21, 119-177.

204. Gross, F. & Turian, H. (1960). Pharmacology of hypertensin and synthetic analogues. In: Polypeptides which affect Smooth Muscle and Blood Vessels, ed. Schachter, M. pp. 137-151. Oxford: Pergamon.

205. Grosset, A. & Mironneau, J. (1977). An analysis of actions of prostaglandin E_1 on membrane currents and contraction in uterine smooth muscle. J. Physiol., Lond., 270, 765-784.

206. Grun, G., Fleckenstein, A. & Tritthart, H. (1969). Excitation-contraction uncoupling on the rat's uterus by some "Musculotropic" smooth muscle relaxants. Naunyn-Schmidebergs. Arch. Pharmac., 264, 239-240.

207. Haeusler, G. (1972). Differential effect of verapamil on excitation-contraction coupling in smooth muscle and on excitation-secretion coupling in adrenergic nerve terminals. J. Pharmac. exp. Ther., 180, 672-682.

208. Haeusler, G. & Thorens, S. (1976). Pharmacology of vasoactive antihypertensives. In: Vascular Neuro-effector Mechanisms, ed. Bevan, J.A., Burnstock, G., Johansson, B., Maxwell, R.A. & Nedergaard, O.A. pp. 232-241. Basel: Karger.

209. Hagiwara, S. & Nakajima, S. (1966). Difference in sodium and calcium spikes as examined by application of tetrodotoxin, procaine and manganese ions. J. gen. Physiol., 49, 793-805.

210. Hagiwara, S. & Takahashi, K. (1967). Resting and spike potentials of skeletal muscle fibres of salt-water elasmobranch and teleost fish. J. Physiol., Lond., 190, 499-518.

211. Hamon, G. & Worcel, M. (1973). Mechanism of excitation of uterine smooth muscle. Changes in ionic fluxes induced by angiotensin. J. Physiol., Lond., 232, 99-100p.

212. Hamon, G. & Worcel, M. (1977). Action of angiotensin II on the membrane potential and contraction of rat myometrium. In: Excitation-Contraction Coupling in Smooth Muscle, ed. Casteels, R., Godfraind, T. & Ruegg, J.C. pp. 61-69. Amsterdam: Elsevier.

213. Hinke, J.A.M. (1965). Calcium requirements for noradrenaline and high potassium ion contraction in arterial smooth muscle. In: Muscle, ed. Paul, W.M., Daniel, E.E., Kay, C.M. & Monckton, G. pp. 269-285. New York: Pergamon Press.

214. Hinke, J.A.M. & Wilson, M.L. (1962). Effects of electrolytes on contractility of arterial segments in vitro. Am. J. Physiol., 203, 1161-1166.

215. Hinke, J.A.M., Wilson, M.L. & Burnham, S.C. (1964). Calcium and the contractility of arterial smooth muscle. Am. J. Physiol., 206, 211-217.

216. Hodgkin, A.L. (1958). Ionic movements and electrical activity in giant nerve fibres. Proc. R. Soc. B., 148, 1 - 37.

217. Hodgkin, A.L. & Horowicz, P. (1957). Effects of K and Cl on the membrane potential of isolated muscle fibres. J. Physiol., Lond., 137, 30-31p.

218. Hodgkin, A.L. & Katz, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol., Lond., 108, 37-77.

219. Hodgkin, A.L. & Keynes, R.D. (1955). Active transport of cations in axons from sepia and loligo. J. Physiol., Lond., 128, 28-60.

220. Hodgkin, A.L. & Rushton, W.A.H. (1946). The electrical constants of a crustacean nerve fibre. Proc. R. Soc. B., 133, 444-479.

221. Hodgson, B.J. & Daniel, E.E. (1973). Studies concerning the source of calcium for contraction of rat myometrium. Can. J. Physiol. Pharmac., 51, 914-932.

222. Hodgson, B.J., Kidwai, A.M. & Daniel, E.E. (1972). Uptake of lanthanum by smooth muscle. Can. J. Physiol. Pharmac., 50, 730-733.

223. Holman, M.E. (1957). The effect of changes in the sodium chloride concentration on the smooth muscle of the guinea-pig's taenia coli. J. Physiol., Lond., 136, 569-584.

224. Holman, M.E. (1958). Membrane potentials recorded with high-resistance micro-electrodes; and the effects of changes in ionic environment on the electrical and mechanical activity of the smooth muscle of the taenia coli of the guinea-pig. J. Physiol, Lond., 141, 464-488.

225. Horton, E.W. (1969). Hypothesis on the physiological roles of prostaglandins. Physiol. Rev., 49, 122-161.

226. Horton, E.W. (1979). Prostaglandins and smooth muscle. Br. med. Bull., 35, 295-300.

227. Houssay, B.A. & Braun-Menendez, E. (1942). The role of renin in experimental hypertension. Br. med. J., 2, 179-181.

228. Huang, Y.C.H. & Kemp, R.G. (1971). Properties of a phosphodiesterase with high affinity for adeonosine 3',5'-cyclic phosphate. Biochemistry, 10, 2278-2283.

229. Hudgins, P.M. & Weiss, G.B. (1968). Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. J. Pharmac. exp. Ther., 159, 91-97.

230. Hurwitz, L., Fitzpatrick, D.F., Debbas, G. & Landon, E.J. (1973). Localization of calcium pump activity in smooth muscle. Science, 179, 384-386.

231. Hurwitz, L. & Suria, A. (1971). The link between agonist action and response in smooth muscle. Ann. Rev. Pharmac., 11, 303-326.

232. Imai, S. & Takeda, K. (1957). Actions of calcium and certain multivalent cations on potassium contracture of guinea pig's taenia coli. J. Physiol, Lond., 190, 155-169.

233. Inoue, F. & Frank, G.B. (1962). Action of procaine on frog skeletal muscle. J. Pharmac. exp. Ther., 136, 190-196.

234. Johnson, C.C. (1929). The actions and toxicity of sodium nitroprusside. Arch. int. Pharmacodyn., 35, 480-496.

235. Johnson, J.A. & Davis, J.O. (1973). Angiotensin II. Important role in the maintenance of arterial blood pressure. Science, 179, 906-907.

236. Kalsner, S., Nickerson, M. & Boyd, N.G. (1970). Selective blockade of potassium-induced contractions of aortic strips by β-diethylaminoethyldiphenylpropyl-acetate (SKF525A). J. Pharmac. exp. Ther., 174, 500-508.

237. Kao, C.Y (1966). Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. Pharmac. Rev., 18, 997-1049.

238. Kaplan, N.M., Silah, J.G. (1965). Effect of angiotensin II on blood pressure in humans with hypertensive disease. J. clin. Invest., 43, 659-669.

239. Kass, R.S. & Tsien, R.W. (1975). Multiple effects of calcium antagonists on plateau currents in cardiac Purkinje fibres. J. gen. Physiol., 66, 169-192.

240. Keatinge, W.R. (1966). Electrical and mechanical responses of vascular smooth muscle to vasodilator agents and vasoactive polypeptides. Circulation Res., 18, 641-649.

241. Keatinge, W.R. (1968). Sodium flux and electrical activity of arterial smooth muscle. J. Physiol., Lond., 194, 183-200.

242. Keynes, R.D. (1954). The ionic fluxes in frog muscle. Proc. R. Soc. B., 142, 359-382.

243. Khairallah, P.A. & Page, I.H. (1961). Mechanism of action of angiotensin and bradykinin on smooth muscle in situ. Am. J. Physiol., 200, 51-54.

244. Khairallah, P.A. & Page, I.H. (1963). Effects of bradykinin and angiotensin in smooth muscle. Ann. N.Y. Acad. Sci., 104, 212-220.

245. Khairallah, P.A., Page, I.H., Bumpus, F.H. & Turker, R.K. (1966). Angiotensin tachyphylaxis and its reversal. Circulation Res., 19, 247-254.

246. Khairallah, P.A., Vadaparampil, G.J. & Page, I.H. (1965). Effect of ions on angiotensin interaction with smooth muscle. Arch. int. Pharmacodyn., 158, 155-164.

247. Kobayashi, M. (1965). Effects of Na and Ca on the generation and conduction of excitation in the ureter. Am. J. Physiol, 208, 715-719.

248. Kobayashi, M. & Irisawa, H. (1964). Effect of sodium deficiency on the action potential of the smooth muscle cell of ureter. Am. J. Physiol., 206, 205-210.

249. Kohlhardt, M., Bauer, B., Krause, H. & Fleckenstein, A. (1972). Differentiation of the transmembrane Na and Ca channels in mammalian cardiac fibres by the use of specific inhibitors. Pflugers Arch. ges. Physiol., 335, 309-322.

250. Kohlhardt, M., Bauer, B., Krause, H. & Fleckenstein, A. (1973a). Selective inhibition of the transmembrane Ca conductivity of mammalian myocardial fibres by Ni, Co and Mn ions. Pflugers Arch. ges. Physiol. 338, 115-123.

251. Kohlhardt, M., Herdey, A. & Kubler, M. (1973b). Interchangeability of Ca and Sr ions as charge carriers of the slow inward current in mammalian myocardial fibres. Pflugers Arch. ges. Physiol. 344, 149-158.

252. Kohlstaedt, K.G., Helmer, O.M. & Page I.H. (1938). Activation of renin by blood colloids. Proc. Soc. exp. Biol. Med., 39, 214-215.

253. Kondo, K., Manku, M.S., Horrobin, D.F., Boucher, R. & Genest, J. (1977). Potentiation of pressor effects of noradrenaline and potassium ions in the rat mesenteric arteries by physiological concentration of angiotensin II. Effects of prostaglandin E2 and cortisol. Clin. Sci. Molec. Med., 53, 233-239.

254. Kreye, V.A.W., Baron, G.D., Lüth, J.B. & Schnidt-Gayk, H. (1975). Mode of action of sodium nitroprusside on vascular smooth muscle. Naunyn-Schmiedebergs Arch. Pharmac., 288, 381-402.

255. Kreye, V.A.W. & Gross, F. (1977). Drugs acting on arteriolar smooth muscle. III. Nitroprusside. In: 'Antihypertensive Agents', Handbook of Experimental Pharmacology, Vol. 39, ed. Gross, F. pp. 418-461. Berlin-Heidelberg-New York: Springer-Verlag.

256. Kreye, V.A.W. & Lüth, J.B. (1976). Effect of sodium nitroprusside, temperature, and calcium withdrawal on the relaxation speed of vascular smooth muscle. In: Ionic Actions on Vascular Smooth Muscle, ed. Betz, E. pp. 145-149. Berlin-Heidelberg-New York: Springer-Verlag.

257. Kuriyama, H. (1963). The influence of potassium, sodium and chloride on the membrane potential of the smooth muscle of taenia coli. J. Physiol, Lond., 166, 15-28.

258. Kuriyama, H., Ito, Y. & Suzuki, H. (1977). Effects of membrane potential on activation of contraction in various smooth muscles. In: Excitation-Contraction Coupling in Smooth Muscle, ed. Casteels, R., Godfraind, T. & Ruegg, J.C. pp. 25-36. Amsterdam: Elsevier.

259. Kuriyama, H., Osa, T. & Toida, N. (1966). Effect of tetrodotoxin on smooth muscle cells of the guinea pig taenia coli. Br. J. Pharmac., 27, 366-376.

260. Kuriyama, H. & Tomita, T. (1970). The action potential in the smooth muscle of the guinea pig taenia coli and ureter studied by the double sucrose-gap method. J. gen. Physiol., 55, 147-162.

261. Lammel, E. (1977). ^{45}Ca-uptake of stomach smooth muscle during different modes of activation. In: Excitation-Contraction Coupling in Smooth Muscle, ed, Casteels, R., Godfraind, T. & Ruegg, J.C. pp. 274-277. Amsterdam: Elsevier.

262. Laragh, J.H. (1962). Interrelationships between angiotensin, norepinephrine, epinephrine, aldosterone secretion and electrolyte metabolism in man. Circulation, 25, 203-211.

263. Lardy, H.A. & Elvehjem, C.A. (1945). Biological oxidations and reductions. Ann. Rev. Biochem., 14, 1 - 30.

264. Lee, T.B., Kuo, J.F. & Greengard, P. (1972). Role of muscarinic cholinergic receptors in the regulation of guanosine 3', 5' cyclic monphosphate content in mammalian brain, heart muscle and intestinal smooth muscle. Proc. natn. Aced. Sci., U.S.A., 69, 3287-3291.

265. Lettvin, J.Y., Pickard, W.F., McCulloch, W.S. & Pitts, W. (1964). A theory of passive ion flux through axon membranes. Nature, 202, 1338-1339.

266. Levi, H. & Ussing, H.H. (1948). The exchange of sodium and chloride ions across the fibre membrane of the isolated frog sartorious. Acta physiol. scand., 16 232-249.

267. Lloyd, T.D. Jr. (1970). Responses to hypoxia of pulmonary arterial strips in nonaqueous baths. J. appl. Physiol., 28, 566-569.

268. Lüllman, H. (1970). Calcium fluxes and calcium distribution in smooth muscle. In: Smooth Muscle, ed. Bulbring, E., Brading, A., Jones, A. & Tomita, T. pp. 151-165. London: Arnold.

269. Luttgau, H.C. & Niedergerke, R. (1958). The antagonism between Ca and Na ions on the frog's heart. J. Physiol., Lond., 143, 486-505.

270. Main, I.H.M. (1964). The inhibitory actions of prostaglandins on respiratory smooth muscle. Br. J. Pharmac., 22, 511-519.

271. Manku, M.S. & Horrobin, D.F. (1976). Indomethacin inhibits responses to all vasoconstrictors in the rat mesenteric vascular bed. Restoration of responses by prostaglandin E_2. Prostaglandins, 12, 369-376.

272. Marshall, J.M. & Csapo, A.I. (1961) Hormonal and ionic influences on the membrane activity of uterine smooth muscle cells. Endocrinology, 68, 1026-1035.

273. Marshall, J.M. & Kroeger, E.A. (1973). Adrenergic influences on uterine smooth muscle. Phil. Trans. R. Soc. Lond. B., 265, 135-148.

274. Martin, A.R., Wickelgren, O. & Beranek, R. (1970). Effects of iontophoretically applied drugs on spinal interneurones of the lamprey. J. Physiol., Lond., 207, 653-665.

275. Massingham, R. (1973). A study of compounds which inhibit vascular smooth muscle contraction. Eur. J. Pharmac., 22, 75-82.

276. Mayer, C.J., Van Breemen, C. & Casteels, R. (1972). The action of lanthanum and D600 on the calcium exchange in the smooth muscle cells of the guinea pig taenia coli. Pflugers Arch. ges. Physiol, 337, 333-350.

277. McGiff, J.C. & Crowshaw, K. (1970). Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. Circulation Res. Suppl, 26-27, I, 121-130.

278. McGregor, D.D. & Smirk, F.H. (1968). Vascular responses in mesenteric arteries from genetic and renal hypertensive rats. Am. J. Physiol., 214, 1429-1433.

279. McMurtry, F.I., Davidson, A.B., Reeves, J.T. & Grover, R.F. (1976). Inhibition of hypoxic pulmonary vasoconstriction by calcium antagonists in isolated rat lungs. Circulation Res., 38, 99-104.

280. Mendlowitz, M. (1967) Vascular reactivity in essential and renal hypertension in man. Am. Heart J., 73, 121-128.

281. Meyer, P., Papadimitriou, A. & Worcel, M. (1970). Possible existence of different types of angiotensin II receptors. Br. J. Pharmac., 40, 541-542p.

282. Mirroneau, J. (1973). Excitation-contraction coupling in voltage clamped smooth muscle. J. Physiol., Lond., 233, 127-141.

283. Miyazaki, E., Ishazawa, M., Sunano, S., Syuto, B. & Sakagami, T. (1967). Stimulating action of prostaglandins on the rabbit duodenal muscle. In: Nobel Symposium, Vol. 2 pp. 277-282. Stockholm: Almqvist and Wiksells.

284. Moore, P.F., Iorio, L.C. & McManus, J.M. (1968). Relaxation of the guinea-pig tracheal chain preparation by N^6 ,2'-O-dibutyryl, 3',5'-cyclic adenosine monophosphate. J. Pharm. Pharmac., 20, 368-372.

285. Murad, F. (1973). Beta-blockade of epinephrine-induced cyclic AMP formation in heart, liver, fat and trachea. Biochim. Biophys. Acta, 304, 181-187.

286. Murad, F. & Kimura, H. (1974). Cyclic nucleotide levels in incubations of guinea-pig trachea. Biochim. Biophys. Acta, 343, 275-286.

287. Napodano, R.J., Caliva, F.S., Lyons, C., Desimone, J. & Lyons, R.H. (1962). The reactivity to angiotensin of rabbit aorta strips after either alterations of external sodium environment or direct addition of benzydroflumethiazide. Am. Heart. J., 64, 498-502.

288. Nayler, W.G. (1967). Some factors involved in the maintenance and regulation of cardiac contractility Circulation Res. Suppl., 20-21, Part III, 213-221.

289. Nayler, W.G. & Szeto, J. (1972). Effect of verapamil on contractility, oxygen utilization, and calcium exchangeability in mammalian heart muscle. Cardiovas. Res., 6, 120-128.

290. Ng, K.K.F. & Vane, J.R. (1967). Conversion of angiotensin I to angiotensin II. Nature, 216, 762-766.

291. Ng, K.K.F. & Vane, J.R. (1970). Some properties of angiotensin converting enzyme in the lung in vivo. Nature, 225, 1142-1144.

292. Niedergerke, R. & Lüttgau, H.C. (1957). Calcium and the contraction of the heart. Antagonism between calcium and sodium ions. Nature, 179, 1066-1067.

293. Nilsson, K., Djarv, L. & Andersson, R.G.G. (1976). Contraction of glycerol extracted fibres of smooth muscle by acetylcholine and imidazole in the presence of a Ca-binding microsomal fraction from the rabbit colon. Acta physiol. scand., 98, 407-411.

294. Nonomura, Y., Hotta, Y. & Ohashi, H. (1966). Tetrodotoxin and manganese ions: Effects on electrical activity and tension in taenia coli of guinea pig Science, Wash., 152, 97-99.

295. Northover, B.J. (1971). Mechanism of the inhibitory action of indomethacin on smooth muscle. Br. J. Pharmac., 41, 540-551.

296. Northover, B.J. (1972). The effects of indomethacin upon calcium, sodium and magnesium fluxes in various tissues of the guinea pig. Br. J. Pharmac., 45, 651-659.

297. Northover, B.J. (1973). Effect of antiinflammatory drugs on the binding of calcium to cellular membranes in various human and guinea-pig tissues. Br. J. Pharmac., 48, 496-504.

298. Nott, M.W. & Bowman, W.C. (1974). Actions of dantrolene sodium on contractions of the tibialis anterior and soleus muscles of cats under chlorase anaesthesia. Clin. exp. Pharmac. Physiol., 1, 113-122.

299. Ohashi, H., Nonomura, Y. & Ohga, A. (1967). Effects of angiotensin, bradykinin and oxytocin on electrical and mechanical activities in the taenia coli of the guinea pig. Jap. J. Pharmac., 17, 247-257.

300. Ohkawa, W. (1976). Further studies of the action of cyclic AMP on the electrical and mechanical activities of intestinal smooth muscle. Jap. J. Pharmac., 26, 201-207.

301. Oparil, S., Sanders, C.A. & Haber, E. (1970). In vivo andin-vitro conversion of angiotensin I to angiotensin II in the dog blood. Circulation Res, 26, 591-599.

302. Oparil, S., Tregear, G.W., Koerner, T., Barnes, B.A. & Haber, E. (1971). Mechanism of pulmonary conversion of angiotensin I to angiotensin II in the dog. Circulation Res., 29, 682-690.

303. Paiva, A.C.M. & Paiva, T.B. (1960). The importance of the N-terminal end of angiotensin II for its pressor and oxytocic activities. Biochem. Pharmac., 5, 187-191.

304. Paiva, T.B., Aboulafia, J., Nouailhetas, V.L.A. & Paiva, A.C.M. (1976a). Tachyphylaxis and angiotensin receptors. Gen. Pharmac., 7, 185-188.

305. Paiva, T.B., Juliano, L., Nouailhetas, V.L.A. & Paiva, A.C.M. (1974). The effect of pH on tachyphylaxis to angiotensin peptides in the isolated guinea pig ileum and rat uterus. Eur. J. Pharmac., 25, 191-196.

306. Paiva, T.B., Mendes, G.B., Aboulafia, J. & Paiva, A.C.M. (1976b). Evidence against cholinergic mediation of the effect of angiotensin II on the guinea pig ileum. Pflugers Arch. ges. Physiol, 365, 129-133.

307. Paiva, T.B., Mendes, G.B. & Paiva, A.C.M. (1977). Specific desensitization (tachyphylaxis) of guinea pig ileum to angiotensin II. Am. J. Physiol., 232, 223-230.

308. Paiva, A.C.M., Paiva, T.B., Miyamoto, M.E. & Nakaie, C.R. (1977). The role of calcium in the response of rabbit aorta to angiotensin. Mayo Clin. Proc., 52, 427-429.

309. Park, W.K., Regoli, D. & Rioux, F. (1973). Characterization of angiotensin receptors in vascular and intestinal smooth muscles. Br. J. Pharmac., 48, 288-301.

310. Passo, S.S., Assaykeen, T.A., Goldfien, A. & Ganong, W.F. (1971). Effect of α and β-adrenergic blocking agents on the increase in renin secretion produced by stimulation of the medulla oblongata in dogs. Neuroendocrinology, 7, 97-104.

311. Paton, D.M. & Daniel, E.E. (1967). On the contractile response of the isolated rat uterus to prostaglandin E_1. Can. J. Physiol. Pharmac., 45, 795-804.

312. Peach, M.J. (1972). Physiological roles of angiotensin. In: Chemistry and Biology of Peptides, ed. Meinhofer, J. pp. 471-493. Michigan: Ann Arbor.

313. Peach, M.J. (1977). Renin-angiotensin system: Biochemistry and mechanisms of action. Physiol. Rev., 57, 313-370.

314. Peiper, U., Griebel, L. & Wende, W. (1971). Activation of vascular smooth muscle of rat aorta by noradrenaline and depolarization: Two different mechanisms. Pflugers Arch. ges. Physiol., 330, 74-89.

315. Picarelli, Z.P., Kupper, R., Prado, E.S., Prado, J.L. & Valle, J.R. (1954). Assay of renin and hypertensin with the isolated guinea pig ileum. Circulation Res., 2, 354-358.

316. Poch, G. & Kukovetz, W.R. (1971). Papaverine-induced inhibition of phosphodiesterase activity in various mammalian tissues. Life Sci., 10, Part 1, 133-144.

317. Prosser, C.L. (1974). Smooth muscle. Ann. Rev., Physiol., 36, 503-535.

318. Prosser, C.L., Burnstock, G. & Kahn, J. (1960). Conduction in smooth muscle: Comparative structural properties. Am. J. Physiol., 199, 545-552.

319. Prosser, C.L. & Sperelakis, N. (1956). Transmission in ganglion-free circular muscle from the cat intestine. Am. J. Physiol., 187, 536-545.

320. Putney, J.W. & Bianchi, P. (1974). Site of action of dantrolene on frog sartorius muscle. J. Pharmac. exp. Ther., 189, 202-212.

321. Raeymakers, L., Wuytack, F. & Casteels, R. (1974). Na-Ca exchange in taenia coli of the guinea-pig. Pflugers Arch. ges. Physiol., 347, 329-340.

322. Rangachari, P.K., Paton, D.M. & Daniel, E.E. (1972). Aerobic and glycolytic support of sodium pumping and contraction in rat myometrium. Am. J. Physiol., 223, 1009-1015.

323. Rasmussen, H. & Goodman, B.P. (1977). Relationships between calcium and cyclic nucleotides in cell activation. Physiol. Rev., 57, 421-509.

324. Regoli, D., Park, W.K. & Rioux, F. (1974). Pharmacology of angiotensin. Pharmac. Rev., 26, 69-123.

325. Regoli, D. & Vane, J.R. (1964). A sensitive method for the assay of angiotensin. Br. J. Pharmac. Chemother., 23, 351-359.

326. Reid, W.D. & Laragh, J.H. (1965). Sodium and potassium intake, blood pressure and pressor response to angiotensin. Proc. Soc. exp. Biol. Med., 120, 26-29.

327. Reid, I.A., Morris, B.J. & Ganong, W.F. (1978). The renin-angiotensin system. Ann. Rev. Physiol. 40, 377-410.

328. Reuter, H. (1973). Divalent cations as charge carriers in excitable membranes. Prog. Biophys. Mol. Biol., 26, 1 - 43.

329. Reuter, H., Blaustein, M.P. & Haeusler, G. (1973). Na-Ca exchange and tension development in arterial smooth muscle. Phil. Trans. R. Soc. Lond., B., 265, 87-94.

330. Riemer, J., Mayer, C.J. & Ulbrecht, G. (1975). Dual effects of calcium ions on pacemaker activity in guinea pig taenia coli. Pflugers Arch. ges. Physiol., 336, 19-31.

331. Rink, T.J. (1975). The role of the plasma membrane in the regulation of intracellular calcium. In: Calcium Transport in Contraction and Secretion, ed. Carafoli, E., Clementi, F., Drabikowski, W. & Margareth, A., pp. 235-242. Amsterdam: North Holland Publishing Company.

332. Rioux, F., Park, W.K. & Regoli, D. (1973). Application of drug-receptor theories to angiotensin. Can. J. Physiol. Pharmac., 51, 665-672.

333. Ritchie, J.M. & Straub, R.W. (1956). The effect of cooling on the size of the action potential of mammalian non-medullated fibres. J. Physiol, Lond., 134, 712-717.

334. Robison, G.A., Butcher, R.W. & Sutherland, E.W. (1966). Adenyl cyclase as an adrenergic receptor. Ann. N.Y. Acad. Sci., 139, 703-723.

335. Robison, G.A., Butcher, R.W. & Sutherland, E.W. (1971). Cyclic AMP. New York: Academic Press.

336. Robison, G.A. & Sutherland, E.W. (1970). Sympathin E, Sympathin 1, and the intracellular level of cyclic AMP. Circulation Res. Suppl. 27, Part 1, 147-161.

337. Rosenberger, L. & Triggle, D.J. (1978). Calcium, calcium translocation, and specific calcium antagonists. In: Calcium and Drug Action, ed. Weiss, G.B. pp. 3-31. New York-London: Plenum Press.

338. Ruegg, J.C. (1971). Smooth muscle tone. Physiol. Rev., 51, 201-248.

339. Salvetti, A., Arzilli, F., Poli, L., Pedrinelli, R., Sassano, P. & Motolese, M. (1978). Effects of therapy on the renin-angiotensin-aldosterone system of hypertensive patients. In: The Endocrine Function of the Human Adrenal Cortex. Proceedings of the Serono Symposia, Vol. 18, ed. James, V.H.T., Serio, M., Giusti, G. & Martini, L. pp. 431-456. London: Academic Press.

340. Sander, G.E. & Huggins, C.G. (1972). Vasoactive peptides. Ann. Rev. Pharmac., 12, 227-264.

341. Sands, H., Meyer, T.A. & Rickenberg, H.V. (1973). Adenosine 3', 5'-monophosphate-dependent protein kinase of bovine tracheal muscle. Biochim. Biophys. Acta, 302, 267-281.

342. Savino, E.A. & Taquini, A.C. Jr. (1977). Effects of adrenaline, angiotensin, potassium and calcium on lanthanum treated portal veins. Arch. int. Pharmacodyn., 226, 100-108.

343. Schild, H.O. (1949). pAx and competitive drug antagonism. Br. J. Pharmac., 4, 277-280.

344. Shwarz, H., Bumpus, F.M. & Page, I.H. (1957). Synthesis of a biologically active peptide similar to natural isoleucine angiotonin octapeptide. J. Am. Chem. Soc., 79, 5697-5703.

345. Schwyzer, R., Iselin, B., Kappeler, H., Riniker, B., Rittel, W. & Zuber, H. (1957). Synthese von hypertensin-peptiden. Uber die partielle hydrolyse von hypertensin-asp-β-amiden zur der entprechenden dicarbonsaüren. Hypertensin II-analoge. Chimia, 11, 335-336.

346. Shade, R.E., Davis, J.O., Johnson, J.A., Gotshall, R.W. & Spielman, W.S. (1973). Mechanism of action of angiotensin II and antidiuretic hormone on renin secretion. Am. J. Physiol., 224, 926-929.

347. Shanes, A.M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. Pharmac. Rev., 10, 59-273.

348. Shibata, S. & Briggs, A.H. (1966). The relationship between electrical and mechanical events in rabbit aortic strips. J. Pharmac. exp. Ther., 153, 466-470.

349. Shibata, S. & Carrier, O. Jr. (1967). Antagonizing action of chlorpromazine, dibenamine and phenoxybenzamine on potassium-induced contraction. Can. J. Physiol. Pharmac., 45, 587-596.

350. Shibata, S., Carrier, O. Jr. & Frankenheim, J. (1968). Effect of chlorpromazine, dibenamine and phenoxybenzamine on the contractile response of taenia coli to potassium, acetylcholine, angiotensin and barium. J. Pharmac. exp. Ther., 160, 106-111.

351. Shimo, Y. & Holland, W.C. (1966). Effects of potassium on membrane potential, spike discharge, and tension in taenia coli. Am. J. Physiol, 211, 1299-1304.

352. Sigurdsson, S.B., Uvelius, B. & Johansson, B. (1975). Relative contribution of superficially bound and extracellular calcium to activation of contraction in isolated portal vein. Acta physiol. scand., 95 263-269.

353. Singh, B.N. & Vaughan-Williams, E.M. (1972). A fourth class of anti-dysrhythmic action? Effect of verapamil on oubain toxicity, on atrial and ventricular intracellular potentials, and on other features of cardiac function. Cardiovasc. Res., 6, 109-119.

354. Sitrin, M.D. & Bohr, D.F. (1971). Ca and Na interaction in vascular smooth muscle contraction. Am. J. Physiol., 220, 1124-1128.

355. Skeggs, L.T., Lentz, K.E., Kahn, J.R., Woods, K.R. & Shumway, N.P. (1956). The amino acid sequence of hypertensin II. J. exp. Med., 104, 193-197.

356. Skinner, S.L., McCubbin, J.W. & Page, I.H. (1964a). Control of renin secretion. Circulation Res., 15, 64-76.

357. Skinner, S.L., McCubbin, J.W. & Page, I.H. (1964b). Renal baroreceptor control of acute renin release in normotensive, nephrogenic and neurogenic hypertensive dogs. Circulation Res., 15, 522-531.

358. Skou, J.C. (1961). The effect of drugs on cell membrane with special reference to local anaesthetics. J. Pharm. Pharmac., 13, 204-217.

359. Somlyo, A.P. (1972). Excitation-contraction coupling in vertebrate smooth muscle: Correlation of ultrastructure with function. The Physiologist, 15, 338-348.

360. Somlyo, A.V. & Somlyo, A.P. (1966). Effect of angiotensin and beta-adrenergic stimulation on venous smooth muscle. Am. Heart J., 71, 568-570.

361. Somlyo, A.V. & Somlyo, A.P. (1968a). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. J. Pharmac. exp. Ther., 159, 129-145.

362. Somlyo, A.P. & Somlyo, A.V. (1968b). Smooth muscle. Pharmac. Rev., 20, 197-272.

363. Somlyo, A.P. & Somlyo, A.V. (1970). Vascular smooth muscle. II. Pharmacology of normal and hypertensive vessels. Pharmac. Rev., 22, 249-353.

364. Sparrow, M.P., Maxwell, L.C., Rüegg, J.C. & Bohr, D.F. (1970). Preparation and properties of a calcium ion-sensitive actinomyosin from arteries. Am. J. Physiol., 219, 1366-1372.

365. Speden, R.N. (1970). Excitation of vascular smooth muscle. In: Smooth Muscle, ed. Bulbring, E., Brading, A., Jones, A. & Tomita, T. pp. 558-588. London: Arnold.

366. Stampfli, R. (1954). A new method for measuring membrane potentials with external electrodes. Experimentia, 10, 508-509.

367. Stampfli, R. & Straub, R. (1954). Demonstration der Membranpotentialmessung von markhaltigen Nervenfasern mit Hilfe von Aussenelektroden. Helv. physiol. Acta, 12, c 6-8.

368. St-Louis, J., Regoli, D., Barabé, J. & Park, W.K. Myotropic actions of angiotensin and noradrenaline in strips of rabbit aortae. Can. J. Physiol. Pharmac., 55, 1056-1069.

369. Strewler, G.J., Hinrichs, K.J., Guiod, L.R. & Hollenberg, N.K. (1972). Sodium intake and vascular smooth muscle responsiveness to norepinephrine and angiotensin in the rabbit. Circulation Res., 31, 758-766.

370. Stewart, J.M. (1974). Tachyphylaxis to angiotensin. In: Angiotensin, Handbook of Experimental Pharmacology, Vol. XXXVII, ed. Page, I.H. & Bumpus, F.M. pp. 170-184. Berlin-Heidelberg-New York: Springer-Verlag.

371. Suarez-Kurtz, G. & Bianchi, C.P. (1970). Sites of action of SKF525A in nerve and muscle. J. Pharmac. exp. Ther., 172, 33-43.

372. Sullivan, L.J. & Briggs, A.H. (1968). Effects of manganese on the response of aortic strips to angiotensin and norepinephrine contractions. J. Pharmac. exp. Ther., 161, 205-209.

373. Sutherland, E.W. & Robison, G.A. (1966). Metabolic effects of catecholamines. The role of cyclic-3',5'-AMP in responses to catecholamines and other hormones. Pharmac. Rev., 18, 145-161.

374. Sutherland, E.W., Robison, G.A. & Butcher, R.W. (1968). Some aspects of the role of adenosine 3':5'-monophosphate (cyclic AMP). Circulation, 37, 279-306.

375. Suzuki, H., Osa, T. & Kuriyama, H. (1976). On the excitatory action of prostaglandin E_2 on the pregnant mouse myometrium. In: Physiology of Smooth Muscle, ed. Bulbring, E. & Shuba, M.F. pp. 385-393. New York: Raven Press.

376. Taher, M.S., McLain, L.G., McDonald, K.M. & Schrier, R.W. (1976). Effect of beta-adrenergic blockade on renin response to renal nerve stimulation. J. clin. Invest., 57, 459-465.

377. Takagi, K., Takayagi, I. & Tomiyama, A. (1971). Actions of dibutyryl cyclic adenosine monophosphate, papaverine and isoprenaline on the intestinal smooth muscle. Jap. J. Pharmac., 21, 477-483.

378. Taniyama, K. (1974). Modification of the tonus and drug induced contraction of the rat isolated ileum by removal of Na from bathing media. Jap. J. Pharmac., 24, 433-438.

379. Thaemert, J.C. (1959). Intercellular bridges as protoplasmic anastomoses between smooth muscle cells. J. Biophys. Biochem. Cytol., 6, 67-70.

380. Thienes, C.H. (1926). Altered responses of smooth muscle to autonomic drugs produced by physical and chemical changes. Arch. int. Pharmacodyn., 31, 447-500.

381. Thurston, H. & Laragh, J.H. (1975). Prior receptor occupancy as a determinant of the pressor activity of infused angiotensin II in the rat. Circulation Res., 36, 113-117.

382. Tigerstedt, R. & Bergman, P.G. (1898). Niere und Kreislauf. Skand. Arch. Physiol., 8, 223-271.

383. Tomita, T. & Watanabe, H. (1973). Factors controlling myogenic activity in smooth muscle. Phil. Trans. R. Soc. Lond. B., 265, 73-85.

384. Triner, L., Nahas, G.G., Vulliemoz, Y., Overweg, N.I.A., Verosky, M., Habif, D.V. & Ngai, S.H. (1971). Cyclic AMP and smooth muscle function. Ann. N.Y. Acad. Sci., 185, 458-476.

385. Triner, L., Vulliemoz, Y., Schwatz, I. & Nahas, G.G. (1970). Cyclic phosphodiesterase activity and the action of papaverine. Biochem. biophys. Res. Commun., 40, 64-69.

386. Tritthart, H., Volkmann, R., Weiss, R. & Fleckenstein, A. (1973). Calcium-mediated action potentials in mammalian myocardium. Alteration of membrane response as induced by changes of Ca_e or by promoters and inhibitors of transmembrane Ca inflow. Naunyn. Schmiederbergs Arch. Pharmac., 280, 239-252.

387. Turker, R.K. (1965). Action of angiotensin on cardiac arrhythmias induced by G-strophanthine. Experimentia, 21, 707-708.

388. Turker, R.K. & Ercan, Z.S. (1976). The effects of angiotensin I and angiotensin II on the isolated tracheal muscle of the cat. J. Pharm. Pharmac., 28, 298-301.

389. Turker, R.K., Page, I.H. & Khairallah, P.A. (1967). Angiotensin alteration of sodium fluxes in smooth muscle. Arch. int. Pharmacodyn., 165, 394-404.

390. Turker, R.K., Yamamoto, M., Khairallah, P.A. & Bumpus, F.M. (1971). Competitive antagonism of 8-Ala-angiotensin II to angiotensins I and II on isolated rabbit aorta and rat ascending colon. Eur. J. Pharmac., 15, 285-291.

391. Urakawa, N. & Holland, W.C. (1964). ^{45}Ca uptake and tissue calcium in K-induced phasic and tonic contraction in taenia coli. Am. J. Physiol., 207, 873-876.

392. Van Breemen, C. (1969). Blockade of membrane calcium fluxes by lanthanum in relation to vascular smooth muscle contractility. Arch. int. Physiol. Biochim., 77, 710-716.

393. Van Breemen, C. (1975). Transmembrane calcium transport. Second International Symposium on Vascular Neuroeffector Mechanisms, Odense, Denmark. Abstract in: Blood Vessels 12/6, Nr. 4, 1975.

394. Van Breemen, C., Aaronson, P. & Loutzenhiser, R. (1979) Sodium-calcium interactions in mammalian smooth muscle. Pharmac. Rev., 30, 167-207.

395. Van Breemen, C., Farinas, B.R., Casteels, R., Gerba, P., Wuytack, F. & Deth, R. (1973). Factors controlling cytoplasmic calcium concentration. Phil. Trans. R. Soc. Lond. B., 265, 57-71.

396. Van Breemen, C., Farinas, B.R., Gerba, P. & McNaughton, E.D. (1972). Excitation-contraction coupling in rabbit aorta. Studies by the lanthanum method for measuring cellular calcium influx. Circulation Res., 30, 44-54.

397. Van Breemen, C. & McNaughton, E. (1970). The separation of cell membrane calcium transport from extracellular calcium exchange in vascular smooth muscle. Biochem. biophys. Res. Commun., 39, 567-574.

398. Vander, A.J. (1967). Control of renin release. Physiol. Rev., 47, 359-382.

399. Vander, A.J. (1968). Inhibition of renin release in the dog by vasopressin and vasotocin. Circulation Res., 23, 605-609.

400. Vander, A.J. & Miller, R. (1964). Control of renin secretion in the dog. Am. J. Physiol., 207, 537-545.

401. Van der Kloot, W. & Kita, H. (1975). The effects of the "calcium antagonist" verapamil on muscle action potentials in the frog and crayfish and on neuromascular transmission in the crayfish. Comp. Biochem. Physiol., 50C, 121-125.

402. Van Dongen, R. & Peart, W.S. (1974). Calcium dependence of the inhibitory effect of angiotensin on renin secretion in the isolated perfused kidney of the rat. Br. J. Pharmac., 50, 125-129.

403. Vane, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature, New Biol., 231, 232-235.

404. Villamil, M.F., Nachev, P. & Kleeman, C.R. (1970). Effect of prolonged infusion of angiotensin II on ionic composition of the arterial wall. Am. J. Physiol. 218, 1281-1286.

405. Volicer, L. & Hynie, S. (1971). Effect of catecholamines and angiotensin on cyclic AMP in rat aorta and tail artery. Eur. J. Pharmac., 15, 214-220.

406. Voth, V.D., Agsten, M., Schipp, R. & Lubcke, H. (1971). Untersuchungen zur Wirkung von Angiotensin II auf einen autorhythmischen glatten Gefaßmuskel (Pfortader de Ratte). Arch. Kriesl-Forsch., 65, 41-57.

407. Walter, P. & Bassenge, E. (1969). Effect of angiotensin on vascular smooth muscle. Pflugers Arch. ges. Physiol., 307, 70-82.

408. Watanabe, A.M. & Besch, H.R. Jr. (1974). Subcellular effects of verapamil and D600: Comparison with propranolol. J. Pharmac. exp. Ther., 191, 241-251.

409. Webb, R.C. & Bhalla, R.C. (1976). Calcium sequestration by subcellular fractions isolated from vascular smooth muscles: Effect of cyclic nucleotides and prostaglandins. J. Mol. Cell. Cardiol., 8, 145-157.

410. Weber, A. & Herz, R. (1963). The binding of calcium to actomyosin systems in relation to their biological activity. J. biol. Chem., 238, 599-605.

411. Weder, U. & Grün, G. (1973). Ca-antagonistiche elecktromechanische entkoppelung der glatten gefässmuskulatur als wirkingsprinzip vasodilatatorischer nitroverbindugen. Naunyn-Schmiedebergs Arch. Pharmac. Suppl., 277, R_{88}.

412. Weeks, J.R. (1972). Prostaglandins. Ann. Rev. Pharmac., 12, 317-336.

413. Weiss, G.B. (1974). Cellular pharmacology of lanthanum Ann Rev. Pharmac., 14, 343-354.

414. West, T.C., Hadden, G. & Farah, A. (1951). Effect of anoxia on response of the isolated intestine to various drugs and enzyme inhibitors. Am. J. Physiol. 164, 565-572.

415. Weston, A.H. & Golenhofen, K. (1976). Comparison of the excitatory and inhibitory effects of angiotensin and vasopressin in mammalian portal vein. Blood Vessels, 13, 350-360.

416. Whelan, R.F., Scroop, G.C. & Walsh, J.A. (1969). Cardiovascular actions of angiotensin in man. Am. Heart J., 77, 546-565.

417. Widdicombe, J.H. (1975). Ion content and movement in smooth muscles and the effects of some drugs Ph.D. Thesis, University of Oxford.

418. Wilkenfeld, B.E. & Levy, B. (1969). The effects of theophylline, diazoxide and imidazole on isoproterenol induced inhibition of the rabbit ileum. Fedn. Proc. 28, 741.

419. Winegrad, S. (1971). Studies of cardiac muscle with a high permeability to calcium produced by treatment with ethylenediaminetetraacetic acid. J. gen. Physiol., 58, 71-93.