THE MODE OF ACTION OF ANGIOTENSIN

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SUMMARY

The contractile action of angiotensin upon smooth muscle preparations has been studied to try and elucidate the mechanism of action of angiotensin. To distinguish between the primary interaction of angiotensin with the smooth muscle cells and the resulting contraction, responses to acetylcholine were also measured.

On guinea-pig ileum, angiotensin responses were found to be more susceptible to metabolic inhibition than acetylcholine responses and this suggested that the angiotensin response might involve an energy dependent step. In further experiments it was shown that this energy dependent mechanism was associated with the direct component of angiotensin's action in this tissue. However, the existence of an indirect, cholinergic mechanism was a complicating factor and the investigation was therefore extended to rat descending colon and rat uterus where the action of angiotensin was wholly direct.

Conditions which had revealed the energy dependent mechanism on guinea-pig ileum failed to affect these preparations and the use of more severe conditions produced an equal reduction of responses to acetylcholine and angiotensin, the magnitude of which varied from tissue to tissue. A study of the carbohydrate metabolism of these tissues revealed differences both between tissues and during the course of the oestrous cycle which explained the observed variation in tissue responses during metabolic inhibition. The energy dependence of the angiotensin response was demonstrated by the use of 2,4-dinitrophenol, a more specific metabolic inhibitor, and this confirmed that energy was required for the direct interaction of the hormone with the smooth muscle cells. The possible role of a mediator in this action of angiotensin was investigated but from preliminary experiments, it

appeared that neither cyclic AMP nor prostaglandins were involved.

Although the nature of the energy dependent mechanism has not been elucidated it seems that it may involve the movement of inorganic ions. These findings are related to a possible mechanism of action for angiotensin.

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INTRODUCTION

GENERAL INTRODUCTION

The term hormone was first used by Starling in 1905 to describe secretin and gastrin but it was initially suggested by W. B. Hardy. Derived from the Greek (I excite), it is used to describe those cellular secretions which constitute the second regulatory system in higher animals. Hormones act at specific sites but in contrast to the nervous system, there is no direct connection between the region of secretion and the site of action. They are transported in the body fluids, usually the blood, and can therefore act at several sites within the organism where they bring about changes to integrate and co-ordinate cellular function.

The currently accepted hypothesis of hormone action involves an initial hormone-receptor interaction which constitutes the first step in a chain of cellular processes leading to the physiological response. Receptor sites are believed to be macromolecular components of finite number which possess a genetically determined structure and are capable of specific recognition and interaction with hormones with a high degree of specificity and affinity. It is possible to divide hormones into two groups on the basis of their site of action; the low molecular weight steroid hormones which act at intracellular receptor sites and the high molecular weight peptide hormones which act at receptors located on the cell membrane (Rasmussen, 1969). The adrenal cortical hormone aldosterone is an example of a steroid hormone and has been shown to be localised in the cell nucleus where it is thought to stimulate the synthesis of a specific type of m-RNA. It is believed that specific proteins are induced which in some way alter the cells permeability to sodium, although there is controversy over the precise mechanism (see reviews by Sharp & Leaf, 1966; Fanestil, 1969).

The ability of steroid hormones to penetrate the cellular membrane allows a direct interaction with intracellular structures. In contrast, peptide hormones act at the cell membrane and therefore have an indirect effect upon the intracellular environment. Peptide hormones may affect enzyme reactions at the plasma membrane with a consequent alteration in the trans-membrane movement of essential substances or they may exert an allosteric effect upon the membrane resulting in a molecular rearrangement. Alternatively, the action of the hormone might be mediated via an intermediate compound, or messenger, whose formation or destruction is influenced by the hormone. This second messenger hypothesis was proposed by Sutherland and co-workers (Sutherland, Oye & Butcher, 1965) following a study of the role of cyclic AMP in a number of hormone-target systems. They suggested that hormones cause a change in the level of intracellular nucleotide which alters the metabolic behaviour of the cell and in so doing, translates the information originally contained in the hormone. The intracellular concentration of cyclic AMP has been shown to be controlled by two enzymes, adenyl cyclase which catalyses its formation from ATP (Sutherland, Rall & Mennon, 1962; Rall & Sutherland, 1962) and phosphodiesterase which catalyses its hydrolysis to 5-AMP (Butcher & Sutherland, 1962) and since adenyl cyclase has been shown to be membrane bound, it has been suggested as the target site for hormones. Each hormone must act at a site which is specific for that hormone and which is located prior to the point of stimulation of adenyl cyclase. Combined with the specificity conferred by the difference in the individual tissues response to adenyl cyclase, this explains the ability of adenyl cyclase to act as a 'Universal Messenger' for tissue-hormone interactions (Rasmussen, 1970). Further, it raises the interesting possibility that a single hormone may produce a variety of effects in different tissues through a common mode of action.

Since 1965, cyclic AMP has been implicated in the action of many peptide hormones (reviews: Birnbauer, Pohl, Krans & Rodbell, 1970; Sutherland & Robison, 1966; Robison, Butcher & Sutherland, 1965 and 1968; Sutherland, Oye & Butcher, 1965; Rasmussen, 1970), and some steroid hormones (Singhai, 1973). However, not all peptide hormones have been shown to act in this way and furthermore, it has become apparent that the role of cyclic AMP is far more complex than was originally envisaged by Sutherland and co-workers. Many hormones thought to act by increasing intracellular cyclic AMP have been shown to increase the release of prostaglandins from their target tissues (Ramwell & Rabinovitz, 1971; Butcher, 1970) and this has led to the suggestion that prostaglandins may be involved in hormone action. Several workers have proposed that prostaglandins might constitute a negative feedback mechanism by inhibiting adenyl cyclase and thereby terminating hormone action (Bergstrom, 1967; Horton, 1969; Butcher, 1970; Ramwell & Shaw, 1967). An alternative hypothesis is that some peptide hormones may act primarily upon the release of prostaglandins which in turn activates adenyl cyclase and increases intracellular cyclic AMP concentration (Kuehl, Humes, Cirillo & Ham, 1972). Both mechanisms are highly controversial and have to be reconciled with the observation that prostaglandins can both increase and decrease intracellular cyclic AMP depending upon the tissue (Ramwell & Rabinowitz, 1971; Butcher, 1970; Kuehl et al, 1972). An interesting recent development in hormone research has been the suggestion that cyclic GMP may be involved in promoting cellular events that oppose those believed to be mediated via increased intracellular levels of cyclic AMP (Hadden, Hadden, Haddox & Goldberg, 1972; Goldberg, Haddox, Hartle & Hadden, 1973; Hogan & Shields, 1974). Thus the action of a hormone may be a result of its effects upon the synthesis of the mutually antagonistic nucleotides, cyclic AMP and cyclic GMP.

In the present study an attempt has been made to elucidate the mechanism of action of the peptide hormone angiotensin. Early interest in angiotensin centred upon its pressor action and this resulted in extensive investigations of its contractile action upon isolated smooth muscle preparations. More recently, however, it has been recognised that angiotensin has an important role in sodium and water homeostasis which is probably of greater physiological significance than the contractile action. The present study has evolved from an interest in the action of angiotensin upon transepithelial sodium and water movement in isolated sacs of mammalian intestine. Attempts to extend these investigations and elucidate the mechanism of action of angiotensin have proved difficult due to the lack of an adequate method for controlling experiments on this preparation. In the absence of other agents which stimulate sodium transport it has been necessary to compare the increment in transport produced by angiotensin with the basal transport, the mechanism of which is also unknown. In the present study the contractile action of angiotensin has been compared with an agonist of known mode of action. It was hoped that, using this approach, it would be possible to separate an initial interaction of the hormone with the plasma membrane from the resulting activation of the contractile mechanism. A primary aim of this investigation has been to determine whether the contractile action of angiotensin might be related to the actions of the hormone upon ion movement.

INTRODUCTION TO THE LITERATURE REVIEW

The present study has involved an investigation of the mode of action of the hormone angiotensin upon isolated smooth muscle preparations and it is therefore relevant to review our knowledge of the biochemistry and physiological importance of this hormone together with our present knowledge of its mode of action. The literature on the contractile action of angiotensin and the mechanism of smooth muscle contraction has been reviewed in greater detail. A survey of studies on the metabolic requirements of smooth muscle contraction has been included since the present investigation has involved a study of the energy requirements of the angiotensin response. Particular emphasis has therefore been placed upon studies of the energy requirements of individual spasmogens.

The literature review has been presented in four main parts:

1. The renin-angiotensin system and the hormone angiotensin. The biochemistry and physiological role of angiotensin.

2. The mechanism of smooth muscle contraction including the energy requirements.

3. The contractile action of angiotensin upon smooth muscle.

4. Other investigations of the mode of action of angiotensin.

1. THE RENIN-ANGIOTENSIN SYSTEM AND THE HORMONE ANGIOTENSIN

The endocrine role of the kidney was first recognised by Tigerstadt and Bergmann (1898) who discovered that crude saline extracts of rabbit kidney produced a pressor response in un-anaesthetised rabbits. They named the active principal in the extract renin. Many years elapsed before this observation was extended but following increased interest in the 1930's, Page and Helmer (1940) and Braun-Menendez et al (1940) demonstrated that renin was an enzyme producing its effect by the production of a small molecule, subsequently named angiotensin (Braun-Menendez & Page, 1958).

The biochemistry of the renin-angiotensin system has been extensively reviewed (Peart, 1965; Page & McCubbin, 1968; Lee, 1969; Mulrow & Goffnet, 1969; Gross, 1971). Renin is a proteolytic enzyme which is stored and released from the juxta-glomerular cells of the kidney and it acts on an alpha-2 globulin substrate to produce angiotensin I, a decapeptide. The physiologically active hormone is angiotensin II, an octapeptide, which is produced from angiotensin I by means of converting enzyme found in the plasma. In the remainder of this thesis, the term angiotensin will be used to denote the octapeptide and only brief mention will be made of the decapeptide which will be specifically denoted as angiotensin I. The change of angiotensin I to angiotensin II has been shown to entail the enzymic removal of two aminoacids, leucine and histidine from the C terminal end of the peptide. More, recently converting enzyme has been discovered in the lung (Ng & Vane, 1967 and 1968) and it has been suggested that this may be the major site for conversion.

The structure of angiotensin was elucidated in 1956 (Elliot& Peart, 1965; Skeggs et al, 1956) and it was synthesised the following year (Schwarz, Bumpus & Page, 1957; Schwyzer, Iselin, Kappeler, Riniker, Rittel & Zuber, 1957). Angiotensin is rapidly removed from the

circulation by the tissues and by a number of related polypeptidases known as angiotensinases which occur in the plasma. A complete reaction scheme for the formation and destruction of angiotensin has been summarized by Gross (1963).

Many studies have been made of the physiological role of the renin-angiotensin system and it has generally been assumed that the action of renin is solely through release of angiotensin (Gross, 1971). Renin production and release is stimulated by a reduction in renal blood flow or perfusion pressure, by a reduction in the blood volume and by a reduction in the renal sodium load. (See reviews by Lee, 1969; Davis, 1967 and 1971; Mulrow & Goffnet, 1969; Gross, 1971). Generally, acute stimuli result in increased renin release whereas chronic or repeated stimuli have an additional effect upon synthesis and storage. Several control mechanisms have been suggested for renin. The sympathetic nervous system has some influence and may act as a fine control of release. Enhanced sympathetic tone increases renin release and in addition, a diurnal variation in renin release has been shown to parallel the diurnal variation in sympathetic tone (Gordon, Kuchel, Liddle & Island, 1967). Two other mechanisms have been suggested for the control of renin release. In the baroreceptor or stretch hypothesis, the receptor is located in the afferent glomerular arteriole and responds to decreased stretch of the vascular wall by causing increased renin secretion. This hypothesis was first suggested by Braun-Menendez (1946) and Goormaghtigh (1944) and has been developed by Tobian (1960). The macula densa theory, on the other hand, is that renin secretion responds to alteration either in sodium load or in sodium concentration at the macula densa. More recently it has been suggested that the control mechanism may involve both types of receptors (Blaine, Davis & Harris, 1973). Angiotensin has been shown to inhibit the release of renin and this has been

attributed to a direct action upon the juxta-glomerular cells (Shade, Davis, Johnson, Gotshall & Spelan, 1973) which is calcium dependent (Van Dongen & Peart, 1974). This provides a negative feedback control system to work in unison with the positive stimulatory pathways.

Angiotensin was first recognised by its pressor action in the intact animal and has since been revealed as a constrictor agent several times more potent than adrenaline (see reviews by: Gross, 1971; Whelan, Scroop & Walsh, 1969; Lee, 1969). Angiotensin causes a sustained contraction of most isolated smooth muscle preparations and the literature relating to this action will be reviewed in greater detail at a later stage. However, the role of angiotensin in the maintenance of blood pressure remains obscure despite several decades of intensive research and there is still considerable doubt as to the importance of the renin-angiotensin system in the aetiology of clinical hypertension (Reviews: Lee, 1969; McCubbin & Page, 1968; Laragh, Boer, Brunner, Buhler, Sealey & Vaughan, 1972; Peart, 1971). In contrast, there is increasing evidence for an involvement of angiotensin in the control of sodium and water homeostasis and thus of intravascular volume. Furthermore, actions of angiotensin upon the adrenal cortex and upon the kidney are observed with physiological concentrations of the hormone whereas smooth muscle contraction is only observed with higher concentrations.

Angiotensin has been shown to effect the production and release of aldosterone. The first suggestion that aldosterone secretion was controlled by a humoral factor other than ACTH or plasma electrolytes was made in 1959 (Yankopoulas, Davis, Kliman & Peterson, 1959; Denton, Goding & Wright, 1959) and the factor responsible was named aldosterone stimulating hormone (ASH). In a series of ablation experiments, J.O. Davis and co-workers demonstrated that the source of the ASH was the kidney (Davis, 1960; Davis, Carpenter, Ayers, Holman & Bahn, 1961).

At the same time two groups reported that intravenous angiotensin in man stimulated aldosterone secretion (Genest, Koiw, Nowaczynski & Leboeuf, 1958; Laragh, Angers, Kelly & Lieberman, 1960). This provided confirmation for Gross (1958) who, on the basis of earlier work on the interrelationship of the kidney and the adrenal, had suggested that angiotensin could be the stimulus for aldosterone release. There is now considerable evidence to support this hypothesis and this has been reviewed (Lee, 1969; Davis, 1967 and 1971; Boyd & Peart, 1972). However, it is still not clear whether angiotensin is the only factor controlling aldosterone release or whether it has the same importance in all species. It has been reported that in the rat angiotensin only stimulates aldosterone production in sodium deficient animals (Kinson & Singer, 1968) and in the sheep there is doubt as to the physiological importance of angiotensin in the control of aldosterone (Blair-West, Coghlan, Denton, Coding, Wintour & Wright, 1968). The evidence for an aldosterone stimulating factor of non-adrenal origin is reviewed by Davis (1971). Studies upon the action of angiotensin on aldosterone biosynthesis are reviewed by Boyd and Peart (1971) and it would appear that the effect is upon an early stage of the pathway, possibly between cholesterol and pregnenalone. There have been several reports and this action of angiotensin is stimulated by low plasma sodium or high plasma potassium and that this interaction occurs at the zona glomerulosa.

Since renin is produced and released within the kidney, it has long been supposed that the renin-angiotensin system may have an important intra-renal action. There is now considerable evidence that angiotensin does have a direct effect upon the renal tubule in addition to the indirect effect via aldosterone. Reviews by Mulrow & Goffnet (1969), Gross (1971 and 1973) and Lockett (1973) cover most of the work in this field. In experimental animals, the action of angiotensin has been shown to be dependent upon the concentration, low

sub-pressor doses causing an anti-natriuresis and a fall in glomerular filtration rate and larger doses producing a natriuresis unrelated to a temporary fall in glomerular filtration rate and urine production. There has been general agreement that the natriuretic action of angiotensin is due to a direct action upon the renal tubule (Vander, 1963; Leysacc, 1965; Lowitz, Stumpe & Ochwadt, 1969; Worcel & Meyer, 1970) but the anti-natriuretic action has been attributed both to a direct tubular action (Barraclough, 1965; Barraclough, Jones & Marsden, 1967) and to an indirect action via changes in glomerular filtration rate (Heally, Barcena, O'Connel & Schreiner, 1965; Malvin & Vander, 1967; Kover, Balindt & Tost, 1971; Waugh, 1972). Studies in man have yielded equally contradictory data. Normal subjects respond with anti-natriuresis to all dose ranges of angiotensin that can be consistently applied (Laragh, Cannon, Bentzel, Sicinski & Meltzer, 1963) but hypertensive patients respond with natriuresis (Itskovitz, Murphy & Schoenberg, 1967). In both animals and in man, the action of angiotensin is also affected by body sodium status and the functional status of the kidneys (see review by Gross, 1971).

These investigations of the action of angiotensin are complicated by the marked pressor action of the hormone which itself affects renal function. Attempts have been made to devise methods of separating these actions using in vivo techniques, but this has proved to be extremely difficult. In addition to causing a reduction of total renal blood flow, angiotensin also causes a redistribution by producing a more marked vasoconstriction in the cortex than in the medulla (Carriere & Friborg, 1969). Furthermore, angiotensin causes a redistribution of tubular activity with a decreased load in superficial tubules and an increased load in deeper juxta-medullary tubules (Finberg & Peart, 1970 and 1972). To overcome these problems, the action of angiotensin upon sodium and water movement has been

investigated using isolated trans-epithelial transporting systems.

Three types of preparation have been used in these studies; amphibian skin and bladder, mammalian intestine and mammalian kidney tissue. A direct stimulation of active sodium transport has been reported on frog skin (McAfee & Locke, 1967) but this has not been observed on toad skin, bladder or colon (Barbour, Gill & Barther, 1964; Covello & Crabbe, 1965). More recent studies in toad bladder (Coviello, 1972) and toad skin (Coviello & Brauchman, 1973) have revealed a hydrosmotic effect of angiotensin which resembled that produced by vasopressin. No concomitant effect on sodium movement was observed and the action has been attributed to a permeability change produced by a stimulation of adenyl cyclase (Coviello, 1973). This contradicts a previous report (Handler, Butcher, Sutherland & Orloff, 1965) in which angiotensin was found to have no effect upon adenyl cyclase or cyclic AMP levels in the toad bladder and furthermore was not observed to effect sodium movement. These differences may be due to the marked seasonal variation that occurs in the response to angiotensin.

Angiotensin has also been shown to stimulate water and sodium movement in everted sacs of small intestine from adrenalectomised-nephrectomised rats (Crocker & Munday, 1967 and 1970) and intact dioestrous rats (Crocker, 1971). A dose dependent action of angiotensin upon sodim and fluid transport by everted sacs of descending rat colon has been reported by Davies, Munday and Parsons (1969 and 1970) and low doses were found to stimulate transport whereas higher doses inhibited transport. These workers used adrenalectomised-nephrectomised rats in order to reduce the circulating levels of angiotensin but in a recent report (Hornych, Meyer & Milliez, 1973) it has been demonstrated that the biphasic effect of angiotensin is only observed in these animals and in the normal unoperated animal all concentrations of

angiotensin inhibit sodium transport in the descending colon and stimulate it in the ascending colon. They suggest that the action of angiotensin upon sodium movement might be mediated via an effect upon cyclic AMP whereas Munday and co-workers have reported that angiotensin stimulates the second sodium pump (Davies, Munday & Parsons, 1972; Parsons & Munday, 1972). Evidence for an action of angiotensin upon this pump has also been reported from studies on rat kidney cortex slices where angiotensin stimulates active sodium movement (Munday, Parsons & Poat, 1971 and 1972). However, in contrast Leysacc and co-workers have reported an inhibitory action of angiotensin upon sodium movement in this preparation (Leysacc, Lassen, & Hess-Thaysen, 1961). The difficulty in controlling experiments on isolated transepithelial transporting systems may well account for some of these conflicting results.

Angiotensin has been shown to effect the sympathetic nervous system but the physiological significance of this action has yet to be established. There has been controversy over the mechanism of interaction of angiotensin with the peripheral sympathetic nervous system, which has been ascribed both to a potentiation of noradrenaline release (reviewed by Zimmermann, Gomer & Lias, 1972) and an inhibition of re-uptake (reviewed by Khairallah, 1971 and 1972). It is possible that angiotensin may produce both effects depending upon the concentration (Khairallah, 1971 and 1972). Angiotensin also stimulates sympathetic ganglia (Reit, 1972) and has an important action upon the central nervous system (reviewed by Buckley, 1972; and Severs & Daniels-Severs, 1973) which may increase sympathetic outflow and so be involved in the pressor response. Central administration of angiotensin in animals has been shown to cause drinking (Fitzsimons, 1972; Severs, Summy-Long & Daniels-Severs, 1974) and it is therefore possible that

angiotensin may have a physiological role in the control of water intake. Further research is necessary to establish the importance of this action in the overall control of water and sodium homeostasis.

2. THE MECHANISM OF SMOOTH MUSCLE CONTRACTION

Since this study has been concerned with the primary interaction of angiotensin with the smooth muscle cell and not with the contraction mechanism, it is not relevant to make a detailed survey of the vast literature relating to smooth muscle contraction. However, two aspects which are involved in the present investigation will be reviewed: the role of inorganic ions in the contraction process and the metabolic requirements of contraction.

(a) Inorganic Ions and Smooth Muscle Contraction

(i) Introduction

There is now considerable evidence that the mechanism of smooth muscle contraction is closely analagous to the sliding filament system of vertebrate striated muscle (Ebashi, 1969; Ebashi, Endo & Ohtsuki, 1969; Ruegg, 1971). Smooth muscle cells contain actin filaments indistinguishable from those in striated muscle and a very similar tropin-tropomyosin system and although there are several biochemical differences in the myosin component, this does form an aggregated structure under physiological conditions (Hamoir, 1973). The contractile units in vertebrate smooth muscle cells are arranged obliquely with respect to the cell axis and as a result of shortening become progressively more angled (Small, 1974).

(ii) The Role of Calcium Ions

In smooth muscle, as in skeletal muscle, the tone of the contractile proteins is ultimately controlled by the calcium ion (see reviews by Daniel, 1964a; Axelsson, 1970; Hurwitz & Suria, 1971; Ruegg, 1971; Somlyo & Somlyo, 1968). The interaction of actin and myosin, enzymically Mg-activated actomyosin ATP-ase, is regulated by the tropin-tropomyosin system and calcium is a de-repressor at the allosteric site for this regulation, troponin (Ebashi et al, 1969; Ebashi, 1972). Thus an increase in intracellular calcium concentration causes calcium

to bind to troponin and thereby removes the inhibitory effect upon the actin filaments exerted via the mediator tropomyosin.

In the relaxed muscle cell the calcium concentration is less than 10^{-7} mol. and this must increase to around 10^{-6} mol. for threshold contractions and 10^{-5} mol. for maximal contractions (Ebashi et al 1969; Ruegg, 1971; Somlyo & Somlyo, 1968; Caldwell, 1968). The extra cellular and total muscle calcium concentration are greater than 1 mmol (see reviews by: Lullmann, 1970; Ruegg, 1971) and therefore the activator calcium for smooth muscle contraction can be supplied by influx from the extracellular space (see reviews by: Somlyo & Somlyo, 1968; Hurwitz & Suria, 1971; Hurwitz, Hubbard & Little, 1972; Bohr, 1973). However it is well known that in skeletal muscle a major source of activator calcium is the sarcoplasmic reticulum with its associated transverse tubular system (reviewed by Naylor, 1966). For many years it was thought that there was no comparable structure in smooth muscle cells but abundant sarcoplasmic reticulum has now been observed (Somlyo, Devine, Somlyo & North, 1971; Gabella, 1971 and 1973). Most of this is smooth sarcoplasmic reticulum and it is situated adjacent to the plasma membrane in close association with the calviolae, characteristic pit like invaginations of the plasma membrane which may be analogous to the tranverse-t system of skeletal muscle (Gabella, 1973).

Recent work has shown that the sarcoplasmic reticulum in smooth muscle cells is of variable size and ranges from 5% of total cell volume in arterial muscle to 2% in the taenia-coli of the guinea-pig (Devine, Somlyo & Somlyo, 1973). Both the sarcoplasmic reticulum and the mitochondria have been shown to bind calcium and together with the calcium bound at the plasma membrane, this constitutes an intra-cellular store of activator calcium for the contractile process (see reviews by: Somlyo & Somlyo, 1968; Hurwitz & Suria, 1971; Devine et al, 1973; Bohr, 1973; Reuter, 1974).

(iii) The Role of Membrane Depolarisation

It has long been recognised that membrane depolarisation is not essential for contraction of smooth muscle (see reviews by: Hoffman, 1969; Hurwitz & Suria, 1971; Somlyo & Somlyo, 1968). The ability of certain agents to induce contraction in the presence of depolarised membrane has been attributed to a mobilisation of calcium from intracellular stores. Contraction produced by membrane depolarisation is due to release of calcium from superficial membrane sites and influx from the extra-cellular space and is thus dependent upon the extracellular calcium concentration (Hurwitz & Suria, 1971; Sitrin & Bohr, 1972; Bohr, 1973; Reuter, 1974).

(iv) The Regulation of Intracellular Calcium Concentration

Several mechanisms have been implicated in the regulation of cytoplasmic calcium concentration. The cell membrane has a low permeability to calcium and this helps to maintain the large calcium gradient (Van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973; Somlyo & Somlyo, 1968; Hurwitz & Suria, 1971). Calcium is also removed from the cytoplasm by active accumulation in the intracellular binding sites. An ATP dependent calcium uptake has been observed in the mitochondrial and microsomal fraction of uterine muscle (Carsten, 1969; Batra & Daniel, 1971) of intestinal muscle (Andersson, Lundholm & Mohme-Lundholm, 1972; Godfraind & Verbecke, 1973; Devine et al, 1973) and of vascular smooth muscle (Fitzpatrick, Landon, Debas & Hurwitz, 1972; Baudounin et al 1972; Baudounin-Legros & Meyer, 1973) and this has been suggested as the major mechanism responsible for low intracellular calcium (Borle, 1973). There is also extensive evidence that calcium binds to the inner surface of the plasma membrane by an energy dependent process (Devine et al, 1973; Bohr, 1973; Van Breemen, Fanrias, Casteels, Gerba, Wuytack & Deth, 1973; Wolowyck, 1971; Rothstein, 1968) and attempts have been made to separate a plasma

membrane fraction and demonstrate calcium fixation (Derynck et al, 1973). There is also evidence for an outwardly directed calcium pump located on the membrane (Tomita & Watanabe, 1973; Casteels, Raeymaikers, Goffin & Wuytack, 1973) which is dependent upon ATP. In addition, several workers have suggested a calcium extrusion mechanism coupled to influx of sodium down its electrochemical gradient (Tomita & Watanabe, 1973; Reuter, Blaustein & Haesler, 1973; Burton & Godfraind, 1973). However, it has been suggested recently that this exchange may be confined to calcium bound at the membrane surface and may not involve intracellular calcium (Villamil, Rettori & Yegatti, 1973; Raeymaikers, Wuytack & Casteels, 1974).

(v) The Effect of Spasmogens upon Calcium Movement

An agent could produce a contraction of smooth muscle by increasing the membrane permeability to calcium or by inhibiting the active removal of calcium from the cytoplasm. It has further been suggested that there may be an inwardly directed calcium pump which is coupled to sodium efflux the activation of which would result in smooth muscle contraction (Bohr, Siedel & Sobieski, 1969; Brading, 1973; Reuter et al, 1973). Competition of sodium and calcium for the superficial anionic binding sites (Bohr et al, 1969; Brading, 1973) would explain the observed protagonistic effects of extracellular sodium and calcium ions on tension development in smooth muscle (Goodford, 1967; Caldwell, 1968; Busselin & Carmeliet, 1973). An increase in cytoplasmic calcium produced by stimulation of this pump would be an active process requiring energy from metabolism as opposed to the passive processes of release from intracellular binding sites or increase in membrane permeability.

It is now clear that many spasmogens can affect calcium release from intracellular binding sites in addition to a stimulation of calcium influx (Hurwitz & Suria, 1971; Bohr, 1973). Two phases may be

distinguished in the contractile responses to such agents, an initial rapid phase due to intracellular calcium release and a sustained slow phase due to an influx of extracellular calcium (Bohr, 1964; Godfraind & Kaba, 1969 & 1972; Hurwitz & Suria, 1974; Sitrin & Bohr, 1971; Van Breemen et al, 1973; Ohashi, Ohga & Saito, 1973; Deth & Van Breemen, 1974).

The relative contributions of extracellular and intracellular calcium to a contractile response can be investigated by the recently developed lanthanum technique (Reviews: Van Breemen, et al, 1973; Weiss, 1974). The trivalent lanthanumion replaces calcium at saturable and superficial membrane sites and prevents movement of calcium across the plasma membrane. Agonists which mobilise intracellular calcium produce one contraction in the presence of lanthanum but subsequent responses are inhibited. Thus intracellular sites which release calcium for contraction are distinct from those that remove it during relaxation. (Van Breemen et al, 1973).

(vi) The Effect of Calcium Ions on Membrane Permeability

In addition to their role in the activation of the contractile mechanism, calcium ions are also important in the control of membrane permeability. An increase in the amount of calcium in the membrane causes stabilisation and a decrease in excitability (reviewed by: Burnstock et al, 1963; Bohr, 1963; Rothstein, 1968; Tomita, 1970; Kuriyama, 1970; Bulbring & Tomita, 1970) by reducing sodium and calcium permeability and increasing potassium permeability. It has been suggested that fluctuations in potassium binding to the membrane due to the outward calcium pump produce changes in potassium permeability which give rise to the characteristic slow wave of smooth muscle membrane potential (Tomita & Watanabe, 1973). Calcium influx has been implicated in the action potential of smooth muscle (Bulbring & Kurijama, 1963; Brading & Tomita, 1968; Bennet, 1967).

(vii) The Effect of other Inorganic Cations

Other cations influence smooth muscle contraction mainly through their effect upon the movement and distribution of calcium ions (Bohr, 1964). Thus magnesium ions compete with calcium ions at extracellular and intracellular binding sites and alter membrane permeability to calcium (Altura & Altura, 1974). Sodium and potassium ions also compete with calcium at anionic binding sites on the plasma membrane and have antagonistic effects upon membrane permeability to calcium, which is increased by high potassium or low sodium (Tomita & Watanabe, 1973).

(viii) Cyclic AMP and Smooth Muscle Contraction

The first suggestion that changes in cytoplasmic cyclic AMP might be involved in smooth muscle contraction and relaxation was made in 1960 (Sutherland & Rall, 1960). There is now considerable evidence that the action of catecholamines is associated with increased cyclic nucleotide concentration (Reviews: Sutherland & Robison, 1966; Andersson, 1972; Marshall & Kroeger, 1973) but the correlation between contraction and cyclic AMP concentration is poor (Andersson, 1972). Cyclic AMP increases calcium uptake by intracellular binding sites and by the plasma membrane (Andersson, 1972) and may increase efflux (Marshall & Kroeger, 1973). However, many agents causing contraction of smooth muscle are associated with an increase in cyclic AMP or a biphasic effect of a decrease succeeded by an increase. This has been attributed to the existence of several distinct pools of the nuleotide within the cell which may change independently and which are controlled by separate phosphodiesterases (Andersson, 1972). The increase in cyclic AMP concentration produced by certain spasmogens may be important in the activation of phosphorylase kinase which is necessary to increase the supply of energy to the contractile process (Andersson, 1972). Alternatively the activation may be the direct result of the

increase in intracellular calcium concentration (Diamond, 1973). Recently both cyclic AMP dependent and independent mechanisms have been suggested for phosphorylase activation in smooth muscle (Gross & Mayer, 1974). It has also been suggested that the contractile state of smooth muscle may be controlled by the relative concentrations of cyclic AMP and cyclic GMP (Dunham, Haddox & Goldenberg, 1974).

(b) The Metabolic Requirements of Smooth Muscle Contraction

Smooth muscle requires a supply of energy both to activate the contraction process and to maintain the integrity of its cellular structure. The earliest studies of the energy pathways in smooth muscle were by Rona and Neukirch (1912) who examined the stimulating action of various sugars on intestinal preparations whose contractile ability had been abolished by exposure to glucose free media. Since then the use of this technique has demonstrated that energy production in smooth muscle is by the Embden-Myerhof pathway (Prasad, 1935a and 1935b; Feldberg & Solandt, 1942; Furchgott & Shorr, 1946; Coe, Detar & Bohr, 1968), the Krebs cycle (Furchgott & Wales, 1951 and 1952; Coe et al, 1968) and by the oxidation of fatty acids (Furchgott & Shorr, 1946; Coe et al, 1968).

(i) The Effect of Metabolic Inhibition upon Tone

Inhibition of oxidative metabolism, whether by chemical agents (Schmidt & Nichol, 1933; Farah, West & Angel, 1950; Born & Bulbring, 1950) or by anoxia (Evans, 1926; Gross & Clark, 1923; Garry, 1928; Prasad, 1935b; West, Hadden & Farah, 1951; Detar & Bohr, 1968) causes a progressive loss of tone and a reduction of induced contractions of smooth muscle preparations which is mostmarked in the absence of exogenous substrate (Prasad, 1935b; West, Hadden & Farah, 1951; Lundholm & Mohme-Lundholm, 1960). A similar effect has been observed with inhibitors of glycolysis which are most active under anaerobic

conditions (Prasad, 1935b; Shibita & Briggs, 1968; Rangachari, Paton & Daniel, 1972). Thus, under normal conditions energy production in smooth muscle is by both anaerobic and aerobic mechanisms.

(ii) The Effect of Metabolic Inhibition upon Contractility

Generally smooth muscle tone is more susceptible to metabolic inhibition than either induced contractions or spontaneous activity and there are marked differences in the sensitivity of individual smooth muscle preparations. Since the loss of contractility due to metabolic inhibition is greatest in tissues undergoing contraction, whether spontaneous or induced (Prasad, 1935b; Coe et al, 1968) it probably relates to the energy demands upon the tissue rather than to the time of exposure.

This close association between contractility and energy supply is due to the very small stores of high energy phosphates present in smooth muscle (Walaas & Walaas, 1950; Lipman, 1951; Cspao & Gergely, 1950; Born, 1956; Daemers Lambert, 1968) which are inadequate for one contraction (Lundholm & Mohme-Lundholm, 1962 and 1965; Furchgott, 1966). Thus, the decline of contractility under conditions of metabolic inhibition can be correlated with a fall in creatine phosphate content of the tissue (Born, 1956; Namm & Zucker, 1973) and is accompanied by a fall in ATP (Born & Bulbring, 1955; Namm & Zucker, 1973). Smooth muscle contraction is supported by a simultaneous increase in cellular energy production. Stimulation of anaerobic glycolysis results in an increased production of lactic acid which is most marked under anaerobic conditions when there is a parallel fall in the tissue content of glycogen (Prasad, 1935a; Axelsson, Hogberg & Timms, 1965). However, some of the lactic acid may be produced by a direct action of the spasmogen distinct from its contractile action (Lundholm & Mohme-Lundholm, 1963a; Namm and Zucker, 1973) and furthermore contraction is not consistently associated with increased glycogenolysis (Lundholm

& Mohme-Lundholm, 1963b). More recently, smooth muscle contraction has been observed to cause an increase in the activity of enzymes of the glycolytic pathway (Bostrom, Hogberg & Johansson, 1973). The activation of phosphorylase kinase has already been mentioned and has been attributed both to increased cyclic AMP and calcium ions.

Increased aerobic metabolism results in an increase in oxygen uptake during contraction (Bulbring, 1953). This is best seen under isometric conditions since the decrease in tissue length during isotonic contraction reduces the muscle surface area and tends to reduce oxygen uptake. Stretching the muscle also enhances the uptake of oxygen (Bulbring, 1953) which confirms the original observation by Lovatt Evans (Evans, 1923) and is compatable with an increase in tonus (Evans, 1926). It is also interesting that spontaneous activity causes a fluctuation in oxygen consumption which has been attributed to variations in tissue metabolism (Bozler, 1948; Bulbring, 1953). Recent work has implicated the calcium pump in this spontaneous rhythm (Tomita & Watanabe, 1973).

(iii) Comparative Studies of the effect of Metabolic Inhibition upon induced contractions

There have been few comparative studies of the effect of metabolic inhibition upon induced contractions of smooth muscle. On rabbit intestine, contractions produced by the direct muscle stimulants potassium chloride and barium chloride are reported to be less sensitive to oxygen lack than the responses to adrenaline and pilocarpine (Gross & Clark, 1923). Similarly, the response of rat uterus and stomach strip to prostaglandin has been shown to be dependent upon energy metabolism (Paton & Daniel, 1967; Coceani & Wolfe, 1966). Exposure of these tissues to conditions of metabolic inhibition preferentially reduced responses to prostaglandin but had little effect upon responses to acetylcholine. More recently, it has been reported that responses

of vascular muscle to angiotensin and histamine, unlike the responses to catecholamines or potassium, require a continuous supply of exogenous glucose. This has been attributed to a change in receptor conformation in substrate free solution (Altura & Altura, 1970).

(iv) The Effect of Metabolic Inhibition upon the Maintenance of induced contractions

It has been reported that removal of glucose or inhibition of oxidative metabolism reduces the ability of smooth muscle preparations to maintain induced contractions, even in the continued presence of the agonist (Feldberg & Solandt, 1942; Born & Bulbring, 1955; Born, 1956; West et al, 1951; Eckenfels & Vane, 1972). The sustained, or tonic, phase of the potassium response has been shown to be energy dependent and this has been attributed to a stimulation of active calcium influx (Urakawa & Holland, 1964) which may be related to sodium efflux (Pfaffman, Urakawa & Holland, 1965). More recently it has been suggested that prostaglandin synthesis may be important for the maintenance of induced contractions of smooth muscle (Eckenfels & Vane, 1972).

(v) The Effect of Metabolic Inhibition upon Ion Distribution in Smooth Muscle Cells

It is evident that the effect of metabolic inhibition upon smooth muscle contraction is not simply due to an interference with the contractile mechanism. The importance of active ion movement, particularly of calcium, has been mentioned and this will be prevented by metabolic inhibition. Similarly intracellular calcium binding is e nergy dependent as is the plasma membrane permeability. Exposure of smooth muscle preparations to metabolic inhibition for an hour induces a progressive, non-specific leakings in the plasma membrane (Casteels, Van Breemen & Wuytack, 1972; Raemaekers et al, 1974). There have been several reports that the energy necessary for the

membrane events may be differentiated from that for the contractile process (Prasad & Macleod, 1969; Bueding, Bulbring, Gercken, Hawkins & Kurijama, 1967; Rangachari et al, 1972). Thus cellular energy may be compartmentalised such that the ATP generated is used only for specific processes (Webb, 1966).

3. ANGIOTENSIN AND SMOOTH MUSCLE CONTRACTION

The literature on the action of angiotensin on smooth muscle is vast and it is therefore necessary to concentrate this review on those aspects relevant to the present study. To facilitate this, the review has been divided into two sections:

(a) The response of smooth muscle preparations to Angiotensin.

(b) The role of inorganic ions in the interaction of angiotensin with smooth muscle and the modification of contractile responses by altered ion concentration.

INTRODUCTION

It has been generally accepted that the angiotensin molecule, like that of most other peptide hormones, is too large to penetrate cellular membranes and therefore interacts with specific receptor sites on the surface of the cell (Rasmussen, 1969). Structural analogues of angiotensin have been used to investigate the features of the angiotensin molecule necessary for receptor binding and activation. Although there have been many comparative studies of angiotensin receptors in different tissues, there is still considerable doubt as to whether there is a universal angiotensin receptor. There have been many reports that angiotensin receptors in smooth muscle differ from those in the adrenal medulla and in various nervous tissues (Khairallah, Toth & Bumpus, 1970; Khairallah, 1972; Peach, 1971; Peach, Bumpus & Khairallah, 1969; Bumpus, 1971) and in a recent study differences have been reported in the angiotensin receptors of three smooth muscle preparations, rat colon, rat uterus and rabbit aortae (Papadimitriou & Worcel, 1974). Alternatively, it has been suggested that apparent tissue differences in the characteristics of the angiotensin receptor may be due to differences in the excitation-contraction coupling mechanisms (Mimran, Hinrichs & Hollenberg, 1974).

(a) THE RESPONSE OF ISOLATED SMOOTH MUSCLE PREPARATIONS TO ANGIOTENSIN

Angiotensin causes a contraction of most isolated smooth muscle preparations which is slow in onset and of long duration. Repeated exposure to angiotensin results in a reduction of the contractile response, a phenomenon which is known as tachyphylaxis. It has been suggested that tachyphylaxis results when angiotensin occupies the receptor sites and prevents further stimulation (Page & Bumpus, 1961). This may be due to an increase in binding of angiotensin molecules or to a decrease in breakdown by angiotensinases (Khairallah, Page, Bumpus & Turker, 1966). Consequently, analogues of angiotensin which are not metabolised, such as the 1-sarcosine derivatives, cause the development of prolonged tachyphylaxis as do analogues which bind strongly to the receptor site (Hall, Khosla, Khairallah & Bumpus, 1974). Isolated vascular smooth muscle is generally slower in response and shows more marked tachyphylaxis than intestinal muscle preparations and this resulted in the early use of intestinal smooth muscle for the investigation of the contractile action of angiotensin.

The action of angiotensin has been investigated upon several intestinal smooth muscle preparations and one of the most useful has been the isolated guinea-pig ileum. This responds to angiotensin on a linear dose response relationship in the range 1 to 10 nmol (Gross & Turrian, 1960; Bisset & Lewis, 1962; Regoli & Vane, 1964) and the responses are regular and repeatable. There is a delay of 30 to 40 seconds between addition of angiotensin and the beginning of the contraction and a maximal response is obtained within 90 seconds. Repeated administration of angiotensin gives rise to a tachyphylaxis which is most marked at high concentrations (Godfraind, Kaba & Polster, 1966; Godfraind, 1968). This may be avoided by increasing the period between successive responses to angiotensin. (Godfraind, Kaba & Polster, 1966). The contractile action of angiotensin upon guinea-pig ileum

has been resolved into two components, a direct action upon the smooth muscle cells and an indirect action mediated through the parasympathetic nervous elements (Khairallah & Page, 1961; Robertson & Rubin, 1962). Godfraind and co-workers have reported that the indirect action of angiotensin gives rise to an initial fast contraction which after a transient fall merges into the slow sustained contraction produced by the direct action of the hormone. Isometric recording allowed a clearer dissociation of the two phases of the angiotensin response than did isotonic recording (Godfraind et al, 1966). Two mechanisms have been suggested for the indirect action of angiotensin upon the isolated guinea-pig ileum, a stimulation of ganglion cells in Auerbach's Plexus (Khairallah & Page, 1961) and a stimulation of the parasympathetic nerve endings (Ross, Ludden & Stone, 1960). Recent studies indicate that the indirect action of angiotensin is due to a stimulation of acetylcholine release from the post-ganglionic nerve endings (Panisset, 1967; Suzuki & Matsumoto, 1965).

Other intestinal tissues which contract in response to angiotensin include rabbit ileum (Robertson & Rubin, 1962), mouse ileum (Goldenberg, 1967), rat small intestine and colon (Bisset & Lewis, 1962) and guinea-pig taenia coli (Regoli & Vane, 1964).

The isolated rat colon is a particularly useful tissue for investigation of the contractile action of angiotensin. It responds to angiotensin in a dose dependent manner over a concentration range of 1 to 10 nmol. (Bisset & Lewis, 1962). In addition, the action of angiotensin is wholly direct (Regoli & Vane, 1964; Ellis & Reit, 1969) which makes it suitable for bioassay of angiotensin. Regoli and Vane selected rat descending colon as the most suitable tissue for the bioassay of angiotensin in plasma and devised methods of increasing the sensitivity and specificity of the preparation. A similar study has been performed more recently by Gagnon and Sirois (1972).

Responses of rat colon to angiotensin will show tachyphylaxis, but as with guinea pig ileum, this may be avoided by increasing the interval between responses.

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 very regular (Bisset & Lewis, 1962; Gross & Turrian, 1960; Paiva & Paiva, 1960). Although slightly less sensitive than guinea pig ileum, responses of rat uterus to angiotensin are wholly direct (Khairallah & Page, 1961 and 1963; Regoli & Vane, 1964). Tachyphylaxis to angiotensin is more marked in the rat uterus than in intestinal smooth muscle preparations but can be reduced by allowing sufficient time between successive responses.

Angiotensin also causes contraction of isolated vascular and cardiac muscle and in the whole animal produces a marked pressor effect. The response of vascular smooth muscle and cardiac muscle to angiotensin is much slower than the response of intestinal smooth muscle and tachyphylaxis is very marked. Thus on the spirally cut rabbit aorta, angiotensin contractions take 2 to 4 minutes to maximalise and it is necessary to allow 30 minutes between responses. The contractions of isolated cardiac muscle are even slower and at least 2 hours are necessary between successive responses in order to avoid tachyphylaxis.

Findings from investigations of the action of angiotensin upon cardiac and vascular muscle and upon the cardiovascular system which relate to the present investigation will be discussed in the following section. Apart from this, it is not relevant to pursue a detailed description of the action of angiotensin upon individual vascular smooth muscle preparations and upon the cardiovascular system, details

of which may be found in a recent review by Gross (1971).

(b) THE ROLE OF INORGANIC IONS IN THE CONTRACTILE ACTION OF ANGIOTENSIN

(i) The Effect of Variations in the Extracellular Sodium Concentration

There have been several studies of the effect of extracellular sodium concentration upon the contractile responses of smooth muscle preparations to angiotensin but the results have been conflicting.

Reduction of the sodium concentration by a half caused a greater reduction of the angiotensin response of guinea-pig ileum than either the acetylcholine or bradykinin responses (Khairallah, Vadarampil & Page, 1965) which was attributed to an effect upon the cholinergically mediated indirect action of angiotensin upon this tissue. A similar reduction in sodium concentration produced a smaller but equal reduction of responses to the three agonists on the rat uterus. Blair-West and co-workers failed to confirm these findings with guinea-pig ileum and found that a reduction of the sodium concentration from 144 meq/l to 110 meq/l caused an equal reduction of responses to acetylcholine and angiotensin. However, an increase in the sodium concentration to 200 meq/l caused a potentiation of the angiotensin response while simultaneously depressing the response to acetylcholine (Blair-West, Harding & McKenzie, 1967). Since the potentiation also occurred when the indirect component of angiotensin's action was blocked by atropine or tetrodotoxin, they concluded that changes in sodium concentration effected the direct interaction of angiotensin with the smooth muscle cell.

The same group of workers have since reported similar findings on the constrictor action of angiotensin upon the rabbit ear artery (Blair-West, Harding & McKenzie, 1968) and the isolated rat portal vein (Blair-West, McKenzie & McKinley, 1971). An increase in the sodium concentration of 40 meq/l enhanced the response to angiotensin while

a similar decrease in sodium concentration reduced the angiotensin response. Since no sympathetic activity of angiotensin was detected, these effects were attributed to an involvement of sodium ions in the direct interaction of angiotensin with the smooth muscle cells. However, on rat portal vein, decreased sodium concentration only reduced the angiotensin response under hyposmolar conditions and in isosmolar conditions when sucrose was used to maintain the osmolarity, the angiotensin response was unaffected. No explanation of this observation has been advanced.

Alteration in the external sodium concentration has also been reported to effect the inotropic action of angiotensin upon cat papillary muscle (Lefer, 1967). A reduction in sodium concentration of 30 mmol reduced the response to angiotensin and conversely an increase of 30 mmol potentiated the response. This effect of sodium was present after reserpinisation and therefore was attributed to a direct action on the smooth muscle cells. Further, it appeared to be specific for angiotensin since the same changes in sodium concentration failed to affect responses to noradrenaline. The converse effect of altered sodium concentration has been reported on the angiotensin response of spirally cut rabbit aortae (Napodano, Calva, Lyons, DeSimone & Lyons, 1962). A decrease in sodium concentration of 25% enhanced the angiotensin response whereas a 25% increase in concentration depressed the angiotensin response. Changes in osmolarity of the solution were controlled and the effect was attributed to a change in the ratio of the extracellular to intracellular sodium concentration.

These investigations of the effect of the sodium ion upon reactivity of smooth muscle to angiotensin have been extended to include the pressor action of angiotensin in the whole animal. A potentiation of the pressor response to angiotensin has been

reported in rats (Reid & Laragh, 1965) and in dogs (Healy, Suszkiw, Dennis & Schreiner, 1966) maintained on high sodium diet. Similarly pre-treatment of rats with DCA and salt enhances the pressor response to angiotensin and increases the gradient of the dose response curve relative to the normal rat (Gross & Lichtlen, 1958). This effect of DCA and salt has not been observed in studies on the angiotensin response of isolated arteries (Bohr, 1964). Increased sensitivity to angiotensin has also been observed in the early stages of salt hypertension in the rat (Honore & Gardner, 1966). Conversely, negative sodium balance due to sodium deficient diet reduces the pressor response to angiotensin in rats (Reid & Laragh, 1965) and in dogs (Healy,et al, 1966 Davis, Hartcroft, Titus, Carpenter, Ayers & Spiegel, 1962). A similar effect has also been observed in the sheep where sodium depletion reduces the pressor response to angiotensin and adrenaline (Blair-West, Coghlan, Denton, Goding, Munro & Wright, 1962). In man, sodium depletion reduces the pressor response to angiotensin and conversely during retention the response is potentiated (Ames, Borkowski, Sicinski & Laragh, 1966). At least part of these effects of sodium concentration upon the pressor activity of angiotensin may be due to changes in the activity of the renin-angiotensin system and hence of circulation levels of angiotensin. However, there have been several reports that sodium concentration does have a direct effect upon the pressor action of angiotensin in sheep (Blair-West, Coghlan, Denton, Scoggins & Wintour, 1972), the rat (Weinberger, Ramsdell, Rosner & Geddes, 1972), and in man (Brunner, Chang, Wallach, Sealey & Laragh, 1972).

(ii) The Effect of Variations in the Extracellular Calcium Concentration

A reduction in calcium concentration decreases the contractile response of many smooth muscle preparations to angiotensin. The oxytocic action of angiotensin upon rat uterus has been shown to be

dependent upon the presence of calcium (Renson, Barac & Bacq, 1959). However, it has since been reported that a reduction in calcium concentration produces an equal depression of contractile responses of rat uterus to angiotensin, bradykinin and acetylcholine (Khairallah et al, 1965). In a detailed investigation of the effect of inorganic ions upon the contractile response of angiotensin these workers found that rat uterus was far more sensitive to calcium removal than guinea pig ileum. Nevertheless, in both tissues, reduction of the calcium concentration produced an equal inhibition of responses to acetylcholine, angiotensin and bradykinin. Changes in the magnesium concentration had the opposite effect to changes in calcium concentration, thus a doubling of magnesium concentration abolished contractile responses whilst a 25% reduction potentiated responses. They also found that up to 85% of the calcium could be replaced with strontium without effecting induced contractions but that a further 5% replacement abolished all responses. They suggested that strontium while able to replace calcium for the contractile mechanism, is unable to do so for the excitation contraction coupling process. The contractile response of isolated frog heart and stomach strip to angiotensin has been shown to be dependent upon the presence of calcium (Singh, 1964) and the inotropic response of cat papillary muscle to angiotensin is potentiated by high calcium (Beaulness, Nantel & Cardinal, 1965). In contrast, reduction of the calcium concentration from 2.54 to 0.63 mmil failed to alter the responses of cat papillary muscle to angiotensin or noradrenaline (Lefer, 1967).

Variable results have also been reported from studies of the calcium dependence of angiotensin contractions of vascularmuscle. The contraction of rabbit aortic strip has been reported to be abolished in the absence of calcium (Shibita & Carrier, 1967) but

other workers have observed contractile responses to angiotensin in calcium free solutions (Somlyo, Devine, Somlyo & North, 1971). Further, the angiotensin induced contraction of isolated small resistance vessels are depressed by increased calcium concentration (Bohr, 1966) whereas the responses of isolated renal arteries are depressed by a reduction of calcium concentration (Hridina, 1966, cited in Gillespie, 1966).

Recently, it has been suggested that calcium ions are important for binding of angiotensin to its receptor (Stewart & Freer, 1972). A model system has been proposed for the angiotensin receptor where calcium ions occupy an anionic site adjacent to the point of combination with the histidine residue of the hormone. It is suggested that in the absence of calcium ions, the ionic site would be free to combine with the hormone and the distortion produced would induce tachyphyllaxis. Further experimentation is required to verify this hypothesis.

(iii) The Effect of Angiotensin Upon Ion Movement in Smooth Muscle

Not only is the angiotensin response effected by changes in the ionic environment, but angiotensin itself causes movement of ions across membranes and so alters the cellular ionic distribution. In recent years this property has been extensively investigated in the hope of elucidating the mechanism of action of angiotensin. Angiotensin, like other vasoconstrictors, has been reported to move sodium into and potassium out of the smooth muscle cells of perfused arteries (Friedman & Friedman, 1964 and 1965). In contrast, the same workers have reported an increased outflow of sodium from isolated arteries with no effect on potassium flux (Friedman & Friedman, 1967). This has been confirmed by Borive and Hagemeijer (1966) who observed an increased potassium efflux from isolated aortic strip in response to noradrenaline but only a rapid, transient rise in response to large concentrations of angiotensin. It seems

probable that the difference in these results reflects the different experimental techniques employed rather than fundamental differences in cellular effects.

In the isolated aorta and rat uterine muscle angiotensin has been shown to stimulate sodium efflux without altering the influx Turker, Page & Khairallah , 1967). Serotonin and bradykinin were found to have no effect upon sodium efflux but vasopressin and oxytocin caused a stimulation. However, the effect of angiotensin upon sodium movement was abolished by ouabain in concentrations which also inhibited the contractile action, whereas with vasopressin and oxytocin, ouabain only effected the stimulation of sodium efflux. It was concluded that angiotensin acts at the cell membrane to produce a change in ionic movement which then initiates the contractile process. This suggestion that the sodium pump might be involved in the contractile action of angiotensin directly contradicted an earlier report. In an investigation of the effect of angiotensin upon the isolated sodium loaded aorta, Daniel (1965) found no evidence for an action of angiotensin upon active sodium movement. More recently, angiotensin has been reported to increase the sodium and calcium content of the smooth muscle cells of dog carotid artery (Villamil, 1972). An increase in sodium efflux was also observed and this was attributed to the increased size of the intracellular sodium pool. The primary action of angiotensin was attributed to an alteration in cellular sodium permeability and it was suggested that the enhanced intracellular calcium concentration might then arise through a stimulation of a sodium calcium pump. Metabolic inhibition abolished these effects of angiotensin.

A possible action of angiotensin upon a sodium calcium pump has also been suggested for the longitudinal muscle of guinea pig

ileum (Godfraind, 1970). In preparations desensitised to acetylcholine, angiotensin induced a sodium efflux which in a low calcium solution was coupled with net calcium influx. It was suggested that this might indicate an action of angiotensin upon an outward movement of sodium which was coupled to inward movement of calcium.

Angiotensin has been reported to cause an increased calcium uptake by guinea pig taenia coli (Shibita, Carrier & Frankenheim, 1968). However, agents which inhibited the contractile response to angiotensin failed to effect the stimulation of calcium uptake and further the inhibition of contraction was not reversed by increased extracellular calcium. The authors concluded that unlike potassium, angiotensin does not induce contraction simply by increasing calcium influx. In the aortic strip, chlorpromazine and dibenamine inhibit neither the angiotensin contraction nor the stimulation of calcium influx (Shibita & Carrier, 1967) although removal of extracellular calcium inhibits the contractile response. It has been suggested that calcium lack indirectly inhibits the angiotensin induced contraction by an action on the contractile mechanism. In another study, manganese has been shown to inhibit the contractile responses of rabbit aortae to angiotensin and noradrenaline (Sullivan & Briggs, 1968). It was concluded that the angiotensin response was due to a movement of calcium across the cell membrane but that an additional mechanism might be involved in the noradrenaline response since, unlike that of angiotensin, this was reduced by membrane depolarisation. Recently a stimulation of calcium entry has been shown to be involved in the contractile response of rabbit artae to both noradrenaline and angiotensin (Deth & Van Breemen, 1974). However, it was also suggested that the response to both spasmogens might involve an initial rapid phase due to release of intracellular calcium.

(iv) The Effect of Angiotensin upon Calcium Binding at Intracellular Sites

The possibility that angiotensin could release calcium from intracellular binding sites has been investigated by Meyer and co-workers using a microsomal membrane preparation derived from the intimal-medial layers of rabbit aorta. These microsomal membranes have been shown to bind calcium by an energy dependent process which is stimulated by Mg-ATP (Baudouin, Meyer, Fermandjian & Morgat, 1972; Baudouin-Legros & Meyer, 1973). Angiotensin reduces this binding capacity and increases the release of calcium by combining with specific receptors which have the same reaction kinetics as the angiotensin receptors found in the aortae (Baudouin, Meyer & Worcel, 1971; Baudouin et al, 1972; Baudouin-Legros & Meyer, 1973). Conversely, cyclic-AMP increases the binding of calcium to the membranes (Baudouin-Legros & Meyer, 1973). Although many peptide hormones act via changes in intracellular cyclic-AMP concentration, there is no evidence that the nucleotide is involved in the action of angiotensin upon calcium binding. Thus in the rat uterus, angiotensin in concentrations sufficient to cause contraction has no effect on the activity of phosphodiesterase (Angles D'Auriac, 1973a), adenyl cyclase (Angles D'Auriac & Meyer, 1972) or on the intracellular concentration of cyclic-AMP (Angles D'Auriac & Meyer, 1973b). Angiotensin has been shown to decrease the activity of calcium ATPase and of the alkaline phosphatase associated with the sarcoplasmic reticulum (Limas & Cohn, 1973) and it is therefore probable that the reduction of calcium binding is produced by a direct inhibition of the calcium transporting mechanism. Although the angiotensin binding parameters for the receptors in microsomal membranes are the same as those for the receptors in smooth muscle preparations, it is difficult to relate the two effects. The microsomal membrane fraction is of mixed origin and contains membrane fragments from the sarcoplasmic reticulum,

caveoli and plasma membrane but angiotensin, like other peptide hormones, is considered to act solely at the plasma membrane. In addition, it has recently been reported that the homogenisation and fractionation processes necessary to prepare the microsomal membranes preparation cause a disruption of the plasma membrane into different diverse types of vesicles each bearing a different complement of the intact membrane surface (Devynck, Pernollet, Meyer, Fermandjian & Fromageot, 1973). Thus angiotensin binding was observed in all of the purified membrane fractions derived from the crude microsomal fraction and it was not possible to identify the site of action of angiotensin.

These investigations have now been extended by the development of a method for the solubilisation of angiotensin receptors in the plasma membrane of rabbit aorta (Devynck, Pernollet, Meyer, Fermandjian, Fromageot & Bumpus, 1974). This technique may provide definitive evidence of angiotensin binding at the plasma membrane. It has also been claimed that specific binding sites for angiotensin have been observed in the plasma membrane from guinea-pig ileum (Olivera & Holzhacker, 1974).

(v) The Effect of Angiotensin upon the Membrane Potential of Smooth Muscle Cells

An alternative method of investigating the interaction of a spasmogen with the smooth muscle membrane is to measure the effect on the membrane potential. Angiotensin has been shown to cause a depolarisation of arterial smooth muscle (Keatinge, 1966) and intestinal smooth muscle (Ohashi, Wonomura & Ohga, 1967) but this does not appear to be essential to the contractile action, which is unaffected by complete membrane depolarisation with potassium (Shibita & Briggs, 1966; Khairallah et al, 1965; Shibita et al, 1968, Sullivan & Briggs, 1968). It has therefore been suggested that the contractile action of angiotensin is unrelated to changes in sodium and potassium flux across the membrane and may instead be due to calcium movement. (Sullivan & Briggs, 1968). However, this observation is difficult to reconcile with the marked resistance of the angiotensin response of guinea pig ileum to calcium removal from the Tyrode solution (Khairallah et al, 1965). Recently angiotensin has been shown to cause an increase in sodium influx in polarised and depolarised rat uterus (Hamon & Worcel, 1973). These workers observed a stimulation of potassium and chloride efflux in the polarised preparation which was not detected in the depolarised preparation and they attributed this to the depolarisation produced by angiotensin. A difference in the action of angiotensin upon depolarised and polarised rat uterus has also been reported by Freer (1974). He found that the angiotensin response of rat uterus was inhibited by the calcium antagonist verampil in both depolarised and polarised preparations but that addition of 45 mmol lithium selectively inhibited the response of the polarised preparation. Since ouabain affected neither preparation, he concluded that the action of lithium was unrelated to an effect on the sodium potassium pump.

4. OTHER INVESTIGATIONS OF THE MECHANISM OF ACTION OF ANGIOTENSIN

(a) The Secondary Messenger Hypothesis

Many of the large molecular weight peptide hormones have been shown to act via the second messenger cyclic AMP, but there is no conclusive evidence for an involvement of the nucleotide in the action of angiotensin. Investigations of the role of the nucleotide in the angiotensin response will be reviewed. Prostaglandins have also been suggested as being involved in the action of hormones either as mediators or as negative feedback inhibitors on the action of cyclic AMP and the relationship between angiotensin and prostaglandins will also be considered.

(i) Cyclic AMP

The hydroosmotic effect of angiotensin upon the toad bladder has been ascribed to an increase in intracellular cyclic AMP produced by stimulation of adenyl cyclase (Coviello, 1973). A similar action has also been suggested as a result of preliminary experiments on the angiotensin induced stimulation of water and sodium movement in rat colon (Hornych et al, 1973). In contrast, Munday and co-workers found no evidence for an involvement of cyclic AMP in the action of angiotensin upon ion and water transport in rat colon (Davies, Munday & Parsons, 1972) or rat kidney cortex (Munday, Parsons & Poat, 1972). Neither does other work on the action of angiotensin upon toad bladder support an involvement of cyclic AMP in the interaction of the hormone (Handler et al, 1965).

More recently the action of angiotensin upon steroidogenesis in isolated adrenal cells has been quantitatively related to an increase in intracellular cyclic AMP (Peytremann, Nicholson, Brown, Liddle & Hardmann, 1973) and an increase in cyclic AMP has been demonstrated during stimulation of the neurohypophysis by angiotensin (Gagnon & Hessler, 1974).

The contractile action of angiotensin upon vascular smooth muscle has been associated with a decrease in intracellular cyclic AMP which was attributed to inhibition of adenyl cyclase (Volicer & Hynie, 1971). However, Meyer and co-workers have shown that angiotensin has no effect upon the adenyl cyclase activity (Angles D'Auriac & Meyer, 1972), the phosphodiesterase activity (Angles D'Auriac & Meyer, 1973a) or the intracellular cyclic AMP concentration (Angles D'Auriac & Meyer, 1973b) of rat uterus. This work on the relationship of cyclic AMP and calcium to the contractile action of angiotensin upon smooth muscle has already been mentioned in greater detail.

(ii) Prostaglandins

Angiotensin has been reported to stimulate the release of prostaglandin-like substances from the dog kidney (McGiff, Crowshaw, Terrigno & Lonigro, 1970; Aiken & Vane, 1971) and the effect is dose related (Aiken & Vane, 1973; Gagnon, Gauthier & Regoli, 1974). It has been suggested that prostaglandins may be involved in the autoregulation of the kidney and that their release by angiotensin may attenuate the action of the hormone (Aiken & Vane, 1973; Gagnon et al, 1974). This may explain the biphasic effect of angiotensin upon renal function in which low doses cause anti-natriuresis and higher doses natriuresis (McGiff et al, 1970). In contrast, Aiken and Vane (1973) were unable to detect the release of prostaglandin like substances during hindlimb vasoconstriction on the dog.

Angiotensin has also been reported to release prostaglandins from the spleen (Douglas, Johnson, Marshall, Joffe & Needeman, 1973; Ferreira, Moncada & Vane, 1973) and the myocardium (Limas, 1974) but the latter effect was attributed to the intermediate release of noradrenaline. Prostaglandins have been shown to potentiate the centrally mediated pressor effect of angiotensin when administered simultaneously into the vertebral artery (Gyang, Deuben & Buckley, 1973). This has been attributed to an action on calcium metabolism.

(b) The Effect of Angiotensin upon Protein Synthesis

There have been several reports that inhibitors of protein synthesis affect the response of certain tissues to angiotensin. These studies have relied upon the differential specificity of inhibitors of transcription, such as actinomycin D, and inhibitors of translation such as cyclohexamide and puromycin.

It has been reported that angiotensin localises in the nuclei of cardiac cells and exerts its action via a stimulation of the transcription stage of protein synthesis (Robertson & Khairallah, 1971). The angiotensin induced stimulation of aldosterone production in the rabbit adrenal gland (Cosby, Sartorelli & Roth, 1971) and of noradrenaline production in guinea pig atria (Roth & Hughes, 1972) is also accompanied by increased protein synthesis due to stimulation of the translation process. Similarly, the stimulation of fluid transport in rat distal colon (Davies, Munday & Parsons, 1972) and of ion transport in rat kidney cortex slices (Munday, Parons & Poat, 1972) is inhibited by cyclohexamide and puromycin but not by actinomycin D. These workers attributed the effect of angiotensin upon ion movement to the synthesis of proteins which are associated with sodium transport by the potassium independent sodium pump. Evidence for an active sodium transport system which is independent of potassium and insensitive to ouabain has been reported (Whittembury & Proverbio, 1970; Robinson, 1970) and Munday and co-workers have shown that the diuretic ethacrynic acid, a specific inhibitor of this pump (Whittembury & Proverbio, 1970; Proverbio, Robinson & Whittembury, 1970) abolishes the angiotensin induced stimulation of sodium movement in rat kidney cortex slices (Munday, Persons & Poat, 1971; Parsons & Munday, 1972) and rat descending colon (Parsons & Munday, 1972).

An action of angiotensin through protein synthesis has to be

reconciled with the generally accepted view that angiotensin is unable to penetrate the plasma membrane. Furthermore, such an action would be expected to result in a latent period before a response to the hormone could be observed. Although a delay of 3 minutes has been reported for the action of angiotensin upon kidney cortex slices, it is doubtful whether this would be sufficient to allow the synthesis of new protein. The experimental evidence that this protein is associated with the second sodium pump is also open to criticism and this will be discussed later in the context of findings from the present study.

METHODS

1. PREPARATIONS

Male albino guinea pigs (200-300 g) or white Wistar rats (150-200 g) were starved for 12 hours prior to experimentation and were killed by cervical dislocation. Intestinal preparations (2-3 cm) were taken from the terminal ileum of the guinea-pig or the terminal 5cm of rat colon immediately adjacent to the rectum and were flushed through with Tyrode solution prior to mounting. Uterine preparations consisted of the whole uterine horn removed from rats at oestrus or dioestrus, as determined by examination of a vaginal smear.

Preparations were suspended in 20 ml organ baths containing Tyrode solution bubbled with air. Intestinal preparations were maintained at 32°C and uterine preparations at 28°C. The composition of the normal Tyrode solution was as follows: NaCl 137 mmol, KCl 2.7 mmol, $MgSO_4.7H_2O$ 1.1 mmol, $NaH_2PO_4.2H_2O$ 0.42 mmol, $NaHCO_3$ 11.9 mmol, $CaCl_2.2H_2O$ 1.8 mmol, glucose 5.6 mmol. For experiments with lanthanum chloride a modified Tris-buffered solution was used to prevent precipitation of the insoluble phosphate and carbonate salts of lanthanum: NaCl 137 mmol, KCl 2.7 mmol, $MgCl_2.6H_2O$ 1.0 mmol, $CaCl_2.2H_2O$ 1.8 mmol, tris 5.0 mmol, glucose 5.6 mmol.

In experiments involving anoxia the Tyrode solution was placed under vacuum for 4 hours and then the vacuum was replaced by oxygen free nitrogen. The Tyrode reservoir and the organ bath were then gassed with nitrogen instead of air. To expose preparations to carbon monoxide, the gas was bubbled through the organ bath in the absence of air. Coceani and Wolfe (1966) used simultaneous administration of air and carbon monoxide during experiments upon isolated rat uterus but although this technique was investigated, it failed to yield consistent results. Carbon monoxide was supplied by BDH and was of 99.5% purity.

2. RECORDINGS

In the early experiments with guinea pig ileum, contractions were recorded on a kymograph using an isotonic frontal-writing lever working against a load of 1-4 g and with a magnification ratio of 15:1. Subsequently this apparatus was replaced with an electronic recording system consisting of Devices 2LD01 isotonic transducers coupled to Devices M2 recorders.

It has been reported that isometric recording reveals more information about the individual components contributing to a muscle response than isotonic recording (Paton, 1961; Paton and Rothschild, 1965; Wilkie, 1962). Furthermore, the energy required for an isometric response is greater than that for an isotonic response and thus isometric recording is more likely to reveal an impairment of contraction due to changes in tissue metabolism (Lundholm and Mohme-Lundholm, 1965). Therefore, in experiments with rat colon, responses were recorded isometrically with Devices 2ST02 transducers and an applied resting tension of 1g. Uterine preparations deteriorated rapidly under isometric conditions and these responses were recorded with the isotonic transducers (Devices 2LD01) using an applied load of 1g.

All tissues were equilibrated for 1.5 h and then the contractile responses were recorded at the maximal sustained deviation from the baseline. A tissue contact time of 90s, which is sufficient to record both components of the guinea-pig ileum response to angiotensin (Godfraind et al, 1966), was employed for both acetylcholine and angiotensin. In all experiments, concentrations of acetylcholine and angiotensin were selected which produced responses approximately equal to the 50% maximal acetylcholine response. A dose cycle of 10 or 15 m was used to avoid tachyphyllaxis of angiotensin responses or potentiation of acetylcholine responses.

3. ELECTRICAL STIMULATION

In some experiments, guinea-pig ileum was stimulated electrically through co-axial electrodes (Paton, 1965) with supra-maximal (usually about 30V) rectangular wave pulses. Repetitive stimuli at a frequency of 20Hz were used to produce sustained contractions of 10s duration. A constant pulse width of 3ms was employed.

4. GLYCOGEN ASSAY

The glycogen content of rat colon and uterus muscle samples was assayed by the method of Lo, Russel and Taylor (1970). In this method the tissue is denatured by boiling with alkali and the glycogen is precipitated and converted to glucose by addition of concentrated sulphuric acid. The glucose is then condensed with phenol in the presence of the acid and the yellow-orange colour is measured spectrophotometrically at 490 m. This technique is reported to be superior to the older anthrone method for glycogen assay (Lo et al, 1970).

PRACTICAL PROCEDURES

White Wistar rats (150-200 g) were starved for 12 hours prior to experimentation and were killed by cervical dislocation. Female rats were selected at oestrus or dioestrus by examination of the vaginal smear immediately prior to killing. Tissues for glycogen assay were removed and placed in a petri dish containing aerated Tyrode solution at 28°C. Preparations of rat colon were flushed through with Tyrode solution before sampling. Approximately 50 mg samples were cut from the tissues and dried on filter paper. All visible fat and connective tissue was then removed with a probe and forceps. No samples were taken from the exposed cut end of tissue since glycogen levels here would not be representative of overall levels. Instead

the cut end was trimmed by 0.25 cm immediately before removing the sample. After weighing the sample on a Class A analytical balance, it was transferred to the bottom of a pyrex centrifuge tube which was then immersed in dry ice and alcohol. Samples were maintained deep frozen until several had been collected and it was convenient to continue the assay.

0.5ml of 30% KOH saturated with Na_2SO_4 was added to the tubes which were then capped and placed in a boiling water bath for 30m. When a homogenous solution was obtained, the glycogen was precipitated from the alkaline digest with 95% ethanol (1.1-1.2 volumes). The tubes were then placed in ice for 30m and subsequently centrifuged at 840g for 30m, after which the supernatent was carefully aspirated. The glycogen precipitate was then dissolved in 3mls of distilled water and a 1ml aliquot was transferred to a 150x20mm test tube. One ml of phenol solution was added followed by 5ml of 98% sulphuric acid which was directed in a stream at the liquid surface to ensure good mixing. After allowing the tubes to stand for 10m they were placed in a shaking water bath at 25-30°C for 20m. The absorbance was then measured using a Pye Unicam SP500 series OI spectrophotometer against blanks prepared with 1ml of bi-distilled water. To ensure accuracy, three aliquots were prepared from each tissue sample and the absorbance was calculated as the mean of these readings.

A standard curve was prepared using triplicate samples of standard glycogen solutions containing from 5 to 100 g of glycogen. The mean slope calculated from the 33 glycogen standard samples was then used to estimate the tissue content of glycogen:

$$\text{Tissue Glycogen Content (g/100g tissue)} \quad \frac{A490}{K} \times \frac{V}{v} \times \frac{10^{-4}}{W}$$

V: total volume of glycogen solution (3ml)

v: volume of aliquot used in colour reaction (1ml)

A490: absorbance at 490m

W: weight of tissue sample (g)

K: slope of standard curve (absorbance per microgram of glycogen)

The colour in this assay is proportional to the concentration of glucose and the concentration of phenol and therefore one standard curve was constructed for each phenol solution.

5. DRUGS AND CHEMICALS

Angiotensin was the commercial Hypertensin (Ciba): Asp $(NH_2)^1$-Val^5-Angiotensin II. This was prepared in a sterile solution (10^{-5}mol) in bi-distilled water and stored frozen in 1 ml ampoules. Acetylcholine chloride (BDH) and histamine acid phosphate (BDH) were stored in a dessicator at 0-5°C and solutions were prepared immediately before the start of an experiment. Final dilutions of all agonists were made in Tyrode solution and a constant test volume of 0.2 ml was added to the bath. All reagents used to modify induced responses were added to the reservoir of Tyrode solution to give the final concentration. All chemicals were of analytical grade.

COMMERCIAL DRUG PREPARATIONS

Ouabain: Supplied by Sigma. Stock solutions were prepared in bi-distilled water immediately before use. To achieve a concentration of 10^{-3}mol the appropriate volume of stock solution was added directly to the organ bath.

Ethacrynic Acid: Supplied as a pure powder by Merk, Sharpe and Dohme. Stock solutions were prepared in bi-distilled water immediately before use.

Indomethacin: Supplied as a pure powder by Merk, Sharpe and Dohme. Stock solutions were prepared in ethyl alcohol (90%) immediately before use.

6. CALCULATION OF RESULTS

All results have been expressed as a percentage reduction of the control response taken in normal Tyrode solution and gassed with air. The control responses were measured when three successive exposures to the selected concentration of spasmogen had induced equal sized contractions. For each treatment, the reported results are the means of values obtained from at least 5 tissues. The effect of the experimental treatments have been analysed using Student's 't' test.

RESULTS

SECTION 1: A COMPARATIVE STUDY OF THE METABOLIC REQUIREMENTS FOR ANGIOTENSIN AND ACETYLCHOLINE INDUCED CONTRACTIONS OF SMOOTH MUSCLE

A. GUINEA-PIG ILEUM

Guinea-pig ileum is very sensitive to angiotensin and has been widely used for investigating the contractile action of the hormone. In this study the tissue was subjected to conditions which interfered with its energy metabolism and the effect upon responses to angiotensin and acetylcholine was determined. Although the metabolic requirements for smooth muscle contraction have been extensively investigated, there have been very few attempts to define the individual requirements of specific spasmogens. It was hoped that this approach would separate the primary interaction of angiotensin with the smooth muscle cells from the resultant activation of the contractile mechanism and so provide information about the mechanism involved.

(1) The Effect of Altering the Glucose Concentration of the Tyrode Solution

The glucose concentration of the Tyrode solution was increased or decreased from the normal concentration of 5.6mmol and the responses to acetylcholine and angiotensin were recorded. It was found that progressively increasing the glucose concentration to 44.8mmol had no effect on either agonist, but that reducing the concentration had marked effects. Table 1 shows the percentage reductions of the acetylcholine and angiotensin responses produced by a 30 minute equilibration in Tyrode solution containing 2.8 mmol and 1.4 mmol glucose. At the lower concentration, the angiotensin response was reduced by $47.9 \pm 3.5\%$ ($n=5$) and the acetylcholine response by $11.8 \pm 1.7\%$ ($n=5$) and this difference was highly significant. ($p < 0.001$).

TABLE 1

The percentage reductions of responses of guinea-pig ileum to angiotensin due to reducing the glucose concentration of the Tyrode Solution, compared to the percentage reductions of responses to acetylcholine.

Glucose Concentration	Percentage Redn. of Angio Resp.	Percentage Redn. of Acetylcholine Resp.	p
2.8 mmol	24.4 ± 12.9 (5)	10.8 ± 3.6 (5)	NOT SIGNIF.
1.4 mmol	47.9 ± 3.5 (5)	11.8 ± 1.7 (5)	$p < .001$

Number of observations in parenthesis.

Results are Mean ± S.E. of mean.

It appeared, therefore, that angiotensin was more dependent upon the availability of glucose for the initiation of a contraction than was acetylcholine.

(2) The Effect of Removing Glucose from the Tyrode Solution

Since a lowering of glucose concentration produced a preferential reduction of the angiotensin response, the effect of complete glucose removal was investigated with respect to time. To avoid any possible effects due to changes in osmolarity of the Tyrode solution, mannitol (5.6 mmol) was substituted for glucose. It was found that during a 20 minute exposure to glucose free Tyrode solution, there was a consistent difference between the percentage reduction of the responses to acetylcholine compared with those to angiotensin (see fig. 1). After 10 minutes the angiotensin response was reduced by 29.0 ± 8.1% (n=6) and after 20 minutes by 72.4 ± 5.4% (n=6), while the corresponding reductions in the acetylcholine response were 0.7 ± 3.5% (n = 6) and 34.1 ± 8.8% (n=6) respectively. The difference between the percentage reductions of the acetylcholine responses compared with the percentage reduction of the angiotensin responses was significant throughout the exposure to glucose free solution ($p < 0.01$), and also during the first ten minutes of recovery.

(3) Inhibition of Oxidative Metabolism

Glucose is a readily utilised energy source for smooth muscle contraction and thus the greater dependence of the angiotensin response upon glucose availability compared with the acetylcholine response might be due to an additional energy requirement for the angiotensin contraction. To investigate further the possible energy dependence of the angiotensin response, ileal preparations were exposed to conditions which prevented energy production by oxidative metabolism.

a. Nitrogen Induced Anoxia

Figure 2 shows the percentage reductions of acetylcholine and

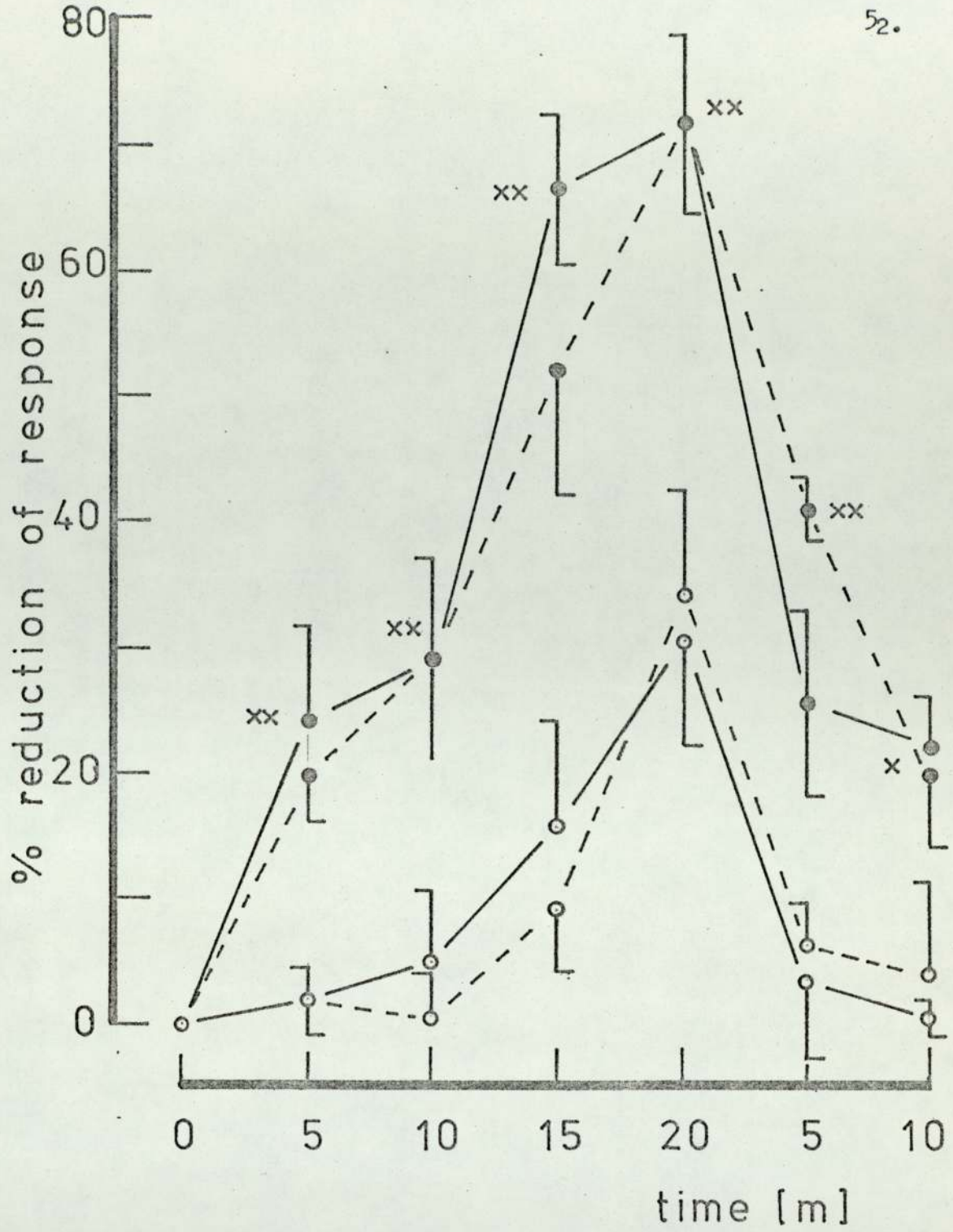

FIGURE 1. The effect of a 20 minute exposure to glucose free Tyrode solution upon responses of guinea-pig ileum to angiotensin and acetylcholine. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean. Solid lines denote the inhibition pattern produced by glucose removal and broken lines that produced when glucose was replaced by an osmo-equivalent of mannitol.

xx : $0.001 < p < 0.01$ x : $0.01 < p < 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

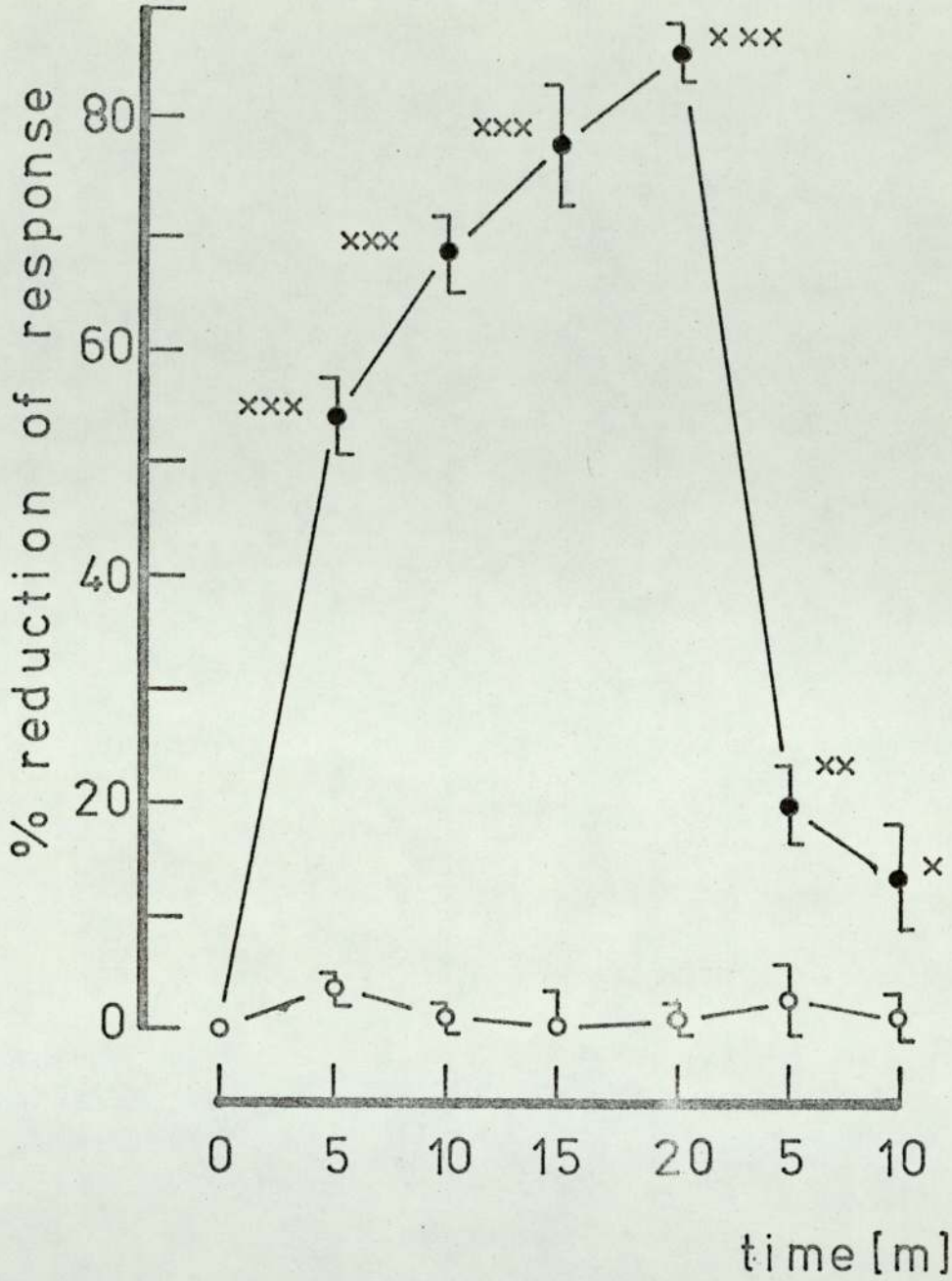

FIGURE 2. The effect of a 20 minute exposure to nitrogen-induced anoxia upon responses of guinea-pig ileum to angiotensin and acetylcholine. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

xxx : $p < 0.001$ xx : $0.001 < p < 0.01$ x : $0.01 < p < 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

angiotensin responses which occurred when preparations of guinea-pig ileum were suspended in oxygen free Tyrode and gassed with nitrogen. There is a marked difference between the percentage reductions of the angiotensin and acetylcholine responses which is highly significant ($p < 0.001$) throughout the 20 minute exposure to anoxic conditions. The sensitivity of the angiotensin response to oxygen deprivation contrasted with the absence of any consistent effect upon the acetylcholine response. Thus after 10 minutes the angiotensin response was reduced by 68.9± 3.7% (n=5) and after 20 minutes by 86.3± 2.7% (n=5), while the corresponding reductions of the acetylcholine response were 0.6± 1.6% (n=5) and 0.7± 1.6% (n=5) respectively.

b. Carbon Monoxide

Figure 3 shows the percentage reductions of the angiotensin and acetylcholine responses which occurred during a 16 minute exposure of ileal preparations to carbon monoxide, a specific inhibitor of the electron transport chain. The angiotensin response was reduced by 47.6± 6.5% (n=6) after 9 minutes and by 66.6± 9.4% (n=6) after 16 minutes whereas small but consistent percentage increases in the response to acetylcholine were observed throughout the exposure to carbon monoxide. The difference between the percentage reductions of the two agonists was at all times highly significant ($p < 0.001$).

These findings with nitrogen and carbon monoxide suggested that oxidative pathways of metabolism might be involved in the mechanism by which angiotensin elicited a contraction of smooth muscle.

(4) The Effect of Hyoscine

In all the preceding experiments there was a marked difference in the percentage reductions of the angiotensin and acetylcholine evoked contractions. However, the angiotensin response of guinea-pig ileum is known to consist of two components. Therefore , hyoscine

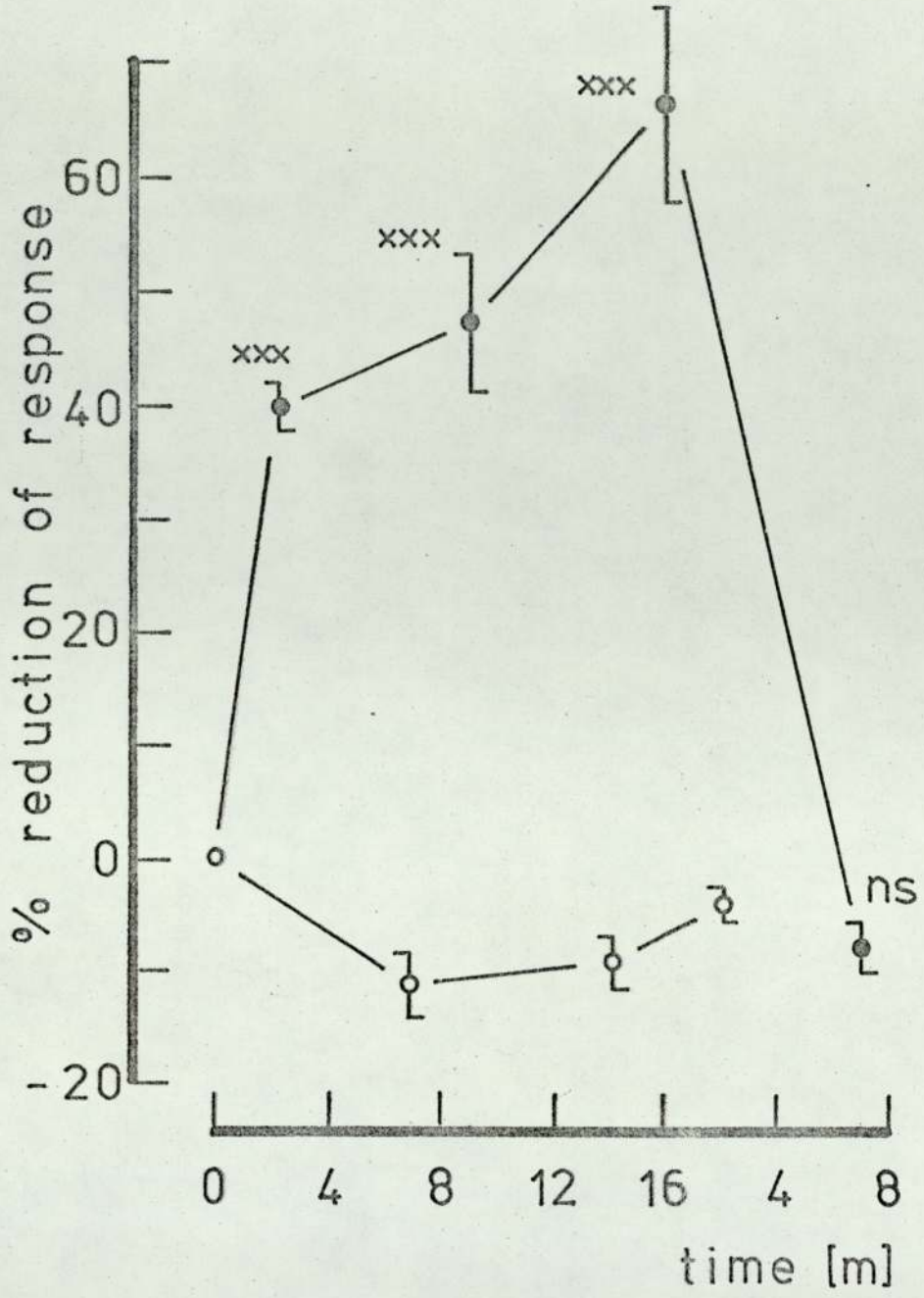

FIGURE 3. The effect of a 16 minute exposure to carbon monoxide upon the responses of guinea-pig ileum to angiotensin and acetylcholine. Mean % reduction of the responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

xxx: $p < 0.001$ ns: $p > 0.05$ when comparing the percentage reduction of the acetylcholine response with that of the immediately preceding angiotensin response.

hydrobromide was added to the Tyrode solution in order to block the indirect, cholinergic component of the response. The effect of glucose lack upon the remaining direct component was then investigated. Figure 4 shows the percentage reductions of angiotensin responses during a 30 minute exposure to glucose free Tyrode containing 2×10^{-7} mol hyoscine hydrobromide. This concentration was sufficient to abolish contractions produced by acetylcholine when added in four times the concentration necessary to elicit a response equal to the angiotensin control response. Histamine was used as a control in these experiments with hyoscine and the corresponding reductions of these responses are also shown in figure 4. It may be seen that the direct component of the angiotensin response was very sensitive to glucose lack, and was reduced by 29.5 ± 3.6% (n=8) after 10 minutes and by 86.7 ± 3.0% (n=8) after 30 minutes. Responses to histamine were only slightly reduced, and decreased by 14.2 ± 2.9% (n=6) after 30 minutes. The difference in the percentage reductions of the two agonists was highly significant ($p < 0.001$) throughout the phase of glucose absence and also during the first 30 minutes of recovery.

(5) Transmural Electrical Stimulation and Glucose Lack

Table 2 shows the percentage reductions of responses to sustained electrical stimuli when ileal preparations were exposed to glucose free Tyrode solution for 30 minutes. The percentage reduction was 7.0 ± 1.9% (n=5) after 10 minutes, and increased to 20.3 ± 4.2% (n=5) after 30 minutes. Co-axial electrodes of the type used by Paton (1955) were employed and the responses elicited are reported to be due to stimulation of post-ganglionic, para-sympathetic nerve fibres (Paton, 1955; Day & Vane, 1966). This was confirmed by the use of hyoscine hydrobromide. At a concentration of 2×10^{-7} mol responses to electrical stimulation were reduced by 80 to 90% which demonstrated

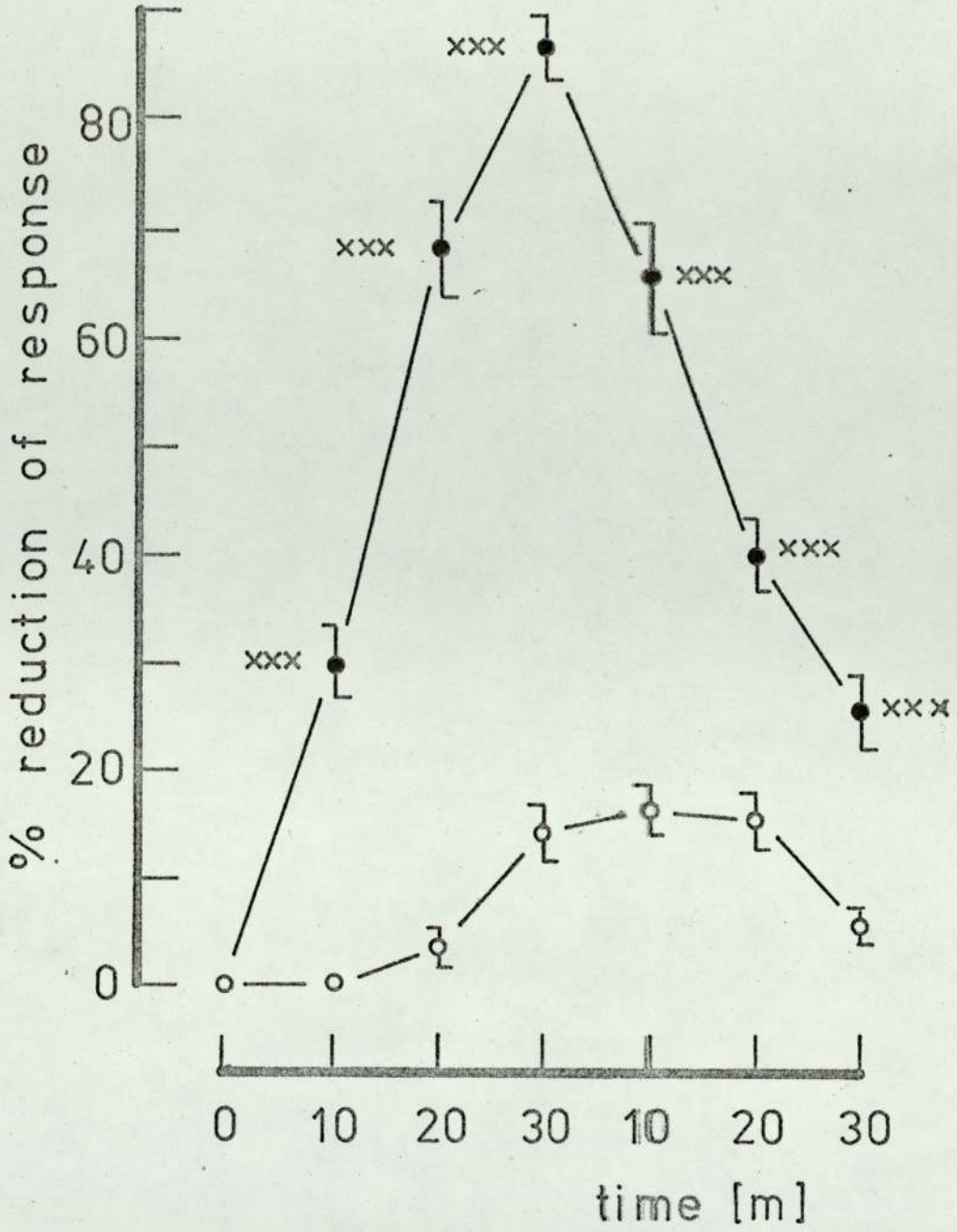

FIGURE 4. The effect upon the responses of guinea-pig ileum to histamine and angiotensin, of a 30 minute exposure to glucose-free Tyrode solution containing 2×10^{-7} mol. hyoscine. Mean % reduction of responses to histamine (O) and angiotensin (●) with the standard error of the mean.

xxx $p < 0.001$ when comparing the percentage reduction of the histamine response with that of the corresponding angiotensin response.

TABLE 2 - Percentage reduction of responses of guinea-pig ileum to co-axial electrical stimulation due to absence of glucose from the Tyrode solution, compared to the percentage reduction of the direct component of the angiotensin response.

Time of Absence of Glucose (m)	% Reduction of electrical response	% Reduction of angiotensin response (direct component)	p
10	7.0 ± 1.9 (7)	29.5 ± 3.6 (8)	< 0.001
20	8.1 ± 2.8 (7)	68.0 ± 4.4 (8)	< 0.001
30	20.3 ± 4.2 (7)	86.7 ± 3.0 (8)	< 0.001

Number of observations in parenthesis.

Results are mean ± S.E. of mean.

that this concentration of inhibitor was sufficient to block endogenously released acetylcholine as well as exogenously applied acetylcholine.

The difference between the percentage reductions of the electrical responses and those of the direct component of the angiotensin response (see previous section) were highly significant ($p < 0.001$) throughout the phase of glucose absence (Table 2).

B. RAT DESCENDING COLON AND RAT UTERUS

The existence of an indirect nervously mediated component to the angiotensin response of guinea-pig ileum was an undesirable complication. Although the direct component may be separated by the use of muscarinic blocking agents it is difficult to control effects due to lack of specificity of these agents. Therefore, these investigations into the energy dependence of the angiotensin contraction were extended to two preparations where the action of angiotensin has been shown to be wholly direct; rat descending colon (Regoli & Vane, 1964; Ellis & Reit, 1962) and rat uterus (Khairallah & Page, 1961; Regoli & Vane, 1964).

(1) The Effect of Glucose Lack and Anoxia, Separately and in Combination

Exposure of preparations of rat descending colon and uterus to glucose free Tyrode solution for periods of up to 2 hours failed to effect their responses to either acetylcholine or angiotensin. Their responses to both agonists were also unaffected by exposure to nitrogen for periods of up to three hours and to carbon monoxide for up to 30 minutes. Since neither glucose lack nor anoxia affected the induced responses, the effect of combining these treatments was investigated.

Figure 5 shows the percentage reductions of angiotensin and acetylcholine responses which occurred during a 30 minute exposure of rat descending colon to glucose free Tyrode and anoxia. There was a progressive reduction of responses to both agonists but at no time was there a significant difference between the percentage reduction of the angiotensin and acetylcholine responses. After 30 minutes the percentage reduction of the angiotensin response was 87.5 ± 4.9 ($n=9$) and that of the acetylcholine response was 76.9 ± 8.1 ($n=9$) which suggested a marked impairment of the contractile process.

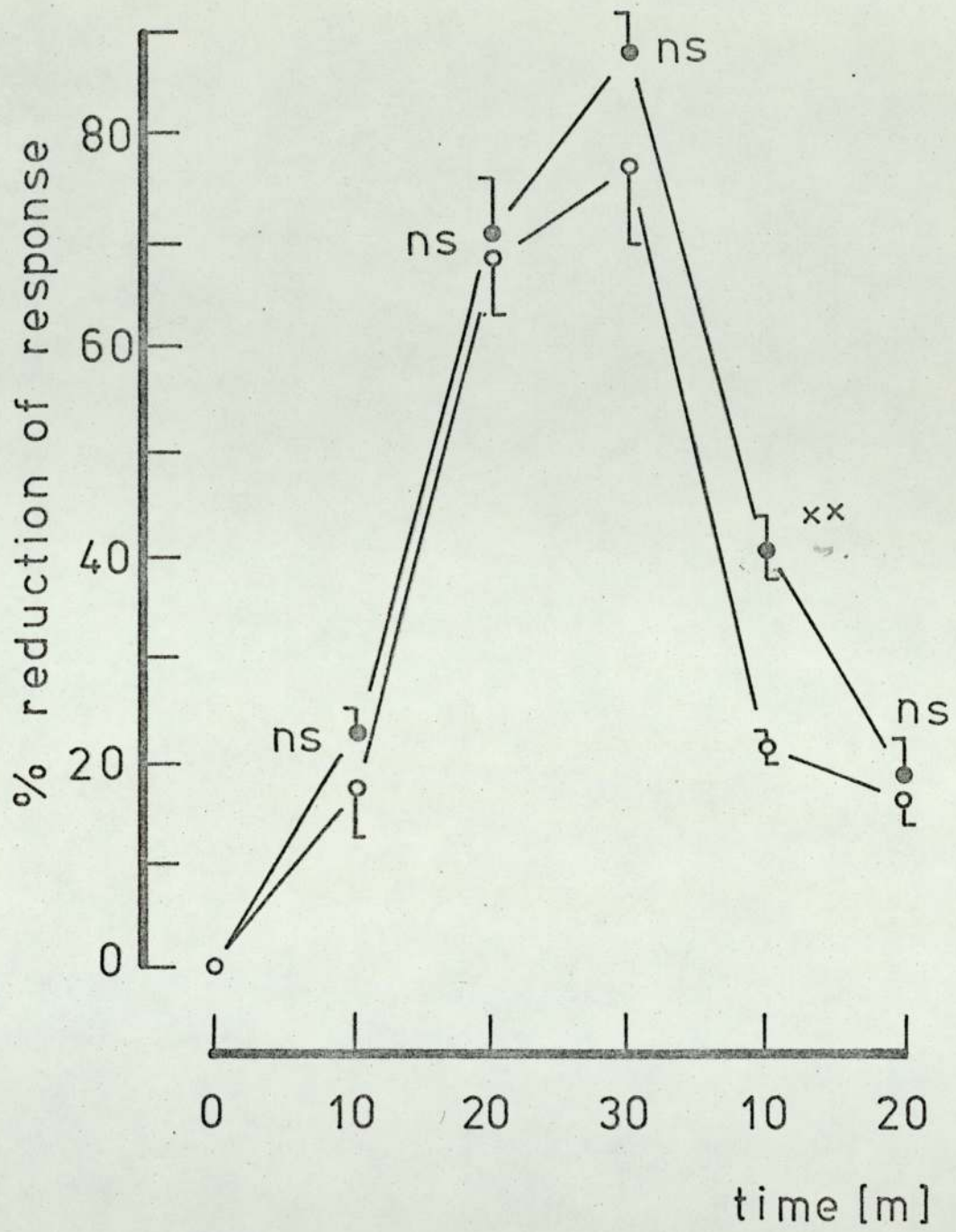

FIGURE 5. The effect of a 30 minute exposure to glucose-free Tyrode solution and anoxia upon responses of rat descending colon to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

xx $0.001 < p < 0.01$ ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

Figure 6 shows the percentage reductions of the angiotensin and acetylcholine responses which occurred during a 45 minute exposure of rat dioestrous and oestrous uterus to glucose free Tyrode solution and anoxia. With dioestrous uterus the results were similar to those obtained with descending colon (fig. 5). There was a progressive reduction of responses to both agonists with no significant difference between the percentage reduction of the angiotensin responses and the acetylcholine responses. After 40 minutes, acetylcholine responses were reduced by 85.8 ± 4.6% and after 45 minutes angiotensin responses were reduced by 86.9 ± 4.6% (n=6). However, using the same conditions, the response of oestrous uterus to either angiotensin or acetylcholine was unaffected. Prolongation of exposure times for up to five hours failed to affect the ability of this tissue to respond to either of the two agonists.

(2) Measurement of Tissue Glycogen Content

Although there was no difference in the responses of colon and uterus to acetylcholine compared with angiotensin during metabolic inhibition there was a difference between the responses of oestrous and dioestrous uterus which was significant for both angiotensin ($p < 0.001$) and acetylcholine ($p < 0.001$). (see fig. 6) Since under these conditions of metabolic inhibition the tissue was dependent upon energy derived from anaerobic glycolysis of endogenous substrates, the difference in the responses of oestrous and dioestrous uterus might be due to differences in the levels of tissue glycogen.

Figure 7 shows the glycogen content of rat descending colon and rat uterus measured by the method of Lo, Russel & Taylor (1970). Oestrous uterus contained 252.0 ± 5.0 mg glycogen / 100g wet weight of tissue (n=46) and this was significantly higher than the content of dioestrous uterus, 147.0 ± 5.0 mg / 100g wet weight of tissue (n=30).

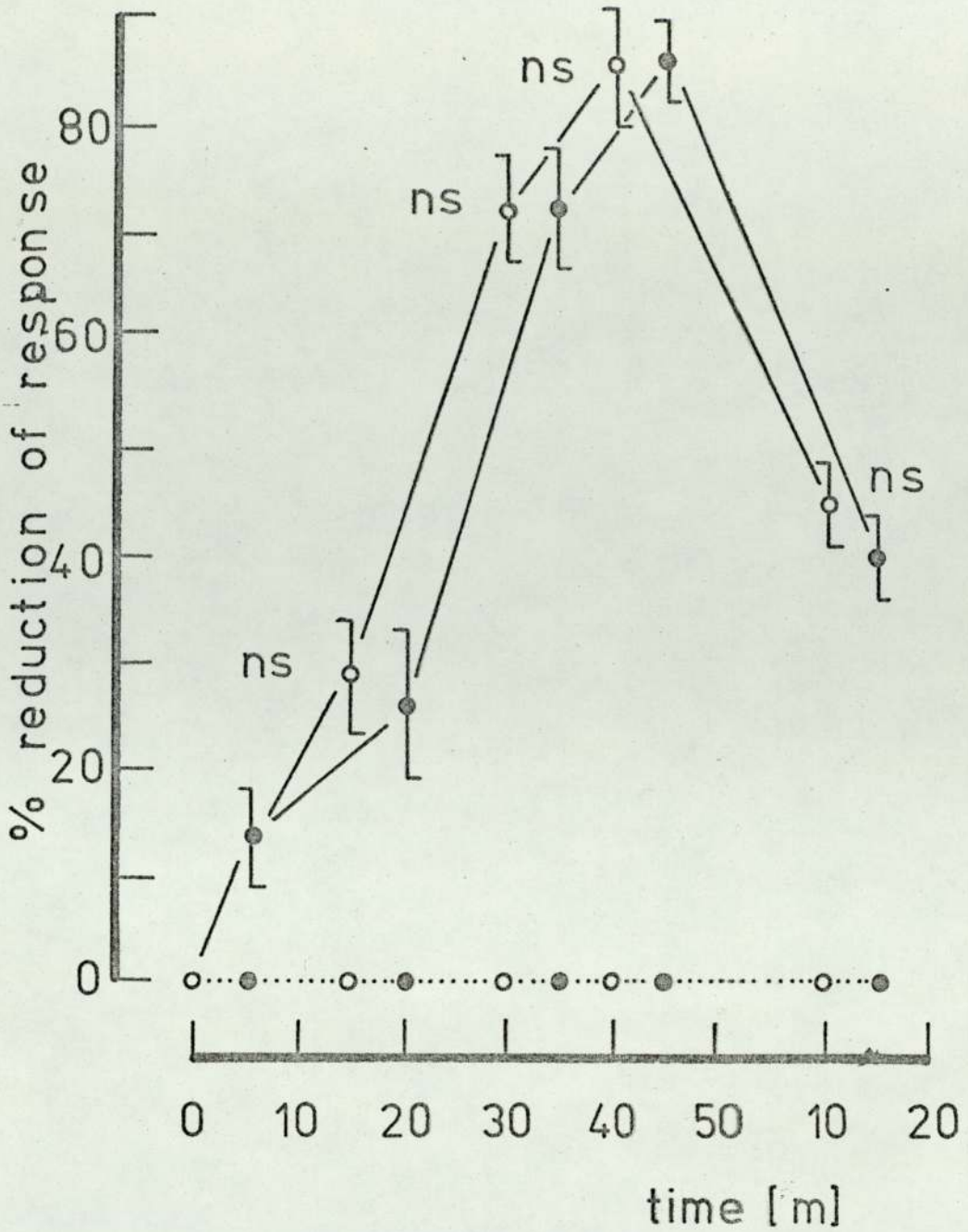

FIGURE 6. The effect of a 45 minute exposure to glucose-free Tyrode solution and anoxia upon responses of rat uterus to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean. Solid lines denote responses of dioestrous uterus and broken lines responses of oestrous uterus.

ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately preceding acetylcholine response.

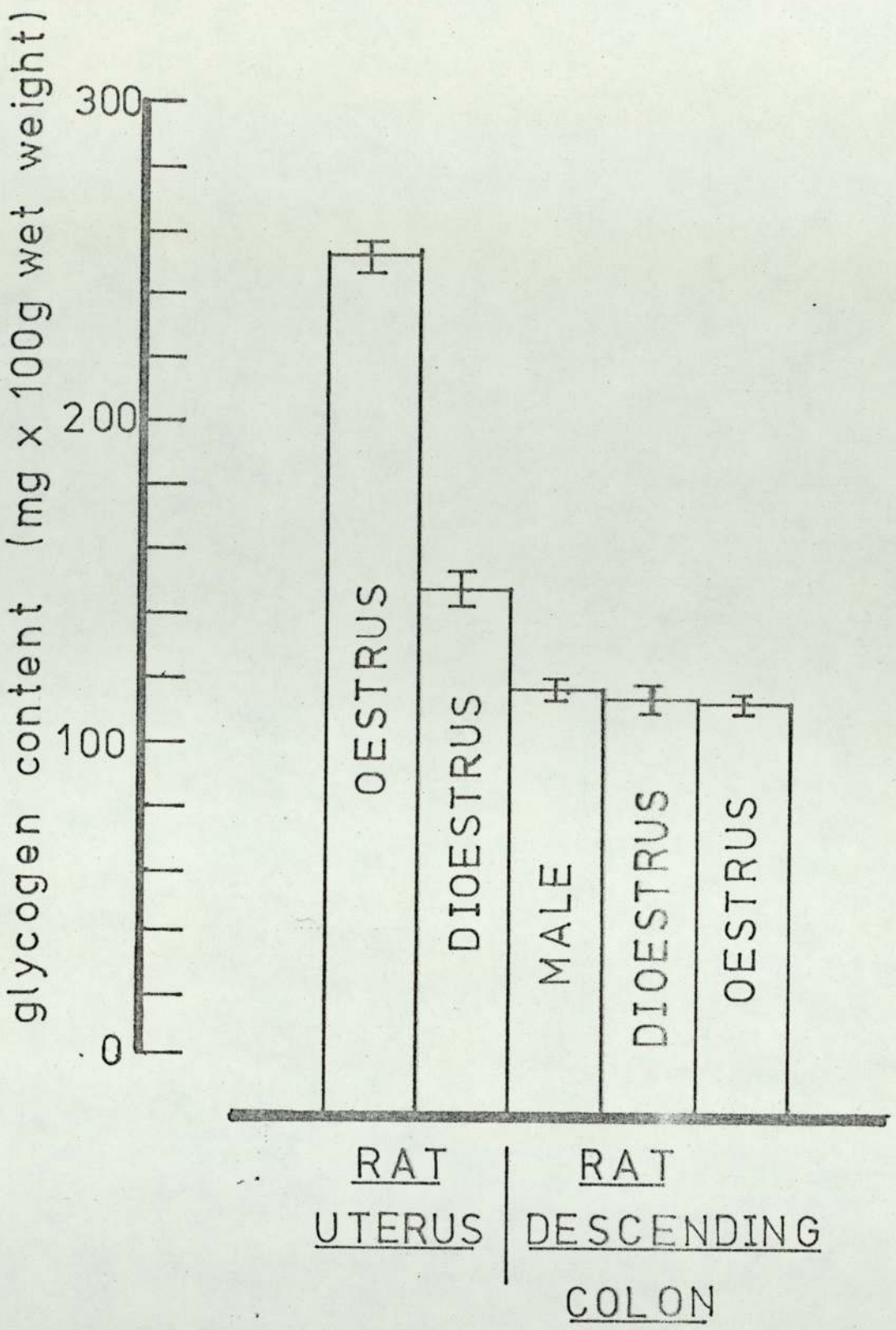

FIGURE 7. The glycogen content of rat descending colon and rat uterus at oestrus and dioestrus. The height of each column denotes the mean glycogen content with the standard error of the mean indicated by brackets.

There was no significant difference between the glycogen content of descending colon taken from male rats, 115.0 ± 3.0 mg / 100g wet weight tissue (n=15), from oestrous rats, 112.0 ± 3.0 mg / 100g wet weight tissue (n=46), or from dioestrous rats, 113.0 ± 2.0 mg / 100g wet weight tissue (n=48).

(3) The Effect of 2,4-Dinitrophenol

To investigate further the importance of anaerobic glycolysis as an energy yielding process, experiments were performed using a Tyrode solution containing 5.6 mmol glucose as substrate and 0.1 mmol 2,4-dinitrophenol. This concentration of 2,4-dinitrophenol has been shown to be effective in uncoupling oxidative phosphorylation in rat uterine tissue (Rangachari, Paton & Daniel, 1972).

Segments of descending colon from oestrous and dioestrous rats were exposed to this solution for 25 minutes and responses to acetylcholine and angiotensin were recorded. There was no difference in the responses obtained from dioestrous compared with oestrous preparations but in both the percentage reduction of the angiotensin response was significantly greater ($p < 0.001$) than that of the acetylcholine response. Figures 8a and 8b show respectively the rapid increase in the percentage reductions of the responses to both agonists when dioestrous and oestrous colon were exposed to 0.1 mmol 2,4-dinitrophenol for up to 25 minutes. After 5 minutes exposure to 2,4-dinitrophenol, the angiotensin response was reduced by 75.2 ± 4.1% (n=5) and the acetylcholine response was reduced by 16.6 ± 4.8% (n=5). The difference between the percentage reduction of the angiotensin response and that of the acetylcholine response was highly significant ($p < 0.001$). Although at 15 minutes both responses were markedly reduced and the angiotensin response almost abolished, there was still a significant difference ($p < 0.001$) between the percentage

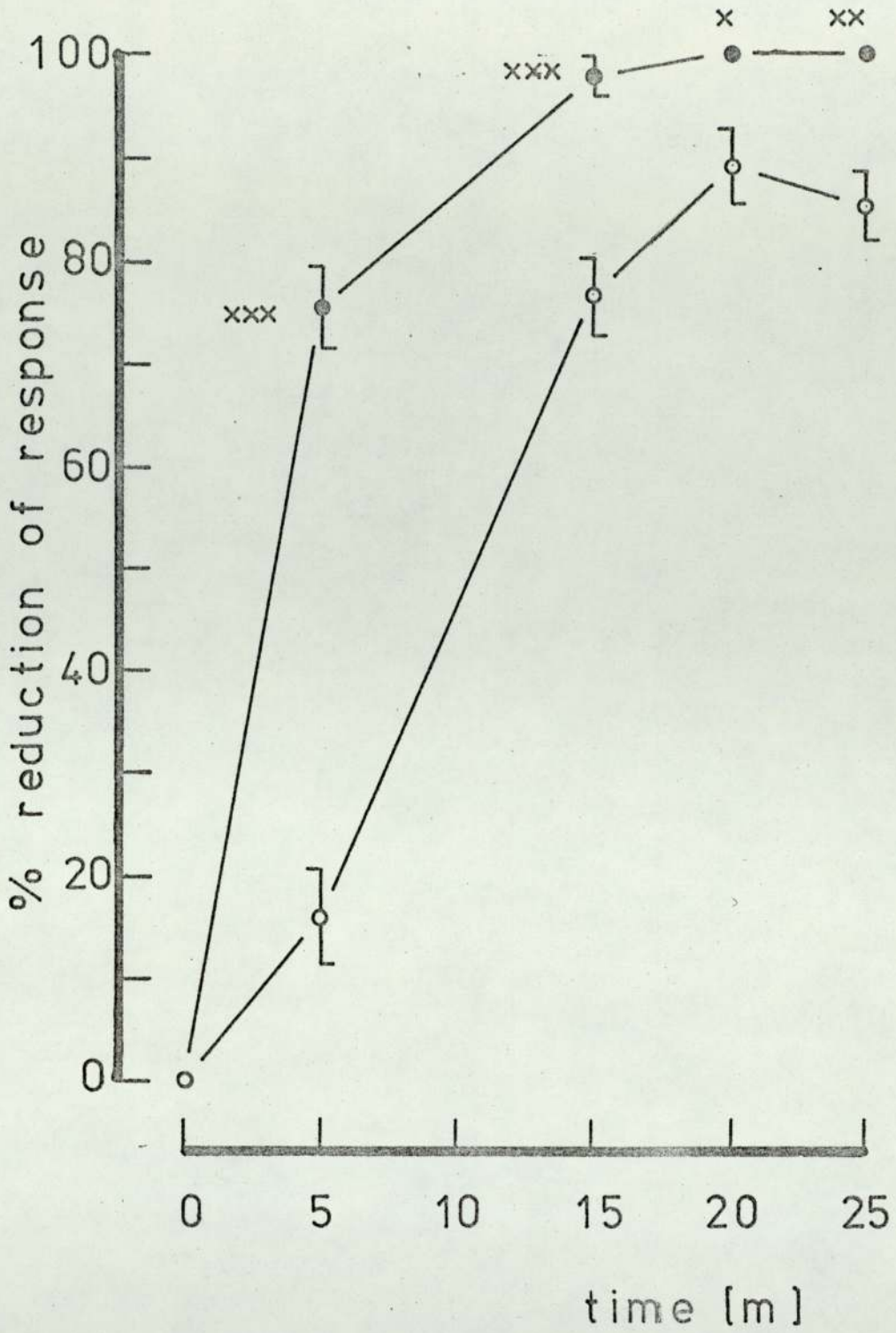

FIGURE 8a. The effect of 0.1 mmol 2,4-dinitrophenol upon the responses of dioestrous rat colon to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

xxx : $p < 0.001$ xx : $0.001 < p < 0.01$ x : $0,01 < p < 0.05$ when comparing the percentage reduction of acetylcholine and angiotensin responses.

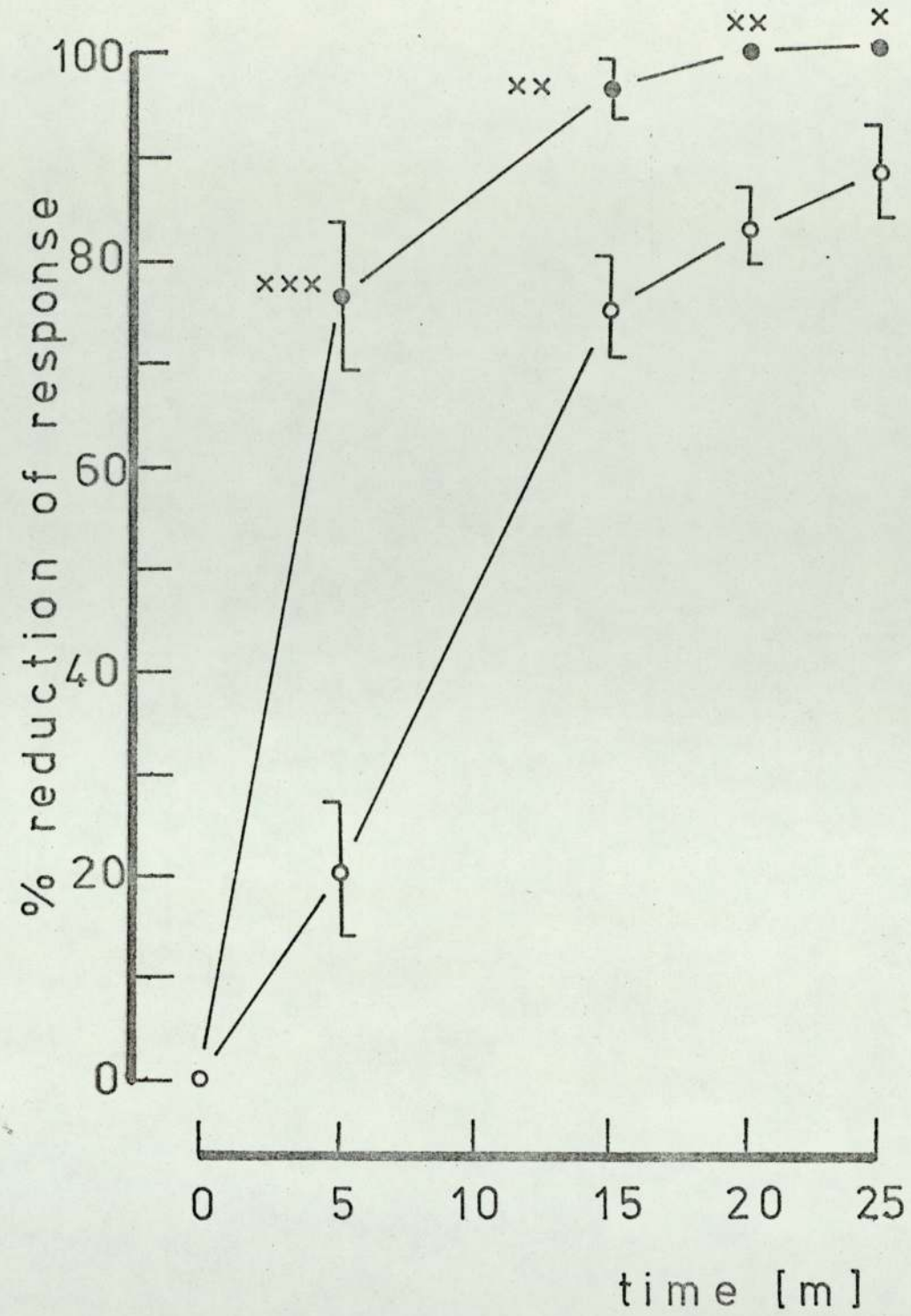

FIGURE 8b. The effect of 0.1 mmol 2,4-dinitrophenol upon the responses of oestrous rat colon to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

xxx : $p < 0.001$ xx : $0.001 < p < 0.01$ x: $0.01 < p < 0.05$ when comparing the percentage reduction of acetylcholine and angiotensin responses.

reduction of the angiotensin response, 98.3 ± 1.6%(n=5), and that of the acetylcholine response, 77.4 ± 4.1% (n=5).

When a lower concentration of 2,4-dinitrophenol (0.05 mmol) was used maximal inhibition occurred within 30 minutes of exposure. The percentage reductions of the angiotensin and acetylcholine responses of dioestrous and oestrous colon produced by this solution are shown in table 3. In both cases, the percentage reduction of the angiotensin response was significantly greater than that of the acetylcholine response ($p < 0.001$).

Figures 9a and 9b show respectively the results obtained when dioestrous and oestrous uterus were exposed to 0.1 mmol 2,4-dinitrophenol. The responses of dioestrous uterus to both angiotensin and acetylcholine were progressively and rapidly reduced during exposure to 24-dinitrophenol but at all times the reduction of the angiotensin response was significantly greater ($p < 0.001$) than that of the acetylcholine response. The rate of reduction of the responses was slower in dioestrous uterus than in descending colon and complete abolition of the angiotensin response on dioestrous uterus was obtained after 35 minutes which compared with 15 minutes for colon.

After 5 minutes the acetylcholine response was reduced by 1.4 ± 0.8% (n=5) and after 20 minutes by 10.2 ± 1.4% (n=5) while the subsequent angiotensin responses were reduced by 25.1 ± 2.1% (n=5) and 70.7 ± 8.5% (n=5) respectively.

The responses of oestrous uterus to both agonists (fig. 9) showed an initial rapid reduction followed by a slower reduction so that after 30 minutes exposure to 0.1 mmol 2,4-dinitrophenol the acetylcholine response was reduced by 50.0 ± 3.3% (n=5) and the subsequent angiotensin response (at 35 minutes) was reduced by 65.7 ± 3.4% (n=5). There was no consistent significant difference

TABLE 3 - Percentage reduction of responses of oestrous and dioestrous rat descending colon to angiotensin produced by a 30 minute exposure to Tyrode solution containing 0.05 mmol 2,4-dinitrophenol, compared to the percentage reduction of responses to acetylcholine.

Preparation	% Reduction of Acetylcholine Responses	% Reduction of Angiotensin Responses	P
Oestrous Colon.	34.4 ± 4.3 (9)	75.6 ± 2.4 (10)	<0.001
Dioestrous Colon.	46.9 ± 2.3 (9)	69.2 ± 2.2 (9)	<0.001

Number of observations in parenthesis.

Results are mean ± S.E. of mean.

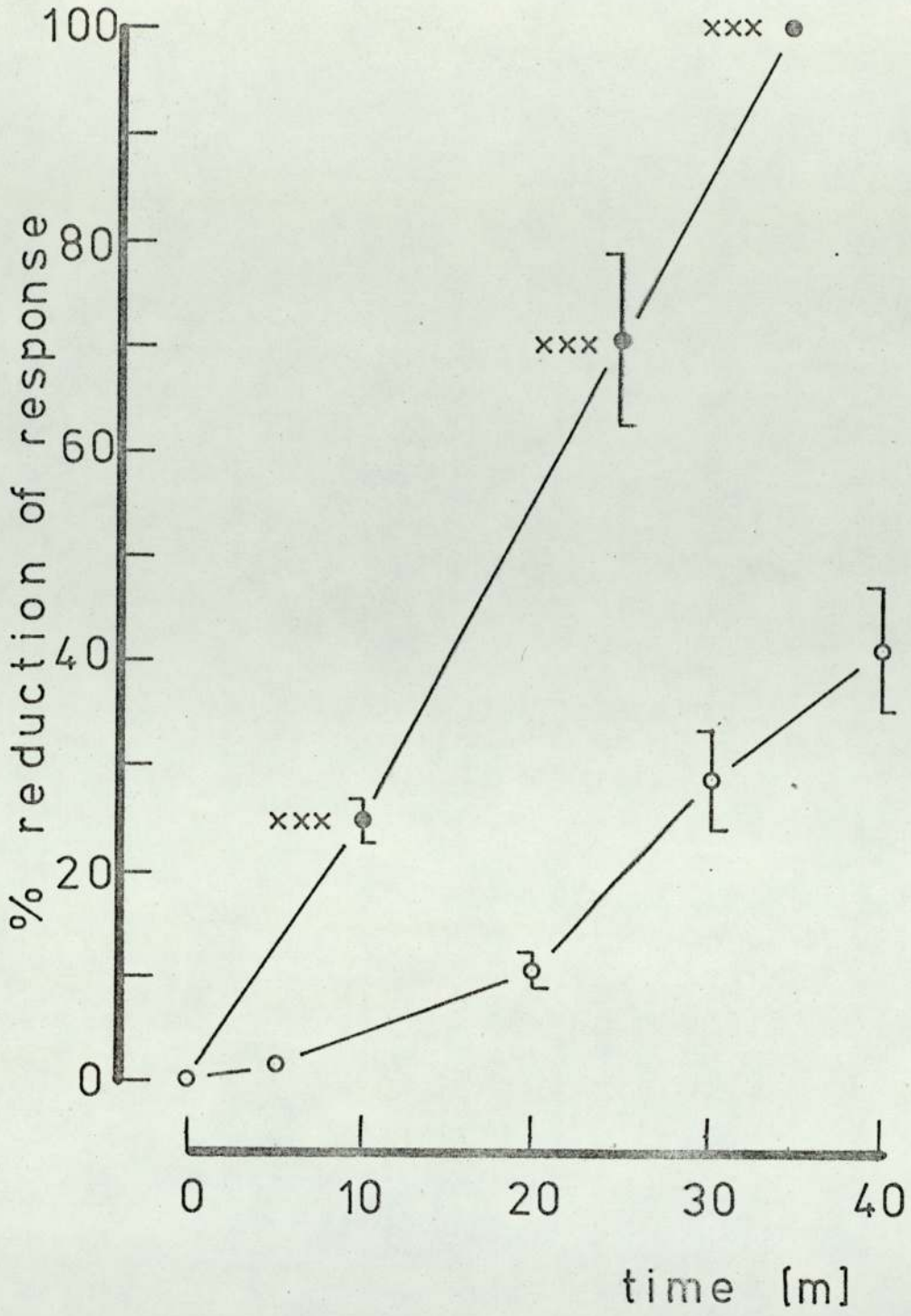

FIGURE 9a. The effect of 0.1 mmol 2,4-dinitrophenol upon responses of dioestrous rat uterus to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

xxx $p < 0.001$ when comparing the percentage reduction of the acetylcholine response with that of the immediately preceding angiotensin response.

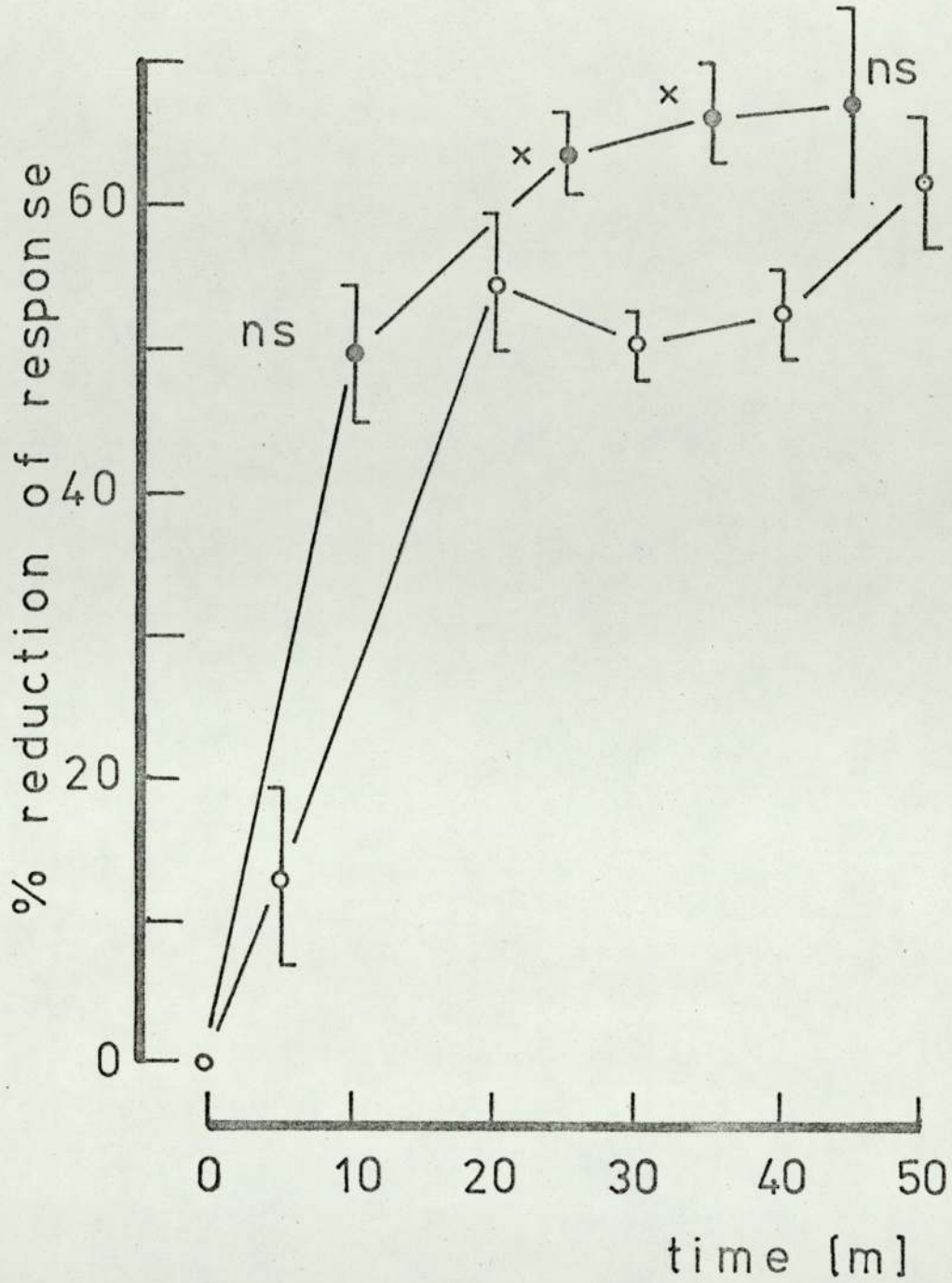

FIGURE 9b. The effect of 0.1 mmol 2,4-dinitrophenol upon responses of oestrous rat uterus to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

x $0.01 < p < 0.05$ ns $p > 0.05$ when comparing the percentage reduction of the acetylcholine response with that of the immediately preceding angiotensin response.

between the percentage reduction of the angiotensin and acetylcholine responses of oestrous uterus during the period of exposure to 0.1 mmol 2,4-dinitrophenol.

SECTION II A STUDY OF THE EFFECT OF METABOLIC INHIBITION UPON THE CONTRACTILE RESPONSES OF RAT DESCENDING COLON TO ANGIOTENSIN AND POTASSIUM (40 MMOL)

It has been reported that metabolic inhibition has a greater effect upon the maintenance of an induced contraction than upon the initial rise in tension (Feldburg & Solandt, 1942; West et al, 1951; Born & Bulbring, 1955; Born, 1956; Eckenfels & Vane, 1972) and it has been suggested that different mechanisms may be involved (Born, 1956). Therefore, in previous experiments contractions to acetylcholine and angiotensin were measured at the maximum sustained level during the drug-tissue contact time. During metabolic inhibition there was a small but variable difference between the maintained acetylcholine response and the initial contraction but there was no detectable change in the maintenance of the angiotensin response. However, the contractile response of isolated smooth muscle preparations to potassium chloride (40 mmol) has been reported to consist of two phases, the second of which being dependent upon energy, is abolished during conditions of metabolic inhibition (Urakawa & Holland, 1964; Pfaffman et al, 1965). Since previous experiments had revealed that the angiotensin response was dependent upon energy, the possible relationship between the angiotensin contraction and the second phase of the potassium contraction was investigated by recording the responses of rat descending colon to potassium under similar experimental conditions.

(a) The Effect of Combined Glucose Removal and Anoxia upon the Potassium Contracture of Rat Descending Colon

Figure 10 shows the percentage reductions of the phasic (fast) and tonic (slow) components of the potassium contracture which occurred during a 30 minute exposure of rat descending colon to glucose free Tyrode and anoxia. The phasic component was the maximum contraction on addition of 40 mmol potassium chloride to the organ bath and the

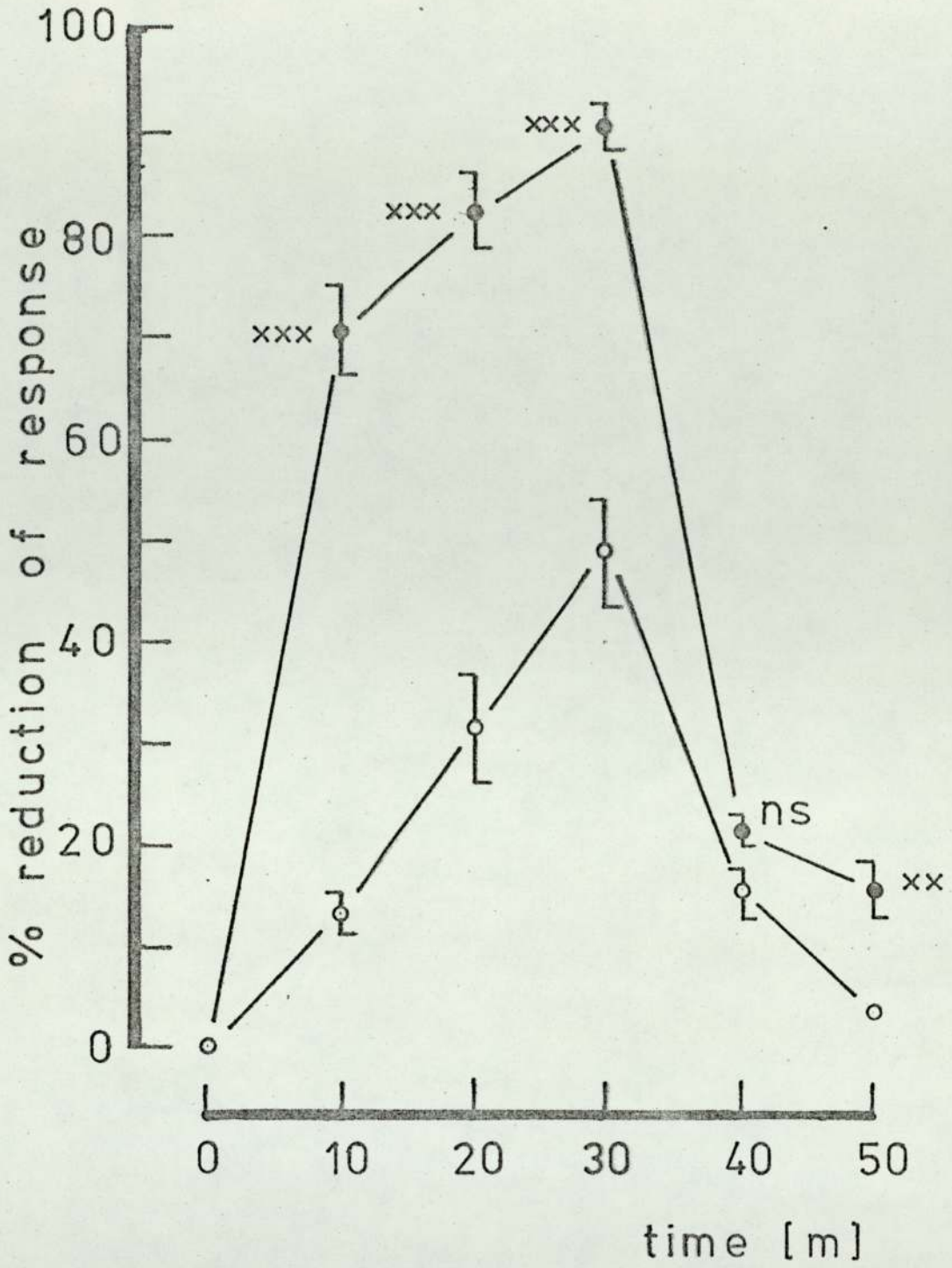

FIGURE 10. The effect of a 30 minute exposure to glucose-free Tyrode solution and anoxia upon responses of male rat descending colon to potassium (40 mmol). Mean % reduction of the phasic (fast) component of the potassium response (●) and the tonic (slow) component of the potassium response (○) with the standard error of the mean.

xxx $p < 0.001$ xx $0.001 < p < 0.01$ ns $p > 0.05$ when comparing the percentage reduction of the tonic component with that of the phasic component.

tonic component was the maintained contraction during the 90 second contact time. The percentage reduction of the tonic component was significantly greater ($p < 0.001$) than the percentage reduction of the phasic component throughout the period of metabolic inhibition. Thus after 20 minutes exposure to glucose free Tyrode and anoxia, the phasic component was reduced by 31.8 ± 5.6% whereas the tonic component was reduced by 82.4 ± 3.7% (n=6).

Table 4 shows the percentage reduction of the tonic potassium response produced by glucose free Tyrode and anoxia compared with the percentage reduction of the angiotensin response when exposed to these conditions. At no time during the exposure of the preparations of rat descending colon to glucose free Tyrode and anoxia was there any significant difference between the percentage reduction of the angiotensin response and that of the tonic potassium response. Thus after 20 minutes exposure to these conditions, the angiotensin response was reduced by 70.6 ± 5.9% (n=6) and the tonic component of the potassium response was reduced by 82.4 ± 3.7% (n=6), and after 30 minutes exposure, the angiotensin response was reduced by 87.5 ± 4.9% (n=6) and the tonic component of the potassium response by 90.8 ± 2.5% (n=6).

(b) The Effect of 2,4-Dinitrophenol upon the Potassium Contractures of Rat Descending Colon

Figure 11 shows the percentage reductions of the phasic and tonic components of the potassium contracture of rat descending colon which occurred during a 35 minute exposure to a Tyrode solution containing 0.1 mmol 2,4-dinitrophenol. Throughout the period of metabolic inhibition, the percentage reduction of the tonic component was significantly greater ($p < 0.01$) than the percentage reduction of the phasic component. Thus, after 15 minutes exposure to 2,4-dinitrophenol the tonic response was reduced by 67.2 ± 6.1% (n=12) and the phasic

TABLE 4 - The percentage reduction of responses of rat descending colon to angiotensin due to exposure to a glucose-free Tyrode solution and anoxia, compared with the percentage reduction of maintained 'tonic' potassium responses.

Length of Exposure (m)	% reduction of angiotensin response	% reduction of maintained potassium response	P.
20	70.6 ± 5.9 (9)	82.4 ± 3.7 (6)	> 0.05 i.e. n.s.
30	87.5 ± 4.9 (9)	90.8 ± 2.5 (6)	> 0.05 i.e. n.s.

Number of observations in parenthesis.

Results are mean ± S.E. of mean.

n.s. - not significant.

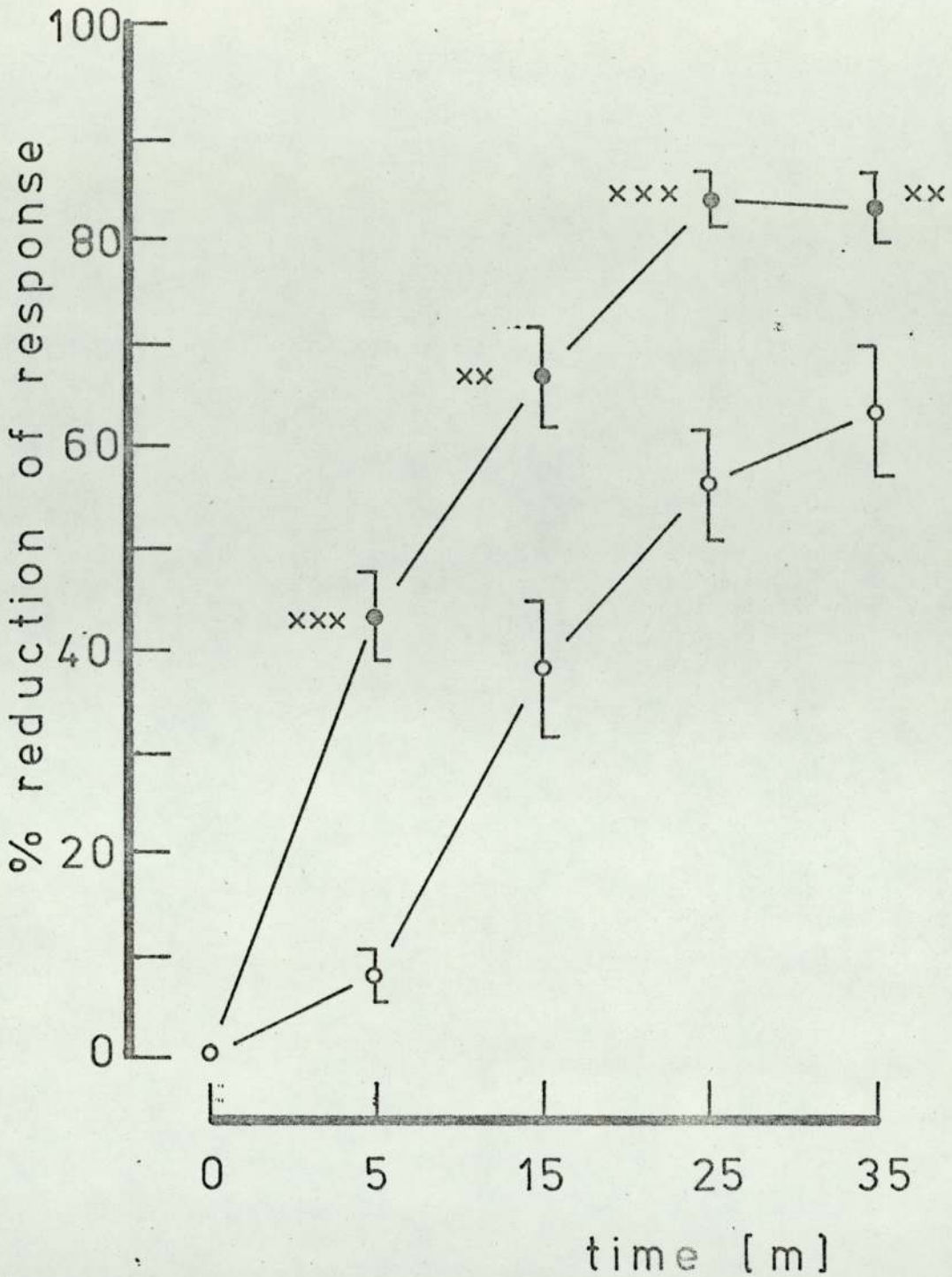

FIGURE 11. The effect of 0.1 mmol 2,4-dinitrophenol upon the responses of male rat descending colon to potassium (40 mmol). Mean % reduction of the phasic (fast) component of the potassium response (●) and the tonic (slow) component of the potassium response (○) with the standard error of the mean.

xxx $p < 0.001$ xx $0.001 < p < 0.01$ when comparing the percentage reduction of the tonic component with that of the phasic component.

response was reduced by 38.4 ± 7.4% (n=12). After 35 minutes of exposure to 2,4-dinitrophenol the percentage reduction of the tonic response increased to 84.0 ± 3.3 (n=10) and the corresponding percentage reduction of the phasic component was 63.6 ± 6 .2 (n=10).

There was a significant difference between the percentage reduction of the tonic potassium response and the angiotensin response during exposure of rat descending colon to 0.1 mmol 2,4-dinitrophenol (Table 5). After 15 minutes the tonic potassium response was reduced by 67.2 ± 6.1% (n=12) while the angiotensin response was reduced by 98.3 ± 1.6% (n=5). After 24 minutes the percentage reduction of the tonic potassium response was 84.6 ± 3.1 (n=12) and the angiotensin response was completely abolished.

It therefore appeared that although the responses to angiotensin and potassium were both energy dependent, the response to angiotensin was more sensitive to inhibition by 2,4-dinitrophenol. This raised the possibility that different mechanisms were involved in the two responses.

(c) The Effect of 2,4-Dinitrophenol upon Prolonged Responses to Angiotensin and Potassium (40 mmol)

To investigate further the relationship between the tonic potassium response and the angiotensin response of rat colon, preparations were exposed to a Tyrode solution containing 0.1 mmol 2,4-dinitrophenol and 3 minute responses to potassium and angiotensin were recorded.

Figure 12 shows the percentage reduction of the phasic and tonic components of the potassium response of rat descending colon produced by an 80 minute exposure to 0.1 mmol dinitrophenol. The percentage reduction of the tonic component was significantly greater than the percentage reduction of the phasic component throughout the period of metabolic inhibition ($p < 0.01$). After 20 minutes the phasic

TABLE 5 - The percentage reduction of responses of rat descending colon to angiotensin due to exposure to Tyrode solution containing 0.1 mmol 2,4-dinitrophenol, compared with the percentage reduction of maintained 'tonic' potassium responses.

Length of exposure (m)	% reduction of angiotensin responses	% reduction of maintained potassium responses	P
15	98.3± 1.6 (5)	67.2± 6.1 (12)	< 0.001
35	100.0 (5)	84.6± 3.1 (12)	< 0.001

Number of observations in parenthesis.

Results are mean ± S.E. of mean.

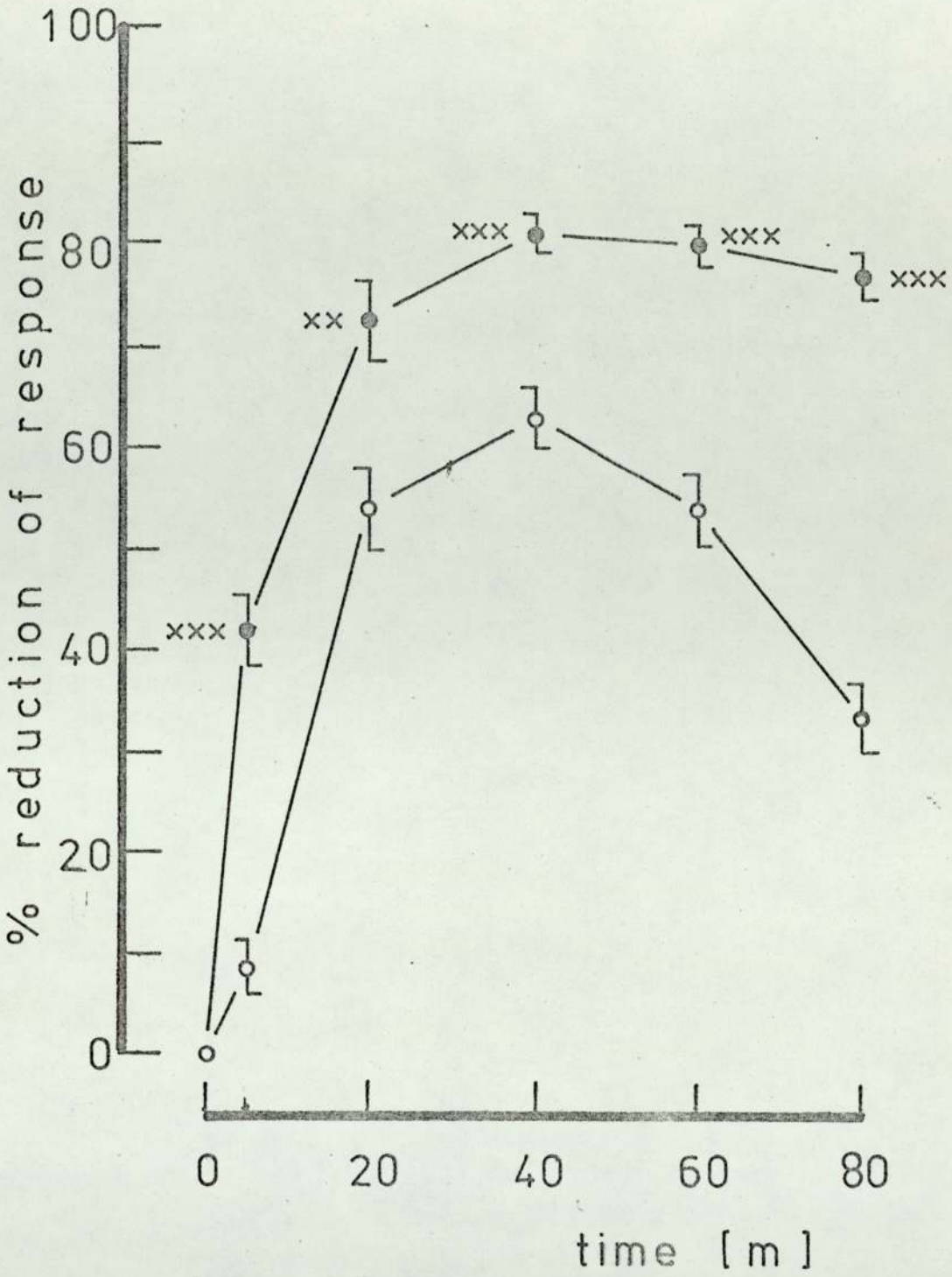

FIGURE 12. The effect of 0.1 mmol 2,4-dinitrophenol upon prolonged responses of 3 minutes duration to potassium (40 mmol). Mean % reduction of the phasic (fast) component of the potassium response (●) and the tonic (slow) component of the potassium response (○) with the standard error of the mean.

xxx $p < 0.001$ xx $0.001 < p < 0.01$ when comparing the percentage reduction of the tonic component with that of the phasic component.

component was reduced by 54.8± 4.2% (n=16) and the tonic component was reduced by 72.9± 4.3% (n=16) and after 40 minutes the percentage reductions were 62.8± 3.1 (n=16) and 81.2± 2.2 (n=16) respectively. Thereafter there was some recovery of both components and after 80 minutes of exposure to 2,4-dinitrophenol the percentage reduction of the phasic component was 37.1± 3.7% (n=16) and the percentage reduction of the tonic component was 77.3± 2.3 (n=16).

Table 6 shows the percentage reduction of responses of three minutes duration to angiotensin which occurred during exposure of preparations of rat descending colon to a Tyrode solution containing 0.1 mmol 2,4-dinitrophenol. The contraction of this tissue to angiotensin is slow and is characterised by a lag period of 20 to 30 seconds before the response is initiated. There was no evidence of any dissociation of the angiotensin contraction into two components during the exposure to 2,4-dinitrophenol and once the maximum contraction was achieved, it was maintained throughout the exposure to angiotensin. Exposure of preparations to 2,4-dinitrophenol caused a progressive reduction of responses to angiotensin. After 20 minutes, the angiotensin response was reduced by 97.6± 1.7% (n=5) and after 40 minutes it was completely abolished. Table 6 compares the percentage reduction of the angiotensin response produced by 2,4-dinitrophenol with the percentage reduction of the tonic phase of the potassium response. Throughout the phase of metabolic inhibition, the percentage reduction of the angiotensin response was significantly greater than the corresponding percentage reduction of the tonic potassium response ($p < 0.01$). Thus after 20 minutes exposure to 2,4-dinitrophenol the angiotensin response was reduced by 97.6± 1.7% (n=5) and the tonic component of the potassium response was reduced by 72.9± 4.3% (n=16). These results confirmed the earlier findings with responses of 90 second duration to angiotensin and potassium

TABLE 6 - The percentage reduction of 3 minute long angiotensin responses of rat descending colon due to exposure to Tyrode solution containing 0.1 mmol 2,4-dinitrophenol, compared with the percentage reduction of the maintained 'tonic' phase of 3 minute long potassium responses.

Length of exposure (m)	% reduction of angiotensin response	% reduction of potassium response (maintained phase)	P.
20	97.6 ± 1.7 (5)	72.9 ± 4.3 (16)	<0.01
40	100.0 (5)	81.2 ± 2.2 (16)	<0.001
60	100.0 (5)	80.4 ± 1.8 (16)	<0.001
80	100.0 (5)	77.3 ± 2.3 (16)	<0.001

Number of observations in parenthesis.

Results are mean ± S.E. of mean.

and it therefore appeared that different mechanisms might be responsible for the energy dependence of the tonic potassium response and the angiotensin response.

(d) The Interaction of Angiotensin and 40 mmol Potassium on Rat Descending Colon

When angiotensin was added to rat colon during a maintained tonic potassium response in normal Tyrode solution, there was a further contraction of the tissue. After 15 minutes exposure to glucose free Tyrode solution and anoxia the tonic potassium response was reduced by 91.8± 0.6% (n=6) and addition of angiotensin failed to elicit a further contraction. Thus conditions which abolished the maintenance of the tonic potassium response also abolished the angiotensin response. The percentage reduction of the tonic potassium response after exposure to glucose free Tyrode and anoxia was significantly greater ($p < 0.001$) than the percentage reduction of the phasic potassium response, 6.7± 2.2% (n=6), which confirmed earlier findings.

In further experiments, preparations of rat descending colon were exposed to a Tyrode solution containing a sub-contractor concentration of angiotensin (10^{-9}mol) and responses to potassium were elicited. There was an increase in both the tonic and phasic components of the potassium contracture compared with control responses in normal Tyrode. The tonic response was increased by 9.4± 2.2% (n=15) and the phasic response was increased by 3.7± 3.2% (n=15) when preparations were exposed to the Tyrode solution containing angiotensin. However, there was no significant difference ($p > 0.05$) between the effect on the tonic response and the effect on the phasic response.

SECTION III THE ROLE OF PROSTAGLANDINS AND CYCLIC AMP IN THE CONTRACTILE ACTION OF ANGIOTENSIN UPON SMOOTH MUSCLE

Many peptide hormones act through a secondary messanger and there have been several reports that angiotensin acts in this way. Such a mechanism could explain the energy dependence of the angiotensin response of smooth muscle observed in the preceding experiments since formation of cyclic AMP or prostaglandins is an energy requiring process. The possible role of cyclic AMP and prostaglandins in the contractile action of angiotensin was therefore investigated.

A. The Role of Prostaglandins

The non-steroidal anti-inflammatory agent indomethacin has been shown to be a potent inhibitor of prostaglandin synthesis (reviews by Vane, 1971; Ferreira, Moncada & Vane, 1971; Flower & Vane, 1974; Flower, 1974) and in concentrations of 3-6 μmol it inhibits prostaglandin synthesis in isolated smooth muscle preparations (Eckenfels & Vane, 1972; Flower, 1974). The effect of this agent was therefore investigated upon responses of rat colon and uterus to angiotensin and acetylcholine.

(1) The Effect of Indomethacin upon Responses of Rat Descending Colon to Angiotensin and Acetylcholine

Exposure of preparations of rat descending colon to a Tyrode solution containing 3 μmol indomethacin had no consistent effect upon responses to angiotensin or acetylcholine. Increasing the concentration to 6 μmol, however, produced a small but significant reduction of the responses to both agonists. Following a 30 minute equilibration in Tyrode solution containing 6 μmol indomethacin, the responses to angiotensin were reduced by 13.6 $\pm$ 2.2% (n=6) and responses to acetylcholine were reduced by 12.8 $\pm$ 3.6% (n=6). There was no significant difference between these percentage reductions ($P > 0.05$). This concentration of indomethacin had no effect upon the spontaneous activity of the smooth muscle preparations and failed to affect the

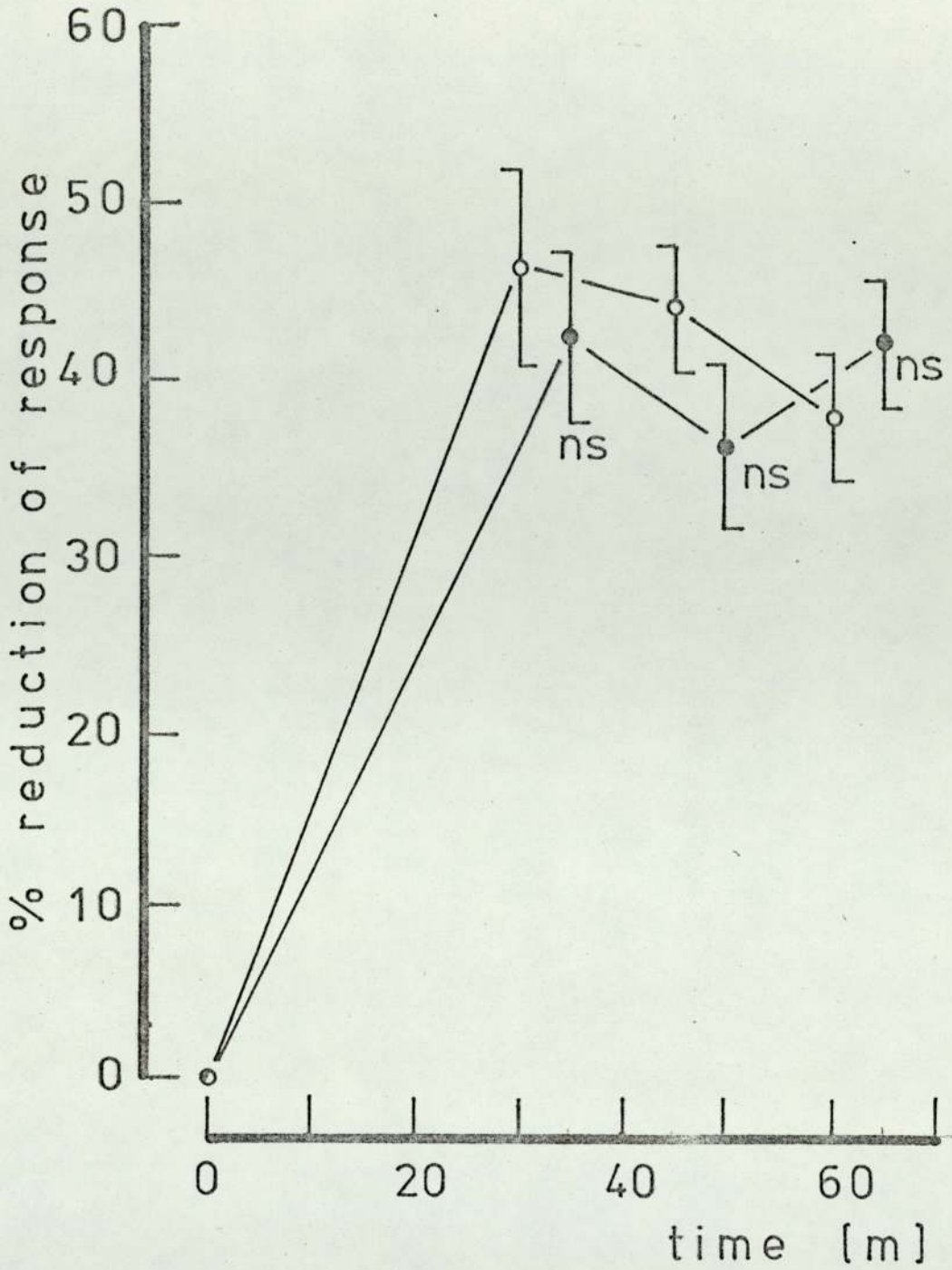

FIGURE 13. The effect of 30 μmol indomethacin upon the responses of male rat descending colon to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine with the standard error of the mean.

ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

maintenance of the induced responses.

When the concentration of indomethacin was increased to 30 μmol the percentage reduction of responses to acetylcholine and angiotensin was increased (see fig. 13). Following a 30 minute equilibration in Tyrode solution containing indomethacin, contractions to acetylcholine and angiotensin were evoked alternately. There was no significant difference between the percentage reduction of the angiotensin responses and the immediately succeeding acetylcholine responses throughout the exposure to indomethacin. Thus after 35 minutes exposure to indomethacin the angiotensin response was reduced by 42.5 ± 5.0% (n=6) and the 45 minute acetylcholine response was reduced by 44.1 ± 3.5% (n=6). The effect of indomethacin was not progressive and the percentage reduction of the acetylcholine response after 65 minutes was not significantly different from the percentage reduction after 30 minutes.

When the concentration of indomethacin was increased to 60 μmol there was no further reduction of responses of rat descending colon to acetylcholine and angiotensin compared with the reductions produced by 30 μmol indomethacin (see fig. 14). Tissues were equilibrated with the solution for 30 minutes before responses to acetylcholine and angiotensin were recorded alternately. There was no significant difference between the percentage reductions of the angiotensin responses and the immediately succeeding acetylcholine responses throughout the exposure to indomethacin. Thus after 35 minutes the angiotensin response was reduced by 36.5 ± 1.0% (n=6) and the 45 minute acetylcholine response was reduced by 39.2 ± 5.9% (n=6) ($p > 0.05$). This concentration of indomethacin abolished all spontaneous activity but had no effect upon the maintenance of induced responses. The reduction of the acetylcholine and angiotensin responses was only slowly progressive and after 60 minutes the percentage reduction of the acetylcholine

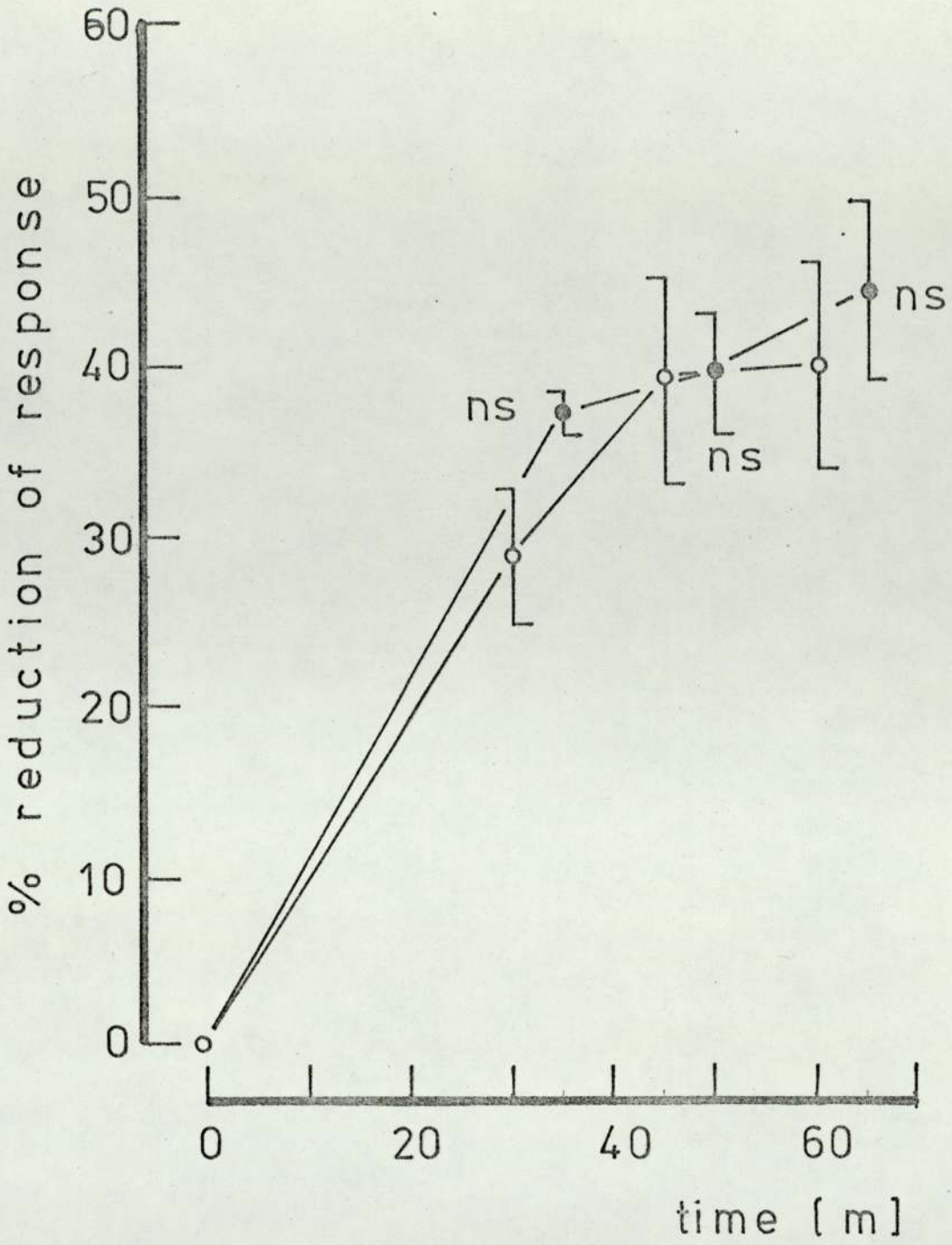

FIGURE 14. The effect of 60 μmol indomethacin upon the responses of male rat descending colon to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the succeeding acetylcholine response.

response was 39.8 ± 6.2%(n=6) compared with 28.8 ± 4.1% (n=6) after 30 minutes.

(2) The Effect of Indomethacin upon the Responses of Rat Dioestrous Uterus to Acetylcholine and Angiotensin

Exposure of preparations of rat dioestrous uterus to a Tyrode solution containing 3 μmol indomethacin had no consistent affect upon responses to acetylcholine and angiotensin. When the concentration was increased to 15 μmol there was a small but significant percentage reduction of responses to acetylcholine and angiotensin (fig. 15). There was no significant difference between the percentage reduction of the angiotensin responses and the succeeding acetylcholine responses and the action of indomethacin was not progressive. After 30 minutes exposure to indomethacin the percentage reduction of the acetylcholine response was 14.7 ± 3.5%(n=6) and after 45 minutes it was 11.2 ± 2.9% (n=6). The immediately succeeding angiotensin responses at 35 minutes and 45 minutes, were reduced by 16.2 ± 4.2% (n=6) and 14.0 ± 3.7% (n=6) respectively.

Figure 15 also shows the percentage reductions of acetylcholine and angiotensin responses of rat dioestrous uterus which occurred during an exposure to a Tyrode solution containing 60 μmol indomethacin. Following a 30 minute equilibration period, alternate contractions were evoked to acetylcholine and angiotensin. The percentage reduction of both agonists was greater than had been observed with 15 μmol indomethacin but there was a significant difference between the percentage reduction of angiotensin responses and the succeeding acetylcholine responses ($p < 0.01$). Thus after 35 minutes exposure to indomethacin the angiotensin response was reduced by 30.5 ± 4.1% (n=6) and the succeeding acetylcholine response at 45 minutes was reduced by 13.2 ± 2.7% (n=6). After 50 minutes exposure to indomethacin the angiotensin response was reduced by 32.4 ± 2.7% (n=6) and since no further contractions were

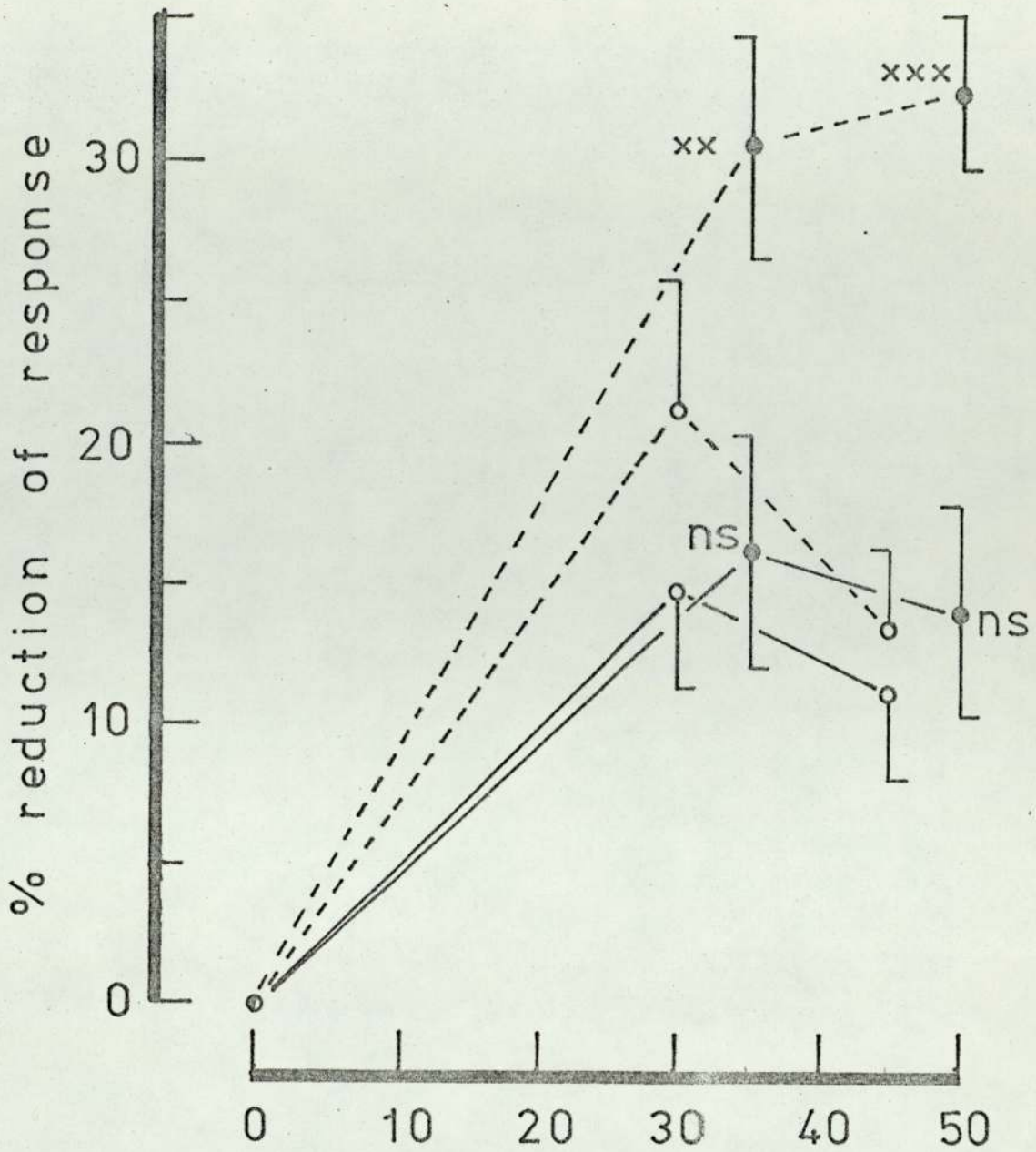

FIGURE 15. The effect of 15 μmol and 60 μmol indomethacin upon the responses of dioestrous rat uterus to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean. Solid lines denote the inhibition pattern produced by 15 μmol indomethacin and broken lines that produced by 60 μmol indomethacin.

xxx $p < 0.001$ xx $0.001 < p < 0.01$ ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

recorded this has been compared with the percentage reduction of the acetylcholine response after 45 minutes. The reduction of responses produced by indomethacin was not progressive and after 45 minutes the percentage reduction of the acetylcholine response, 13.2± 2.7% (n=6), was less than the percentage reduction after 30 minutes, 21.2± 4.5% (n=6).

When the concentration of indomethacin was increased to 0.3 mmol responses to acetylcholine and angiotensin were severely impaired. Following a 30 minute equilibration in Tyrode solution containing indomethacin responses to acetylcholine and angiotensin were evoked alternately. There was a significant difference between the percentage reduction of the acetylcholine responses and the immediately succeeding angiotensin responses ($p < 0.001$). After 35 minutes the angiotensin response was abolished and the following acetylcholine response at 45 minutes was reduced by 65.3± 5.2% (n=6). The reduction of the responses to acetylcholine was progressive and coupled with the severe inhibition of the contractions, this indicated an impairment of the ability of the muscle to contract.

At a concentration of 3 μmol, indomethacin had no effect upon spontaneous activity of preparations of dioestrous rat uterus. In higher concentrations, 15 μmol, 60 μmol and 0.3 mmol, spontaneous activity was inhibited and there was some loss of tone, but there was no evidence of any impairment of the maintenance of induced contractions.

B. The Role of Cyclic AMP

The methylxanthine theophylline has been shown to be an effective inhibitor of phosphodiesterase (Sutherland & Rall, 1960; Butcher & Sutherland, 1962) and it potentiates many actions of drugs that are supposed to be mediated by cyclic AMP (Rall & West, 1963; Robinson, Butcher & Sutherland, 1971). In the concentration range of 1 - 10 mmol, theophylline has been shown to inhibit phosphodiesterase in most preparations including isolated smooth muscle (Davies et al, 1972; Hornych et al, 1973; Pfaffmen & McFarland, 1973). Therefore, the action of theophylline was investigated on the contractions of rat descending colon to acetylcholine and angiotensin.

(1) The Effect of Theophylline upon Responses of Rat Descending Colon to Acetylcholine and Angiotensin

The effect of theophylline upon the responses of rat descending colon to acetylcholine and angiotensin was investigated with respect to time. A concentration of 1 mmol theophylline had no significant effect upon responses to either agonist but higher concentrations caused an equal reduction of induced contractions. Figure 16 shows the percentage reductions of acetylcholine and angiotensin responses which occurred when preparations of rat descending colon were exposed to a Tyrode solution containing 2 mmol theophylline. There was no significant difference ($p > 0.05$) between the percentage reductions of the acetylcholine responses and the percentage reductions of the angiotensin responses during the exposure to theophylline. After 30 minutes the acetylcholine response was reduced by 13.4 ± 1.9% (n=6) and the angiotensin response was reduced by 13.8 ± 1.7% (n=6). The percentage reductions after 50 minutes exposure to theophylline were 14.3 ± 1.7% (n=6) and 19.8±1.9% (n=6) respectively.

When the concentration of theophylline was increased from 2 mmol to 4 mmol there was a further reduction of responses to both

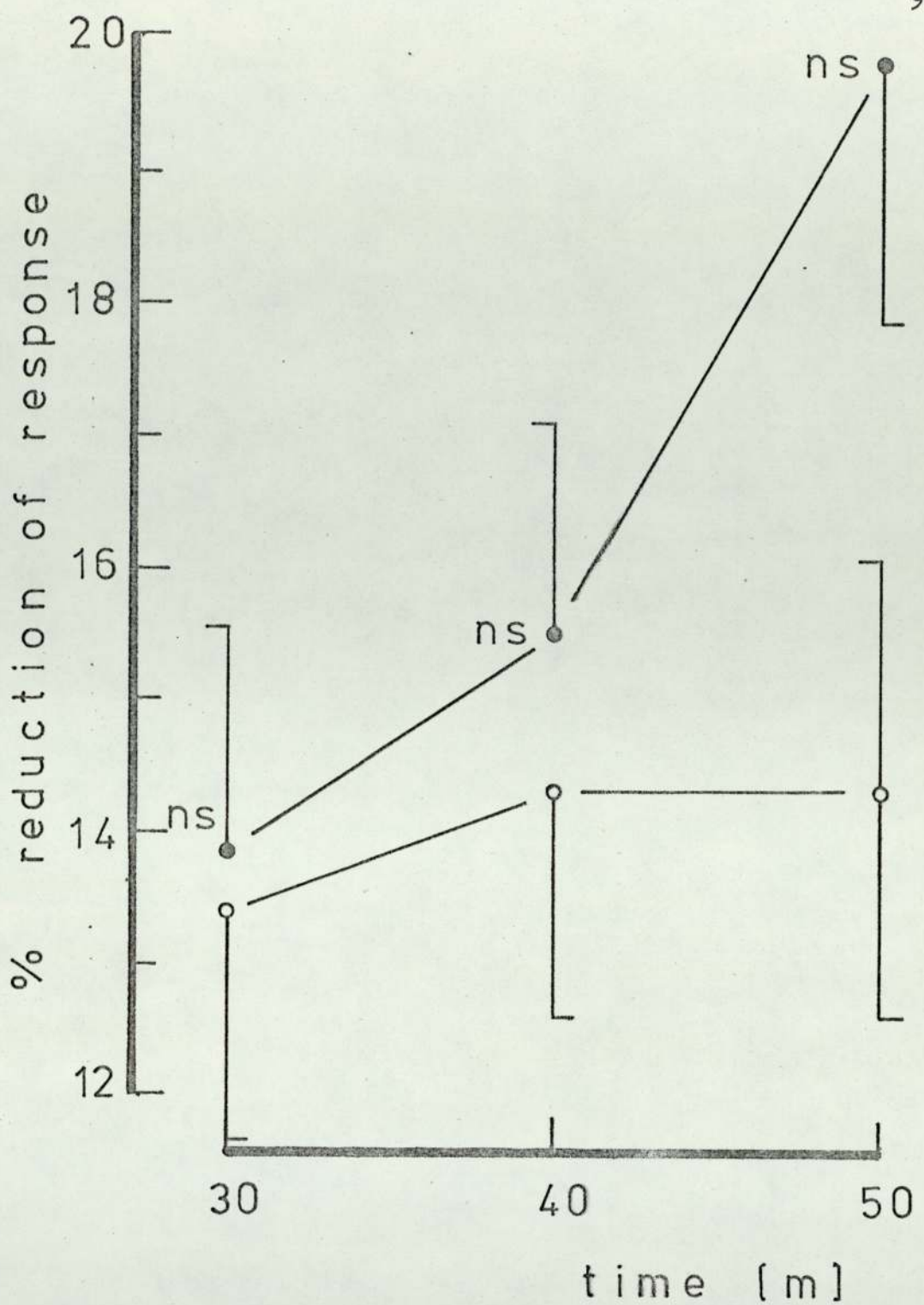

FIGURE 16. The effect of theophylline 0.2 mmol upon the responses of male rat descending colon to angiotensin and acetylcholine. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

agonists but again there was no significant difference between the percentage reduction of the acetylcholine responses and the percentage reduction of the angiotensin responses (Fig. 17). After 30 minutes the acetylcholine response was reduced by 63.7± 3.7% (n=6) and this increased to 73.5± 5.0% (n=6) after 50 minutes. The angiotensin response was reduced by 71.8± 4.7% (n=6) after 30 minutes and by 72.9± 4.7% (n=6) after 50 minutes. Responses of rat descending colon to 40 mmol potassium chloride were also reduced by exposure to Tyrode solution containing 4 mmol theophylline. (fig. 17). Potassium contractures were well maintained in the presence of theophylline and there was no dissociation into phasic and tonic components. The percentage reduction of the potassium response was 65.9± 2.9% (n=6) after 30 minutes exposure to theophylline, and 70.4 ± 3.6% (n=6) after 50 minutes exposure. There was no significant difference between the percentage reductions of the potassium contracture and the corresponding percentage reductions of the angiotensin and acetylcholine responses.

Figure 18 shows the percentage reductions of responses of rat descending colon to acetylcholine and angiotensin which occurred during exposure to Tyrode solution containing 5 mmol theophylline. There was a severe impairment of contractions to both agonists but there was no significant difference between the percentage reductions of the acetylcholine responses and the percentage reductions of the angiotensin responses. After 30 minutes exposure to theophylline, the acetylcholine response was reduced by 79.4± 5.9% (n=6) and the angiotensin response was reduced by 84.9± 4.8% (n=6). After 50 minutes exposure the percentage reductions of the acetylcholine and angiotensin responses were 84.0± 2.9% (n=6) and 92.2 ±1.8% (n=6) respectively.

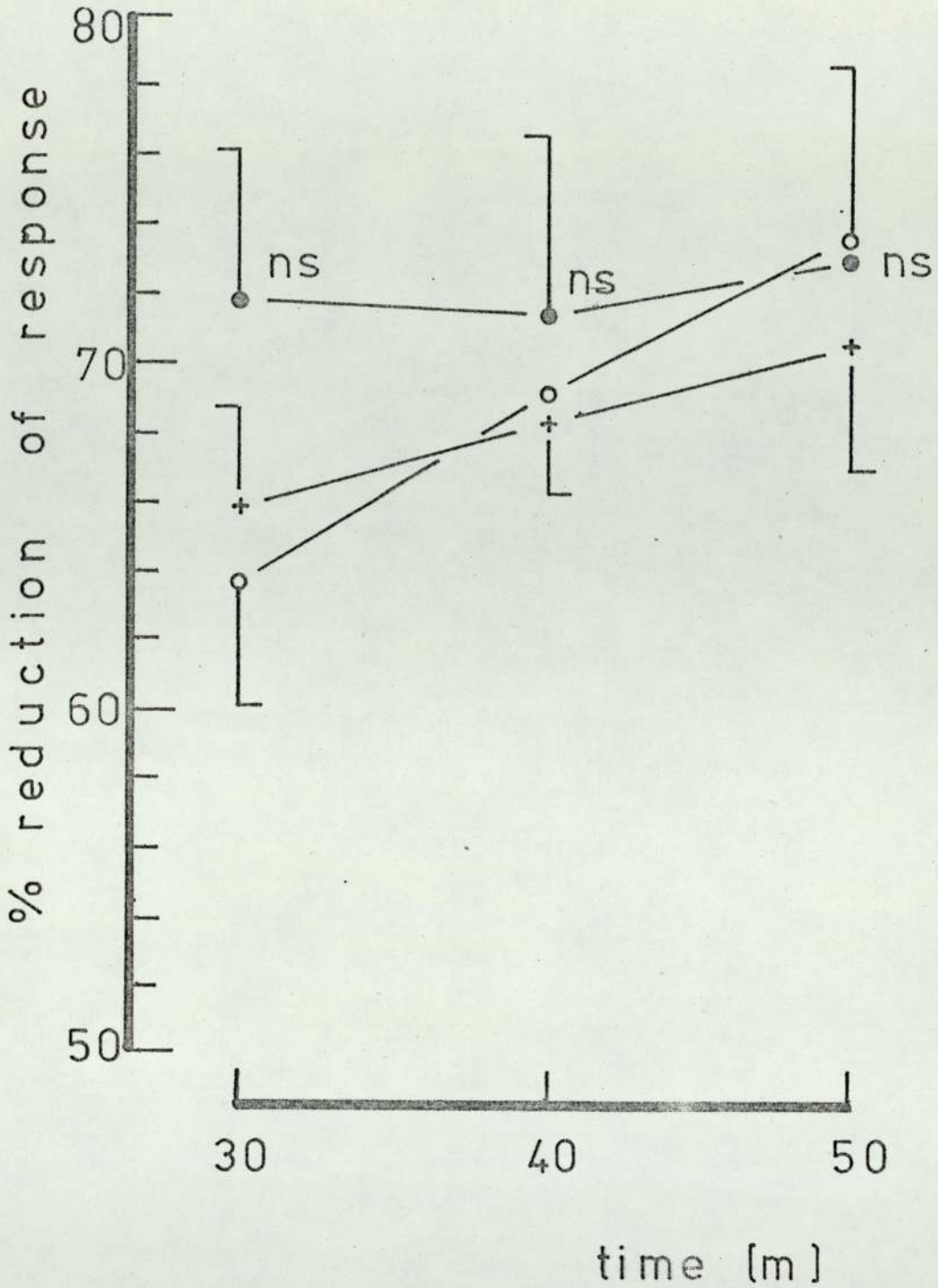

FIGURE 17. The effect of theophylline 0.4 mmol upon the responses of male rat descending colon to angiotensin, acetylcholine and potassium (40 mmol). Mean % reduction of responses to angiotensin (●), acetylcholine (○) and potassium (+) with the standard error of the mean.

ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine or potassium response.

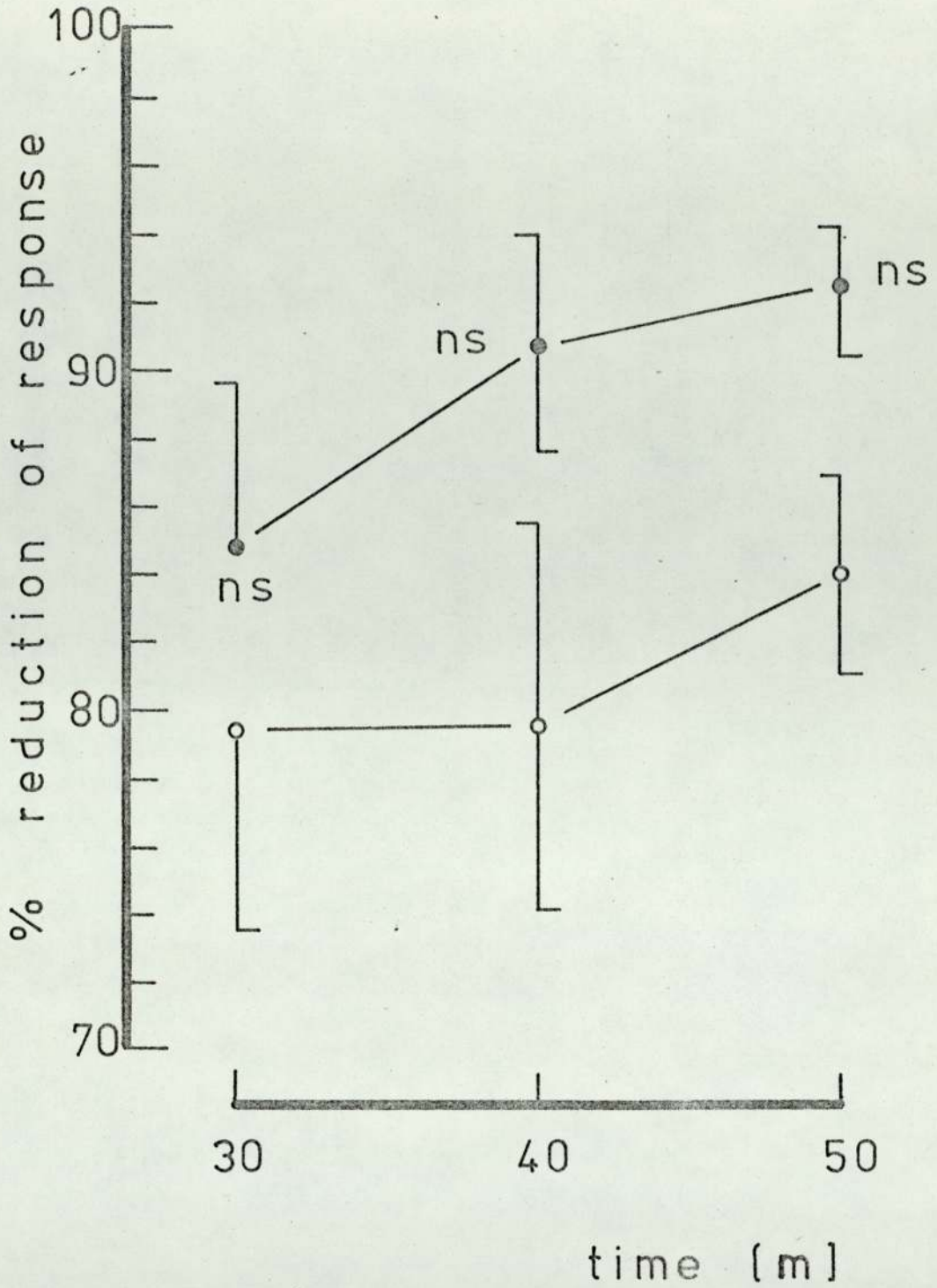

FIGURE 18. The effect of 0.5 mmol theophylline upon the responses of male rat descending colon to angiotensin and acetylcholine. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

SECTION IV THE ROLE OF INORGANIC IONS IN THE ACTION OF ANGIOTENSIN UPON SMOOTH MUSCLE

There have been many reports that the contractile action of angiotensin upon smooth muscle is dependent upon the concentration of sodium and calcium ions in the surrounding solution. However, there are many contradictory reports in the literature which may be due to the diversity in the smooth muscle preparations that have been investigated. Angiotensin also stimulates active sodium transport in isolated epithelial systems and has been reported to have a direct action upon sodium transport in the renal tubule. Since these effects occur with concentrations of angiotensin which are ineffective on smooth muscle preparations, it is possible that the physiological action of angiotensin may be related to an effect upon sodium transport.

Therefore, a systematic study was made of the effect of changes in sodium and calcium concentration upon responses of rat descending colon and rat uterus to angiotensin and acetylcholine. A study was also made of the possible role of the sodium potassium pump and the sodium potassium independent pump in the contractile action of angiotensin since stimulation of active ion movement by angiotensin could explain the energy dependence of the angiotensin response observed in previous experiments.

A. Sodium Ions and the Angiotensin Response

(1) The Effect of Reduced Sodium Concentration upon the Response of Rat Descending Colon to Angiotensin and Acetylcholine

The sodium concentration of the Tyrode solution was decreased from the normal concentration of 137 mmol and the responses to acetylcholine and angiotensin were recorded. (Figure 19). Decreases in sodium concentration were replaced with osmo-equivalent amounts of sucrose. When the sodium concentration was reduced to 120 mmol responses of rat colon to acetylcholine and angiotensin were

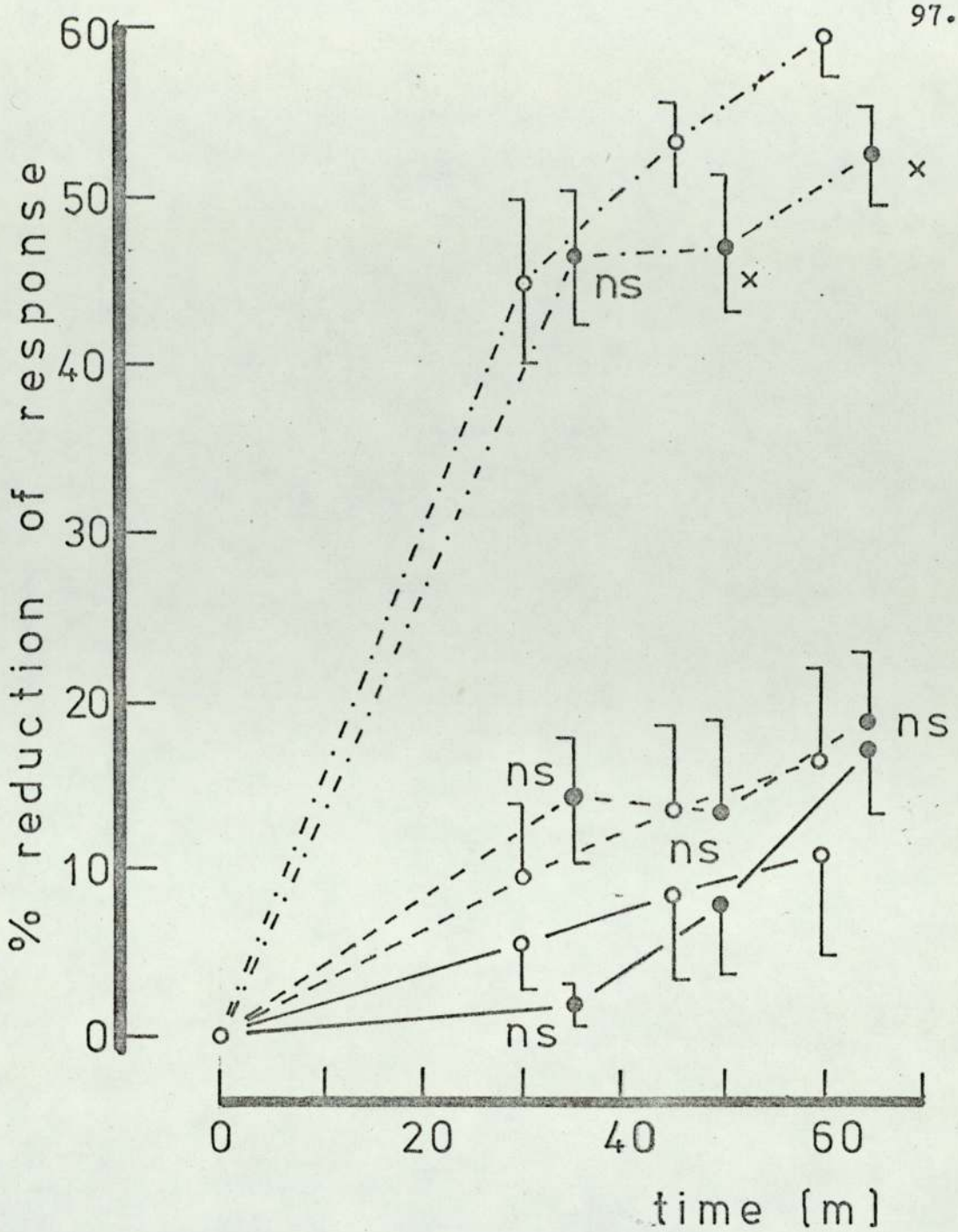

FIGURE 19. The alteration in the response of male rat descending colon to angiotensin and acetylcholine when the sodium concentration was reduced from the normal concentration of 137 mmol. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean. Solid lines denote the inhibition pattern produced by a sodium concentration of 120 mmol, while broken lines and dotted broken lines denote the inhibition patterns produced by sodium concentration of 103 mmol and 68.5 mmol respectively.

x $0.01 < p < 0.05$ ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

progressively reduced but there was no significant difference between the p ercentage reductions of the angiotensin responses compared with the percentage reduction of the immediately succeeding acetylcholine responses ($p > 0.05$). After 35 minutes the angiotensin response was reduced by 2.3 ± 1.4% (n=6) and the 45 minute acetylcholine response was reduced by 8.4 ± 5.3% (n=6). The angiotensin response at 50 minutes was reduced by 8.0 ± 4.6% (n=6) and the 60 minute acetylcholine response was reduced by 11.9 ± 5.8% (n=6).

When the sodium concentration was reduced to 103 mmol there was no further reduction of responses to acetylcholine and angiotensin compared with a sodium concentration of 120 mmol. Responses to acetylcholine and angiotensin were progressively reduced but there was no significant difference between the percentage reduction of the angiotensin responses compared with the percentage reductions of the immediately succeeding acetylcholine responses. After 35 minutes exposure to Tyrode solution containing 103 mmol sodium, the response to angiotensin was reduced by 14.2 ± 3.7% (n=8) and after 50 minutes exposure, by 13.7 ± 5.6% (n=8). The corresponding acetylcholine responses after 45 minutes exposure and 60 minutes exposure were reduced by 13.6 ± 5.3% (n=8) and 16.4 ± 5.7% (n=8) respectively.

After 35 minutes exposure to a Tyrode solution containing 68.5 mmol sodium the angiotensin response was reduced by 47.2 ± 4.1% (n=6) and after 50 minutes the reduction was 46.7 ± 3.6% (n=6). The percentage reduction of the acetylcholine response after 45 minutes was 53.3 ± 2.3% (n=6) and after 60 minutes 59.7 ± 2.5% (n=6). There was no consistent significant difference between the percentage reduction of the angiotensin responses and the immediately succeeding acetylcholine responses. ($p > 0.05$). Responses to both acetylcholine and angiotensin were progressively reduced and the percentage reduction was significantly greater than that produced by 103 mmol sodium.

(2) The Effect of Increasing the Sodium Concentration of the Tyrode Solution upon Responses to Acetylcholine and Angiotensin

The sodium concentration of the Tyrode solution was increased from the normal concentration of 137 mmol. (Figure 20). When preparations of rat colon were exposed to a Tyrode solution containing 171 mmol sodium there was a constant reduction of responses to acetylcholine and angiotensin which did notincrease with time. Thus after 35 minutes the angiotensin response was reduced by 31.5± 5.5% (n=6) and after 50 minutes it was reduced by 30.6 ± 5.4% (n=6). The immediately succeeding acetylcholine responses at 45 minutes and 60 minutes were reduced by 31.4 ± 4.0% (n=6) and 29.6 ± 4.3% (n=6) respectively. There was no significant difference between the percentage reduction of angiotensin responses and the percentage reduction of the succeeding acetylcholine responses during exposure to the high sodium Tyrode.

Figure 20 also shows the percentage reduction of responses to acetylcholine and angiotensin which occurred during exposure of preparations of rat descending colon to Tyrode solution containing 205.5 mmol sodium. The reduction of responses to both agonists was greater than in Tyrode solution containing 171 mmol sodium and was slowly progressive.

After 35 minutes the angiotensin response was reduced by 43.9± 5.8% (n=5) and after 50 minutes by 53.1± 5.3% (n=5), while the immediately succeeding acetylcholine responses (at 45 minutes and 60 minutes) were reduced by 63.1± 5.8% (n=5) and 62.0± 5.4% (n=5) respectively. There was no consistent significant difference between the percentage reduction of the angiotensin responses and the percentage reduction of the succeeding acetylcholine responses during exposure to high sodium Tyrode.

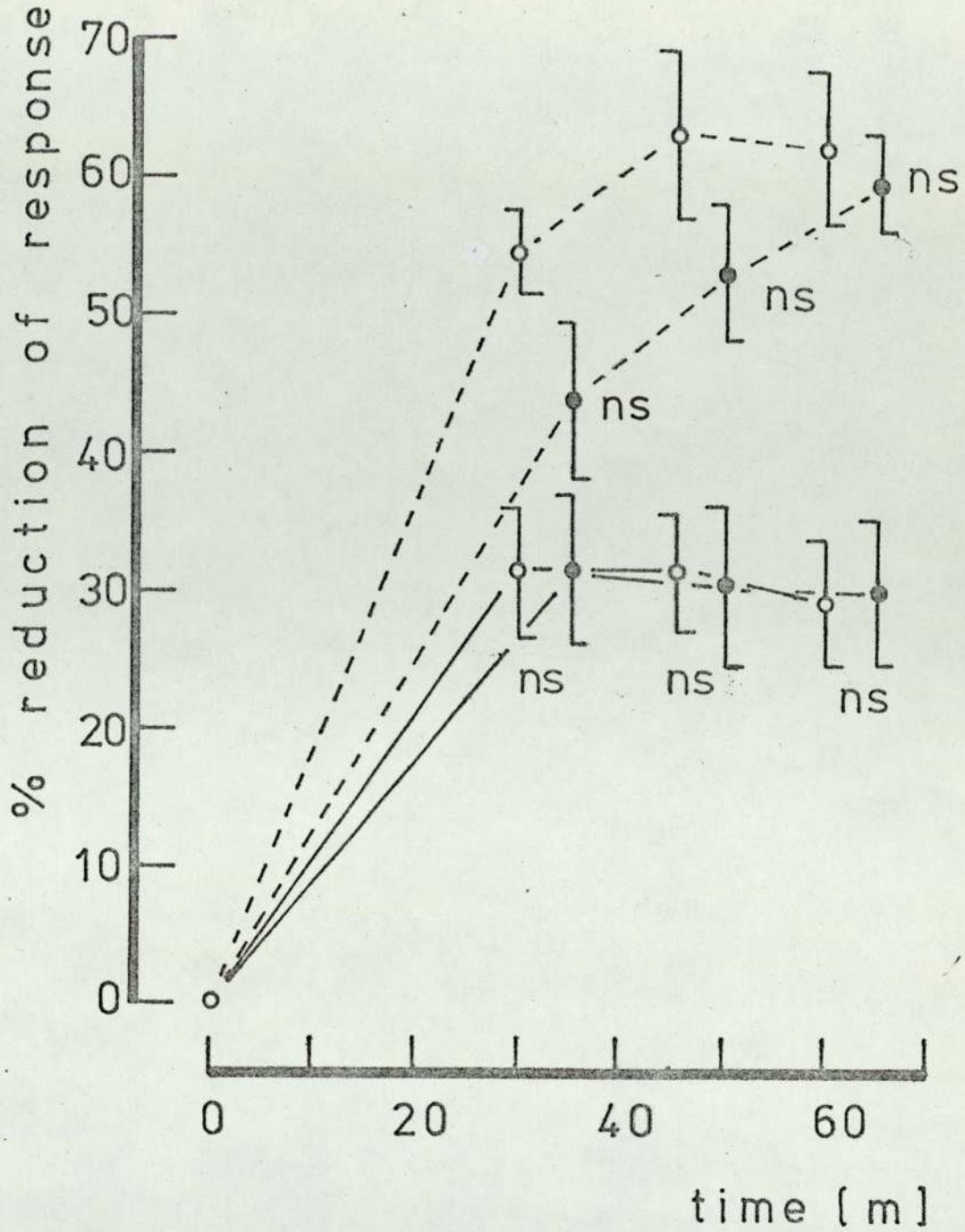

FIGURE 20. The alteration in the response of male rat descending colon to angiotensin and acetylcholine when the sodium concentration of the Tyrode solution was increased from the normal concentration of 137 mmol. Mean % reduction of responses to angiotensin (●) and acetylcholine with the standard error of the mean. Solid lines denote the inhibition pattern produced by a sodium concentration of 171 mmol and broken lines that produced by a sodium concentration of 205.5 mmol.

x $0.01 < p < 0.05$ ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

(3) The Effect of Ouabain upon Responses of Rat Descending Colon and Rat Uterus to Angiotensin and Acetylcholine

Numerous studies have shown that the cardiac glycoside ouabain is a potent inhibitor of the sodium potassium pump, enzymatically $Na^{+}-K^{+}-Mg^{2+}$ activated ATPase (reviews by Glynn, 1957; Glynn, 1964; Caldwell, 1968; Whittam & Wheeler, 1970; Lee & Klaus, 1971). Therefore a study was made of the effect of ouabain upon the contractile responses of rat colon and uterus to angiotensin and acetylcholine.

a. The effect of Ouabain upon Responses of Rat Descending Colon to Acetylcholine and Angiotensin

Ouabain in concentrations of 1 μmol and 10 μmol had no effect upon responses of rat descending colon to either angiotensin or acetylcholine. 0.1 mmol ouabain produced a small but significant reduction in responses to both agonists. The glycoside was incorporated in the Tyrode solution and preparations were equilibrated in the solution for 30 minutes before responses were evoked. Acetylcholine responses showed a mean percentage reduction of 23.0± 1.8 (n=5) and angiotensin responses a mean percentage reduction of 15.9 ± 3.8% (n=5). The effect of ouabain was not progressive and the contractions on each tissue were therefore measured as the mean of three successive responses in the presence or absence of ouabain.

Figure 21 shows the percentage reduction of acetylcholine and angiotensin responses which occurred during a 150 minute exposure of rat descending colon preparations to a Tyrode solution containing 1 mmol ouabain. There was a progressive reduction of responses to both agonists but at no time during the exposure to ouabain, was there any significant difference between the percentage reduction of acetylcholine responses compared with the percentage reduction of angiotensin responses. After 45 minutes, the acetylcholine response was reduced by 22.0± 6.8% (n=6) and after 120 minutes it was reduced by 56.9± 4.0% (n=6). The corresponding percentage reductions of the

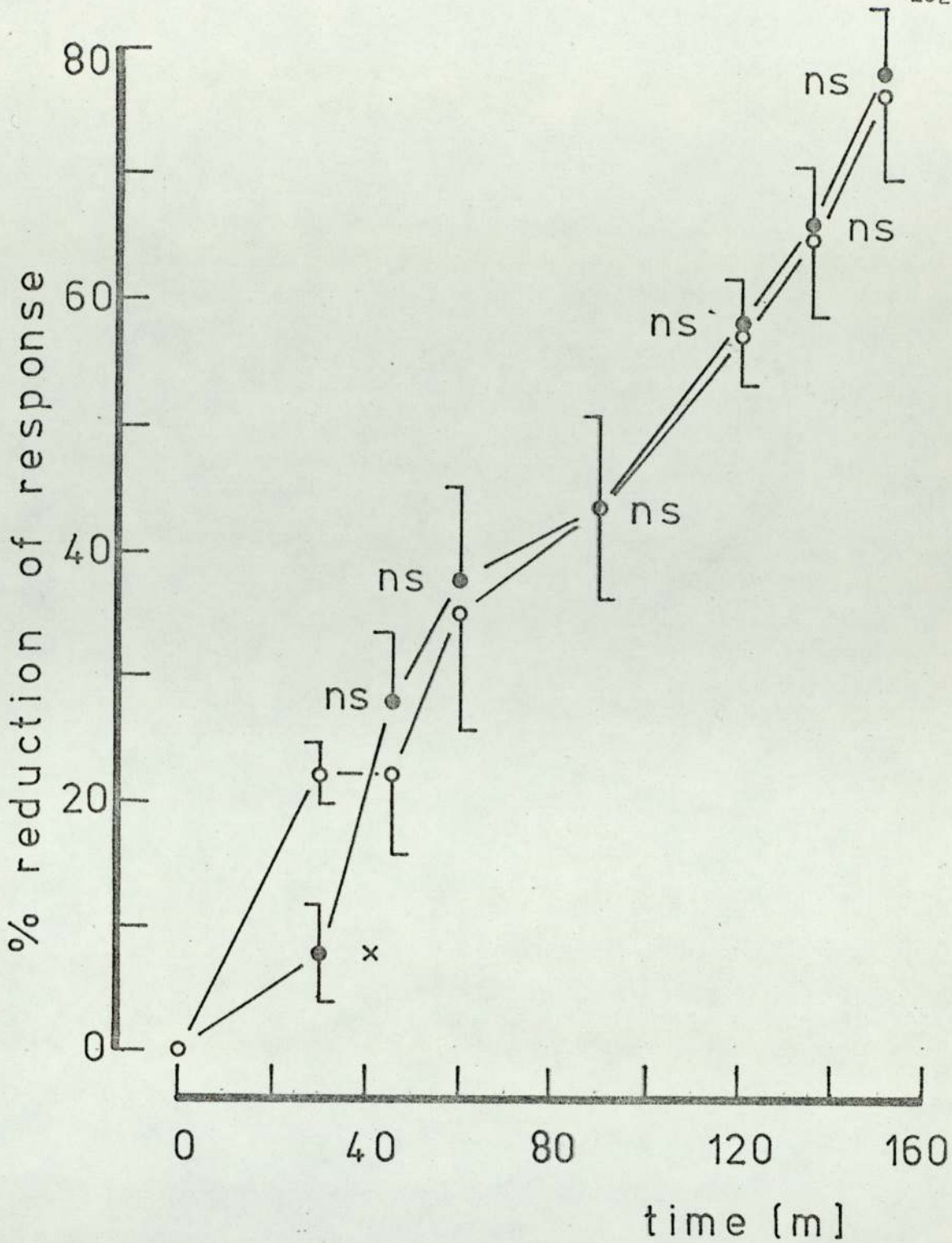

FIGURE 21. The effect of 1.0 mmol ouabain upon responses of male rat descending colon to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

x $0.01 < p < 0.05$ ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

angiotensin responses were 28.0± 5.7% (n=6) after 45 minutes and 57.1 ± 3.5% (n=6) after 120 minutes.

b. The Effect of Ouabain upon Responses of Rat Uterus to Acetylcholine and Angiotensin

Rat dioestrous and oestrous uterus was insensitive to ouabain in concentrations of 1 μmol and 10 μmol. Figures 22a and 22b show respectively the percentage reductions of acetylcholine and angiotensin responses which occurred during a 60 minute exposure of preparations of dioestrous and oestrous uterus to a Tyrode solution containing 1 mmol ouabain. There was a progressive reduction of responses to both agonists but on neither oestrous nor dioestrous uterus was there any significant difference between the percentage reduction of acetylcholine responses compared with the percentage reduction of angiotensin responses. There was no difference in the effect of ouabain upon oestrous uterus compared with the effect upon dioestrous uterus. After 30 minutes the angiotensin response of oestrous uterus was reduced by 33.4± 5.8% (n=5) and after 60 minutes this had increased to 57.8 ± 6.3% (n=5). The corresponding percentage reductions of the acetylcholine response were 22.0± 4.9% (n=5) after 30 minutes and 52.4 ± 3.7% (n=5) after 60 minutes.

The high concentrations of ouabain used in these experiments reflects the well known resistance of rat tissues to this agent. On addition of the glycoside there was a contraction of the smooth muscle preparations which disappeared within the 30 minute equilibration period.

(4) The Effect of Ethacrynic Acid upon the Responses of Rat Descending Colon and Dioestrous Uterus to Acetylcholine and Angiotensin

The diuretic ethacrynic acid has been reported to be a specific inhibitor of the second sodium pump (Whittembury & Proverbio, 1970; Proverbio, Robinson & Whittembury, 1970). Angiotensin has been

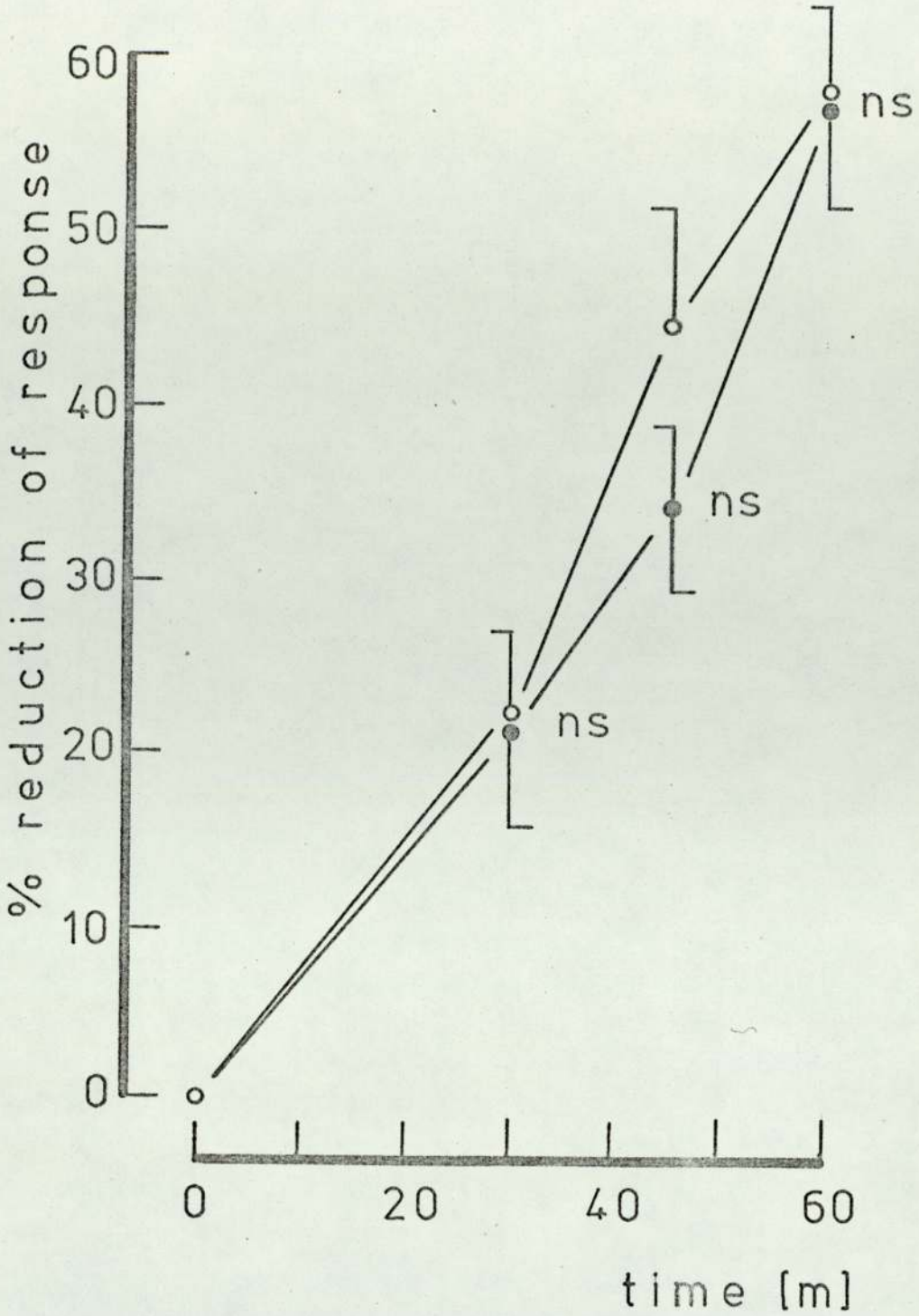

FIGURE 22a. The effect of 1 mmol ouabain upon the responses of dioestrous rat uterus to angiotensin and acetylcholine. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

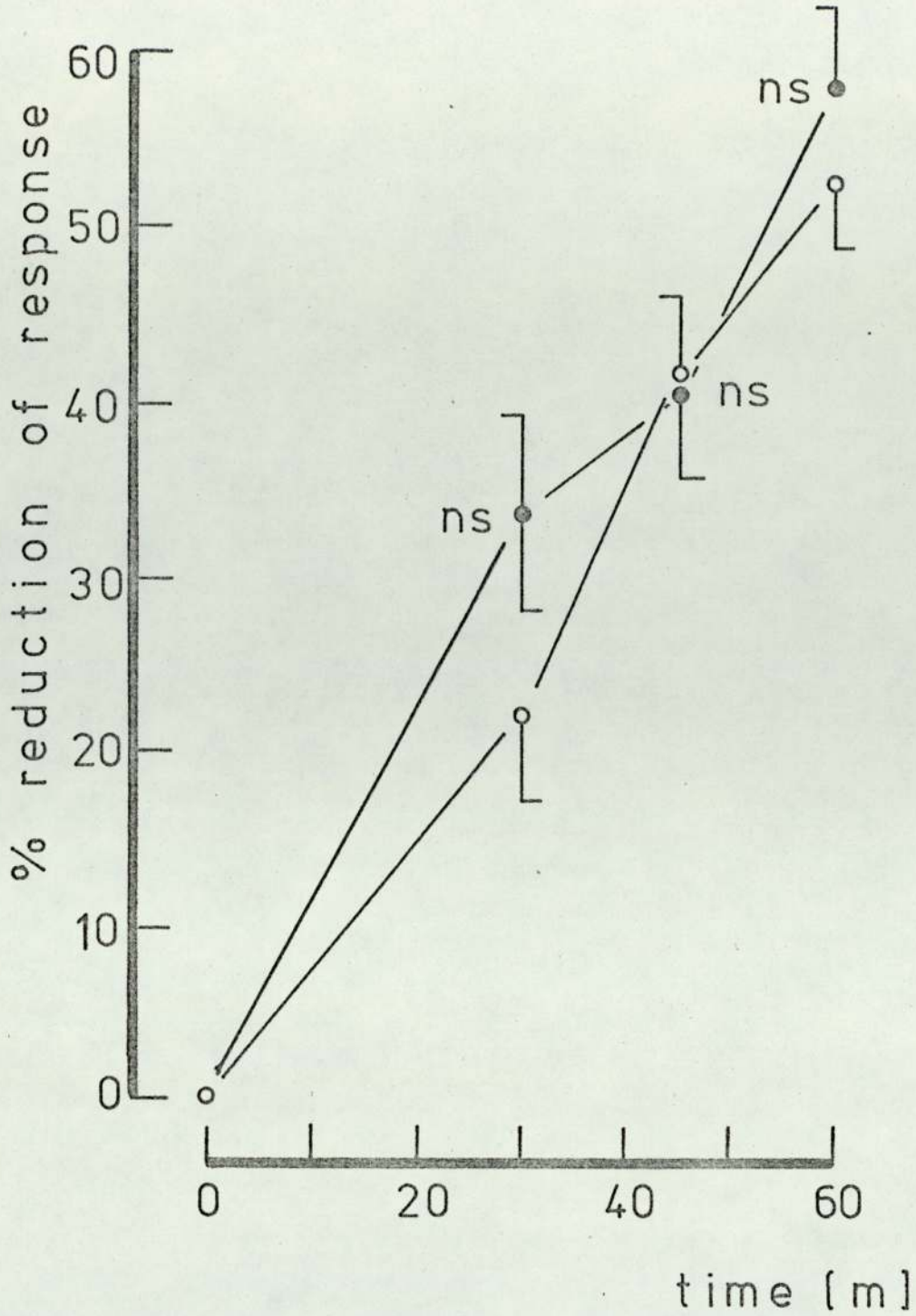

FIGURE 22b. The effect of 1.0 mmol ouabain upon the responses of oestrous rat uterus to angiotensin and acetylcholine. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

reported to stimulate this pump in rat descending colon (Parsons & Munday, 1972) and in rat kidney cortex slices (Munday, Parsons & Poat, 1971; 1972; Parsons & Munday, 1972). Therefore the action of ethacrynic acid was investigated upon the contractile response of rat descending colon and rat uterus to angiotensin.

a. The Effect of Ethacrynic Acid upon the Responses of Rat Descending Colon to Acetylcholine and Angiotensin

Figure 23 shows the percentage reduction of responses to acetylcholine and angiotensin which occurred during exposure of preparations of rat descending colon to Tyrode solution containing 0.1 mmol ethacrynic acid. There was a progressive reduction of responses to both agonists but throughout the exposure to ethacrynic acid the percentage reduction of the angiotensin response was significantly greater than the percentage reduction of the acetylcholine response ($p < 0.001$). After 50 minutes the angiotensin response was reduced by $36.4 \pm 3.3\%$ ($n=6$) and after 65 minutes it was reduced by $42.9 \pm 5.3\%$ ($n=6$). The percentage reductions of the immediately succeeding acetylcholine responses were $13.0 \pm 3.4\%$ ($n=6$) after 60 minutes and $15.4 \pm 0.7\%$ ($n=6$) after 75 minutes.

When the concentration of ethacrynic acid was increased to 0.5 mmol responses to both acetylcholine and angiotensin were severely impaired. Thus after 50 minutes exposure to this solution the angiotensin response was reduced by 100% and the immediately following acetylcholine response was reduced by $89.7 \pm 5.9\%$ ($n=5$). This indicated severe impairment of the contractile mechanism of the muscle.

b. The Effect of Ethacrynic Acid upon Responses of Rat Dioestrous Uterus to Acetylcholine and Angiotensin

Figure 24 shows the percentage reduction of responses to acetylcholine and angiotensin which occurred when preparations of rat dioestrous uterus were exposed to a Tyrode solution containing 0.1 mmol ethacrynic acid. There was a progressive reduction of responses

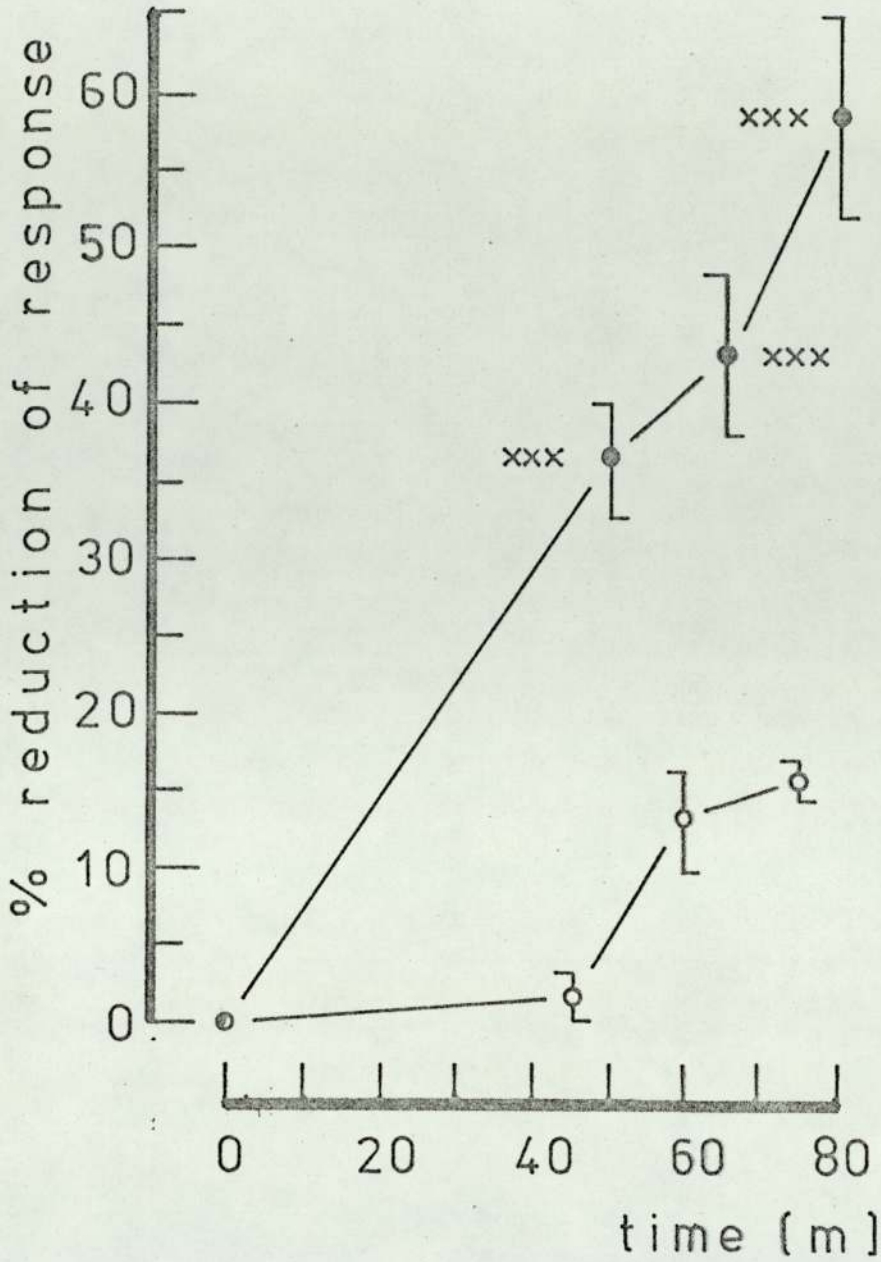

FIGURE 23. The effect of 0.1 mmol ethacrynic acid upon the responses of male rat descending colon to acetylcholine and angiotensin (isometric recording 1g load). Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

xxx $p < 0.001$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

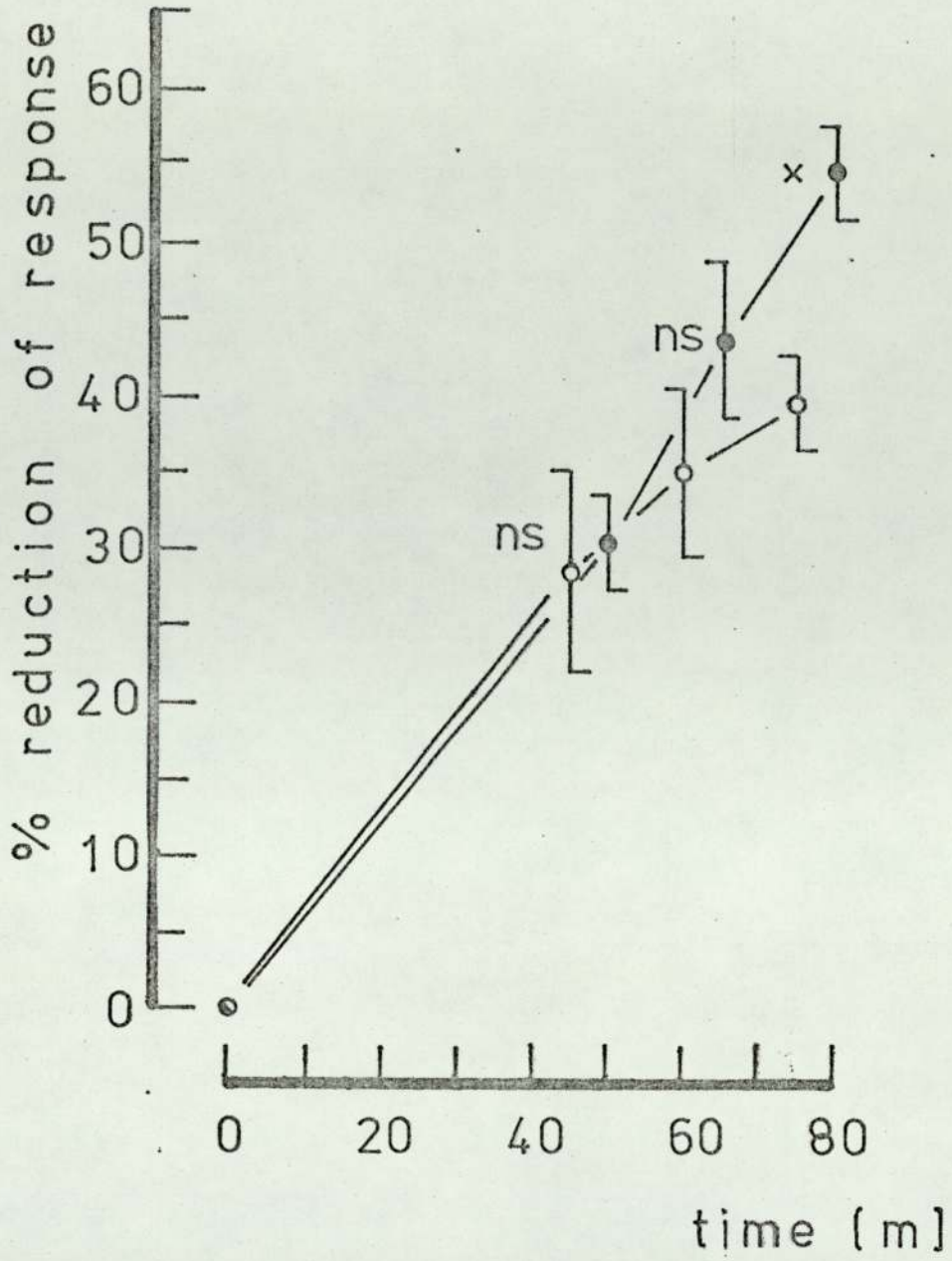

FIGURE 24. The effect of 0.1 mmol ethacrynic acid upon the responses of dioestrous rat uterus to angiotensin and acetylcholine (isotonic recording, 1g load). Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean.

x $0.01 < p < 0.05$ ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

to both agonists but at no time was the percentage reduction of the angiotensin response significantly greater than the percentage reduction of the succeeding acetylcholine response. After 50 minutes the percentage reduction of the angiotensin response was 30.7± 2.8 (n=6) and after 65 minutes 43.6± 5.0 (n=6). The percentage reduction of the acetylcholine response was 35.0± 5.7 (n=6) after 60 minutes and 39.7 ± 3.9 (n=6) after 75 minutes.

These experiments with rat uterus were recorded isotonically with an applied load of 1 g. Experiments with rat colon were recorded isometrically and these preparations showed a preferential reduction of the angiotensin response during exposure to ethacrynic acid. It has been shown that the energy expenditure of a muscle is greater during an isometric contraction than during an isotonic contraction (Axelsson, Hogberg & Timms, 1965; Lundholm & Mohme-Lundholm , 1965). In previous experiments metabolic inhibition has been shown to have a greater effect upon the angiotensin response of smooth muscle than upon the acetylcholine response. Since ethacrynic acid has been reported to cause a non-specific inhibition of cellular energy production (Macknight, 1969; Epstein, 1972 Landon & Fitzpatrick, 1972; Case, Gunther & Cannon, 1973), the preferential reduction of the angiotensin response observed under isometric conditions may be due to a reduction in the available energy. To investigate this possibility, rat dioestrous uterus was exposed to ethacrynic acid under conditions when muscle energy expenditure would be high.

Figure 25 shows the percentage reduction of responses to acetylcholine and angiotensin which occurred during exposure of preparations of rat dioestrous uterus to Tyrode solution containing 0.1 mmol ethacrynic acid. Recordings were made isotonically with an applied load of 3 g. An increase in the isotonic load has been shown to increase muscle energy expenditure during contraction (Axelsson et

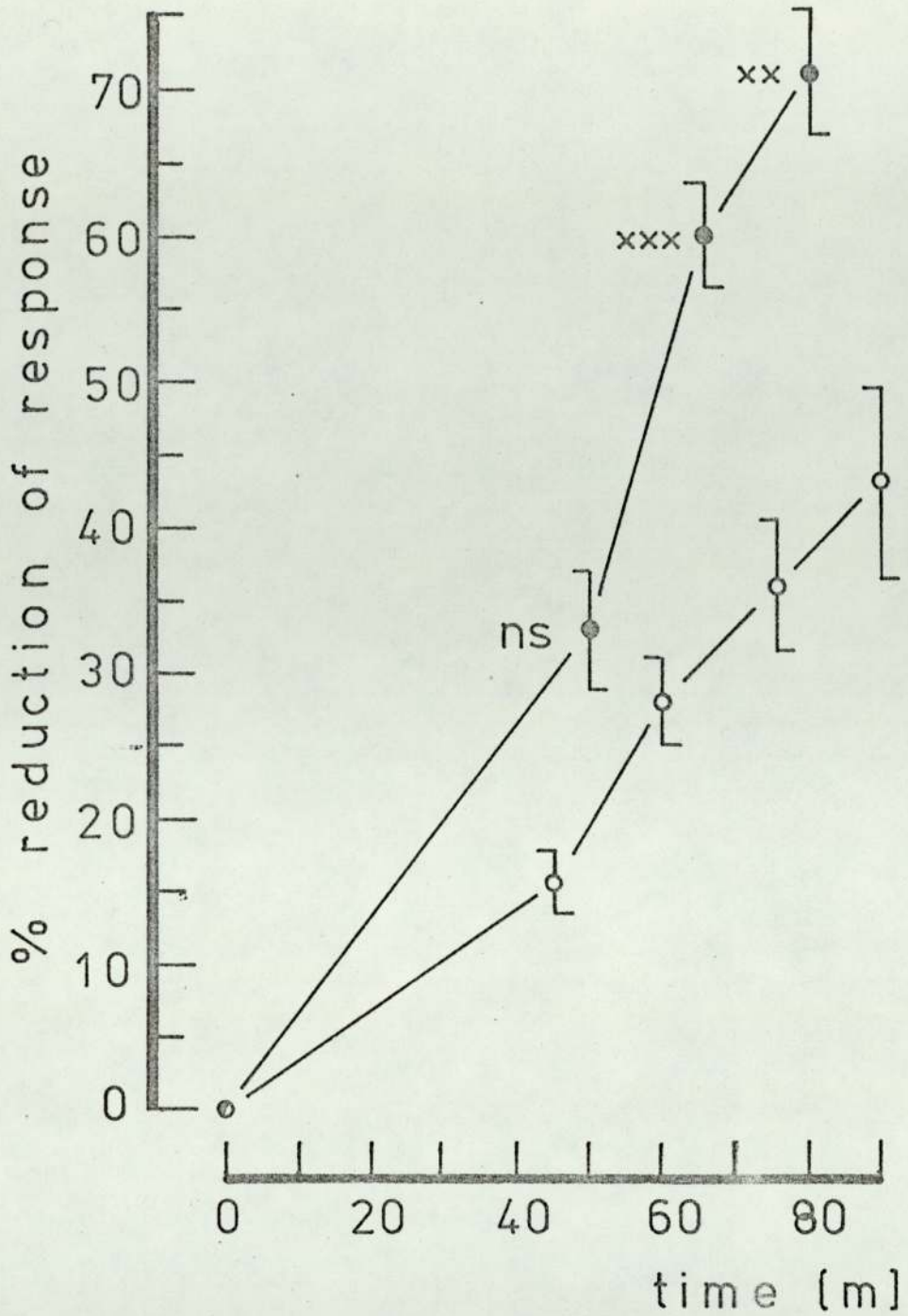

FIGURE 25. The effect of 0.1 mmol ethacrynic acid upon the responses of dioestrous rat uterus to angiotensin and acetylcholine (isotonic recording, 3g load). Mean % reduction of responses to angiotensin (●) and acetylcholine with the standard error of the mean.

ns $p > 0.05$ xx $0.01 > p > 0.001$ xxx $p < 0.001$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

al, 1965). There was a progressive reduction of responses to acetylcholine and angiotensin throughout the exposure to ethacrynic acid. During the later stages of the exposure the percentage reduction of angiotensin responses was significantly greater than the percentage reduction of the following acetylcholine responses ($p < 0.01$). Thus after 65 minutes the angiotensin response was reduced by $60.0 \pm 3.6\%$ (n=7) and the acetylcholine response at 75 minutes was reduced by $35.8 \pm 4.4\%$ (n=7). After 80 minutes the angiotensin response was reduced by $71.0 \pm 3.9\%$ (n=7) and the 90 minute acetylcholine response was reduced by $43.1 \pm 6.4\%$ (n=7).

When the concentration of ethacrynic acid was increased to 0.5 mmol responses to both acetylcholine and angiotensin were severely impaired and there was no significant difference between the percentage reduction of responses to angiotensin compared with the percentage reduction of responses to acetylcholine. Thus after 50 minutes exposure to this solution the angiotensin response was reduced by $88.9 \pm 4.4\%$ (n=6) and the immediately following acetylcholine response was reduced by $91.1 \pm 5.2\%$ (n=6). The large reduction of induced responses indicated a non specific interference with the muscles contractile ability.

These experiments with ethacrynic acid do not support the suggestion that angiotensin acts via stimulation of the 'second' sodium pump. Rather, they support previous findings that the angiotensin response is more dependent upon energy than the acetylcholine response.

B. The Role of Calcium Ions in the Angiotensin Response of Smooth Muscle

(1) The Effect of Reduced Calcium Concentration upon Responses of Rat Descending Colon to Angiotensin and Acetylcholine

The calcium concentration of the Tyrode solution was reduced from the normal concentration of 1.8 mmol and the effect upon responses to acetylcholine and angiotensin was investigated with respect to time. (Figure 26).

When preparations of rat descending colon were exposed to Tyrode solution containing 0.9 mmol calcium there was a significant difference between the percentage reduction of angiotensin responses compared with the percentage reduction of the immediately succeeding acetylcholine responses. After 35 minutes the angiotensin response was reduced by 27.8± 5.0% (n=6) and the following acetylcholine response (at 45 minutes) was reduced by 5.3± 3.5% (n=6) ($p < 0.01$). The effect of low calcium Tyrode solution was not progressive. Thus after 35 minutes exposure the angiotensin response was reduced by 27.8± 5.0% (n=8) and after 65 minutes exposure by 32.9± 6.6% (n=8).

A further reduction of the calcium concentration to 0.18 mmol caused a greater reduction of responses to acetylcholine and angiotensin. Again there was a significant difference between the percentage reduction of angiotensin responses compared with the percentage reduction of the immediately succeeding acetylcholine responses. After 35 minutes the angiotensin response was reduced by 43.5± 1.2% (n=16) and the following acetylcholine response (at 45 minutes) was reduced by 25.5± 5.3% (n=16) ($p < 0.01$). There was a slow but progressive increase in the percentage reduction of responses to both agonists during exposure to the low calcium solution. After 30 minutes the acetylcholine response was reduced by 17.1± 4.2% (n=16) and after 60 minutes the reduction was 28.6± 4.5% (n=16).

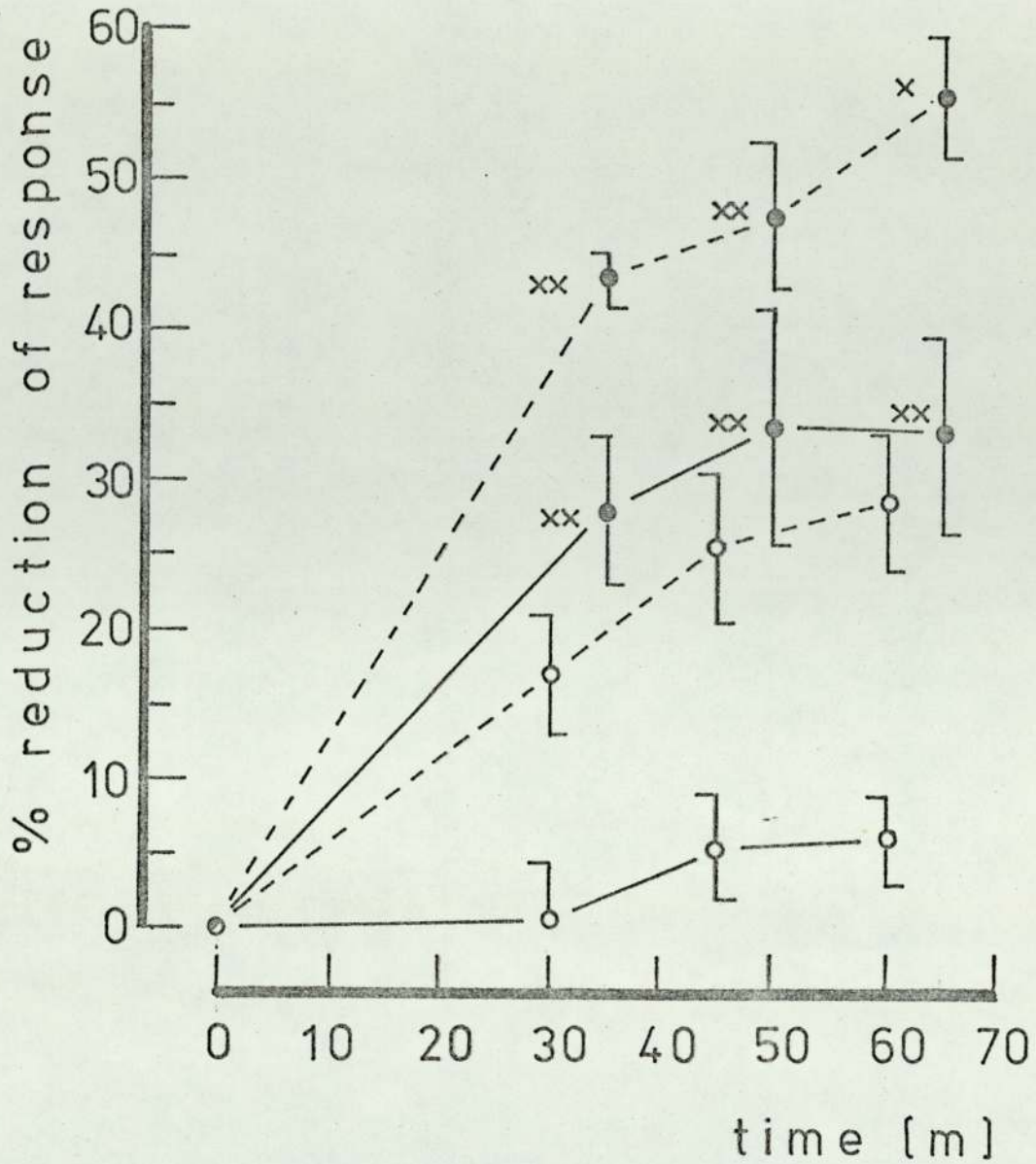

FIGURE 26. The alteration in the isometric responses of male rat descending colon to angiotensin and acetylcholine when the calcium concentration of the Tyrode solution was reduced from the normal concentration of 1.8 mmol. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean. Solid lines denote the inhibition pattern produced by a calcium concentration of 0.9 mmol and broken lines that produced by a calcium concentration of 0.18 mmol.

xxx $p < 0.001$ xx $0.001 < p < 0.01$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

These experiments showed that the angiotensin response was more dependent upon the extracellular calcium concentration than the acetylcholine response. It has been reported that smooth muscle contraction may be evoked by stimulation of an inwardly directed active calcium pump (Bohr et al, 1969; Brading, 1973; Reuter et al, 1973). Since previous experiments had shown that the angiotensin response was energy dependent, the effect of combined glucose removal and low calcium was investigated.

Figure 27 shows the percentage reduction of responses to acetylcholine and angiotensin which occurred during exposure of preparations of rat descending colon to a Tyrode solution containing 0.18 mmol calcium and no glucose. There was a progressive reduction of responses to both agonists but throughout the incubation period, the percentage reduction of responses to angiotensin was significantly greater than the percentage reduction of the immediately succeeding acetylcholine responses. After 35 minutes the angiotensin response was reduced by 48.6 ± 5.2% (n=12) whereas the immediately succeeding acetylcholine response (at 45 minutes) was reduced by 25.8 ± 5.4% (n=12) ($p < 0.01$). There was no significant difference between the responses to acetylcholine and angiotensin during exposure to this solution compared with the responses in the solution containing 0.18 mmol calcium and glucose (fig. 27).

To further investigate the possible role of active calcium movement in the angiotensin contraction, the effect of reduced calcium concentration was studied upon isotonic responses (load 1g) of rat descending colon to acetylcholine and angiotensin. In all previous investigations, isometric recording had been used for rat descending colon. Figure 27 shows the percentage reduction of responses to acetylcholine and angiotensin which occurred during exposure of rat descending colon to a Tyrode solution containing 0.18 mmol calcium

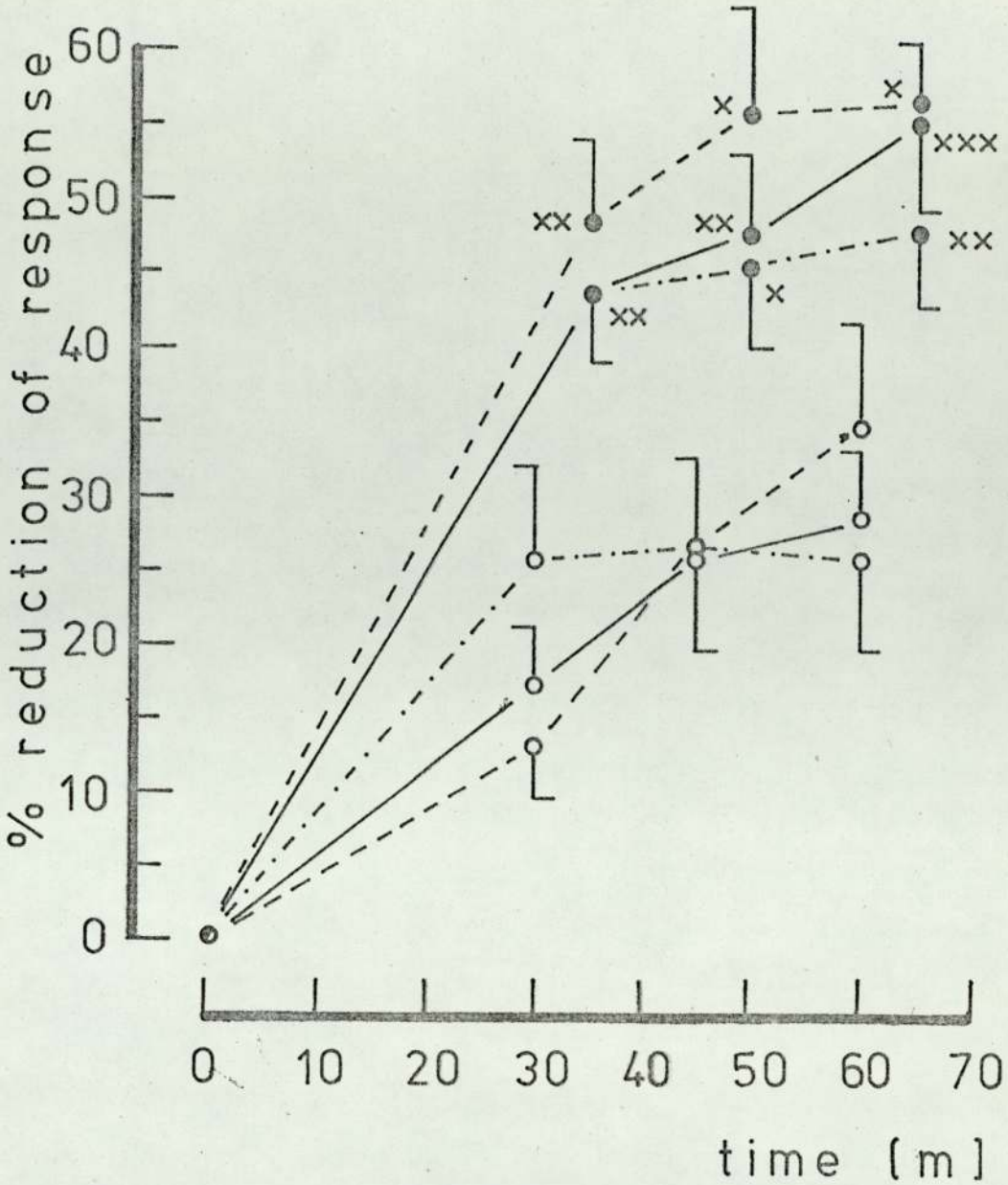

FIGURE 27. The effect of a Tyrode solution containing 0.18 mmol calcium upon the responses of male rat descending colon to angiotensin and acetylcholine in the presence and absence of glucose. Mean % reduction of responses to angiotensin (●) and acetylcholine with the standard error of the mean. Solid lines and dotted broken lines denote respectively the inhibitor patterns of isometric and isotonic responses in the presence of glucose while broken lines denote the inhibition pattern of isometric responses in the absence of glucose.

x $0.01 < p < 0.05$ xx $0.001 < p < 0.01$
xxx $p < 0.001$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

under isotonic conditions. Throughout the exposure to this solution there was a significant difference ($p < 0.01$) between the percentage reduction of angiotensin responses and the percentage reduction of the immediately succeeding acetylcholine responses. After 35 minutes the angiotensin response was reduced by 43.6 ± 5.4% (n=16) while the following acetylcholine response (at 45 minutes) was reduced by 26.0 ± 5.9% (n=16).

The effect of the low calcium solution upon induced responses was not progressive, and there was no significant difference between the percentage reductions of the acetylcholine and angiotensin responses compared with the percentage reductions produced by 0.18 mmol calcium solution under isometric conditions (see fig. 27).

(2) The Effect of Complete Calcium Removal upon Responses of Rat Descending Colon to Acetylcholine and Angiotensin

Figure 28a shows the percentage reductions of responses to acetylcholine and angiotensin which occurred during exposure of preparations of rat descending colon to a Tyrode solution containing no calcium. To prevent effects due to changes in osmotic pressure an osmo-equivalent of sucrose was added to the Tyrode solution in place of calcium. There was a progressive reduction of isometric responses to both agonists but at no time during the experiment was there any significant difference between the percentage reduction of the angiotensin responses and the percentage reduction of the immediately succeeding acetylcholine responses. After 10 minutes the angiotensin response was reduced by 54.6 ± 3.1% (n=5), after 40 minutes by 84.6 ± 4.4% (n=6). The acetylcholine response was reduced by 69.4 ± 7.3% (n=5) after 20 minutes and by 74.8 ± 5.6% (n=5) after 50 minutes.

Figure 28a also shows the percentage reduction of acetylcholine and angiotensin responses caused by exposure to calcium free Tyrode

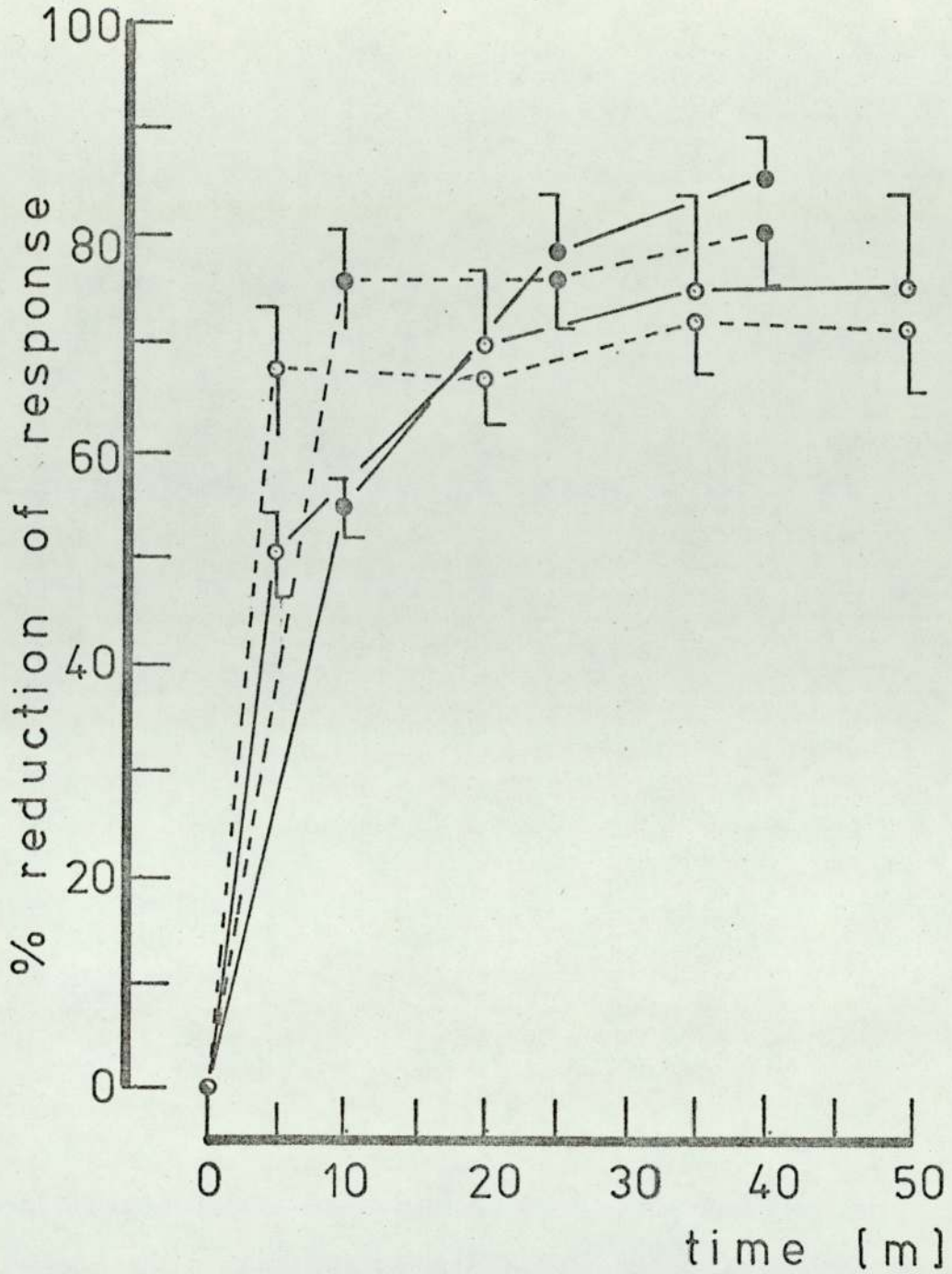

FIGURE 28a. The effect of a calcium free Tyrode solution upon responses of male rat descending colon to acetylcholine and angiotensin. Mean % reduction of responses to angiotensin (●) and acetylcholine with the standard error of the mean. Solid lines denote the inhibition pattern of isometric responses and broken lines that of isotonic responses.

$p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

solution and measured under isotonic conditions. The effect of calcium free Tyrode solution was similar to that observed under isometric conditions. There was a progressive reduction of responses to both agonists and at no time during the incubation period was there any significant difference between the percentage reduction of the angiotensin response and the percentage reduction of the immediately succeeding acetylcholine response. After 10 minutes the angiotensin response was reduced by 75.4 ± 5.3% (n=10) and after 40 minutes it was reduced by 79.8 ± 5.1% (n=10). The acetylcholine response was reduced by 67.4 ± 4.6% (n=10) after 20 minutes and by 70.9 ± 6.4% (n=10) after 50 minutes.

In other experiments preparations of rat descending colon were exposed to a Tyrode solution containing no calcium and no glucose. The percentage reduction of isotonic contractions to acetylcholine and angiotensin during a 50 minute exposure to this solution are shown in figure 28b. There was a progressive reduction of induced responses but at no time was there any significant difference between the percentage reduction of the angiotensin responses compared with the percentage reduction of the immediately succeeding acetylcholine responses. After 10 minutes the angiotensin response was reduced by 70.4 ± 6.4% (n=8) and after 40 minutes by 83.3 ± 4.9% (n=8). The acetylcholine response was reduced by 67.9 ± 5.3% (n=8) after 20 minutes and by 79.9 ± 6.0% (n=8) after 50 minutes. There was no difference between the effect of glucose and calcium free Tyrode upon responses to acetylcholine and angiotensin compared with the effect of calcium free Tyrode with glucose. The osmolarity of both solution was maintained by addition of sucrose to prevent non-specific effects due to changes in osmotic pressure. Exposure of preparations of rat descending colon to Tyrode solution containing calcium but free of glucose had no effect upon induced responses to

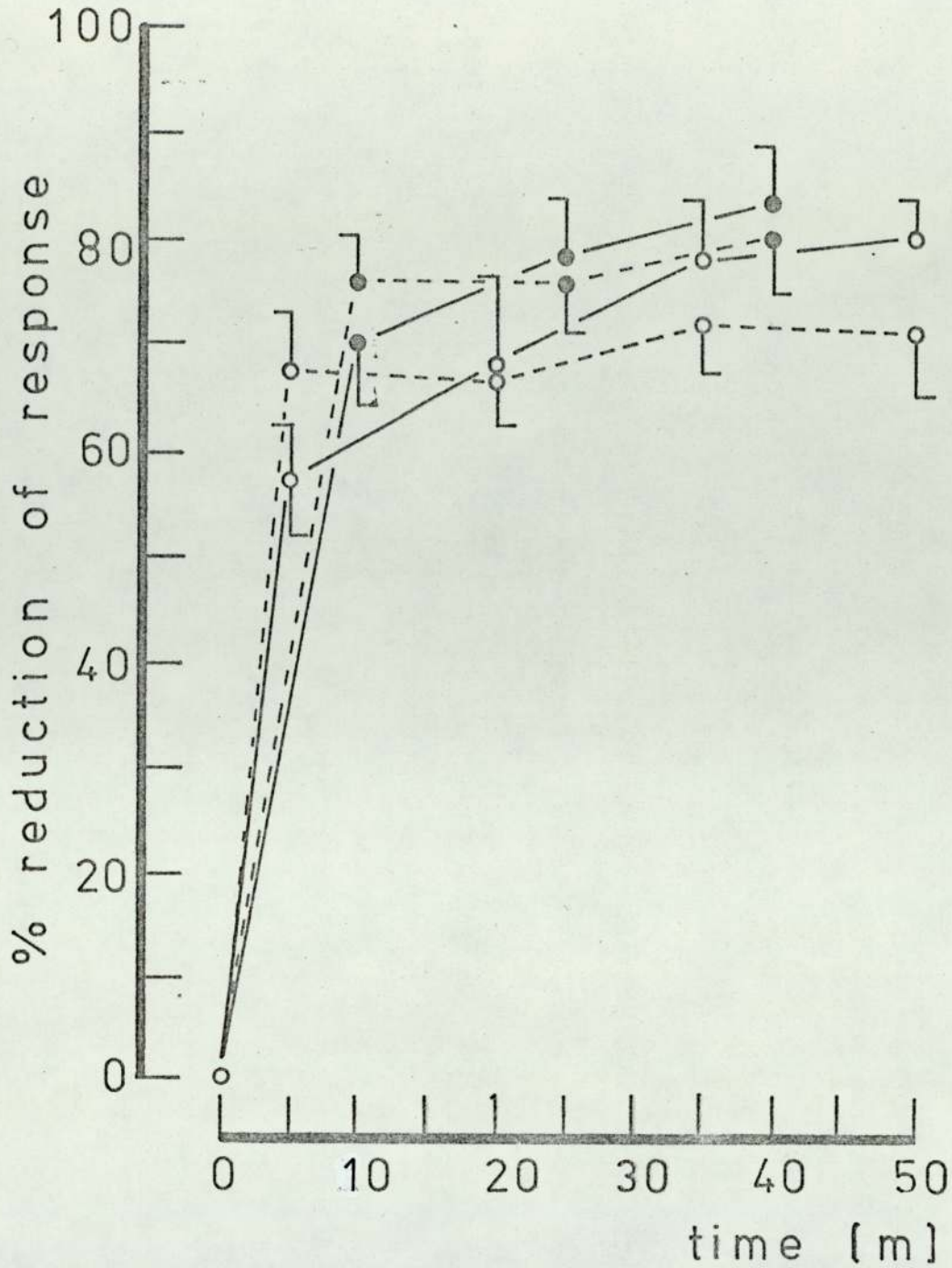

FIGURE 28b. The effect of calcium free Tyrode solution upon isotonic responses of male rat descending colon in the presence and in the absence of glucose. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean. Solid lines denote the inhibition pattern observed in the absence of glucose and broken lines that observed in the presence of glucose.

$p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

acetylcholine and angiotensin and this confirmed earlier observations of the effect of glucose lack and anoxia upon this tissue.

(3) The Effect of Reduced Calcium Concentration upon the Responses of Rat Uterus to Acetylcholine and Angiotensin

The calcium concentration of the Tyrode solution was reduced from the normal concentration of 1.8 mmol and the responses to acetylcholine and angiotensin were recorded on dioestrous uterus (fig. 29) and oestrous uterus (fig. 30).

When the calcium concentration was reduced to 0.9 mmol the percentage reduction of induced responses was greater than had been previously observed with rat descending colon and furthermore, on rat uterus there was no significant difference between the percentage reduction of the angiotensin responses and the succeeding acetylcholine responses. Thus after 35 minutes the angiotensin response of dioestrous uterus was reduced by 51.4 ± 7.4% (n=6) and the following acetylcholine response (at 45 minutes) was reduced by 54.9 ± 3.3% (n=6) ($p > 0.05$). There was no consistent significant difference between the effect of 0.9 mmol calcium Tyrode upon the contractile responses of oestrous uterus (fig. 30) compared with dioestrous uterus (fig. 29).

Similarly when the calcium concentration was reduced to 0.45 mmol the percentage reduction of induced responses was greater than had been observed when rat descending colon was exposed to this solution. Again there was no significant difference between the percentage of angiotensin responses compared with the immediately succeeding acetylcholine responses and there was no significant difference in the responses obtained from dioestrous uterus (fig. 29) compared with oestrous uterus (fig. 30). After 35 minutes the angiotensin response of dioestrous uterus was reduced by 60.2 ± 6.9% (n=6) and the following acetylcholine response (at 45 minutes) was reduced by 65.7 ± 5.2% (n=6). ($p > 0.05$).

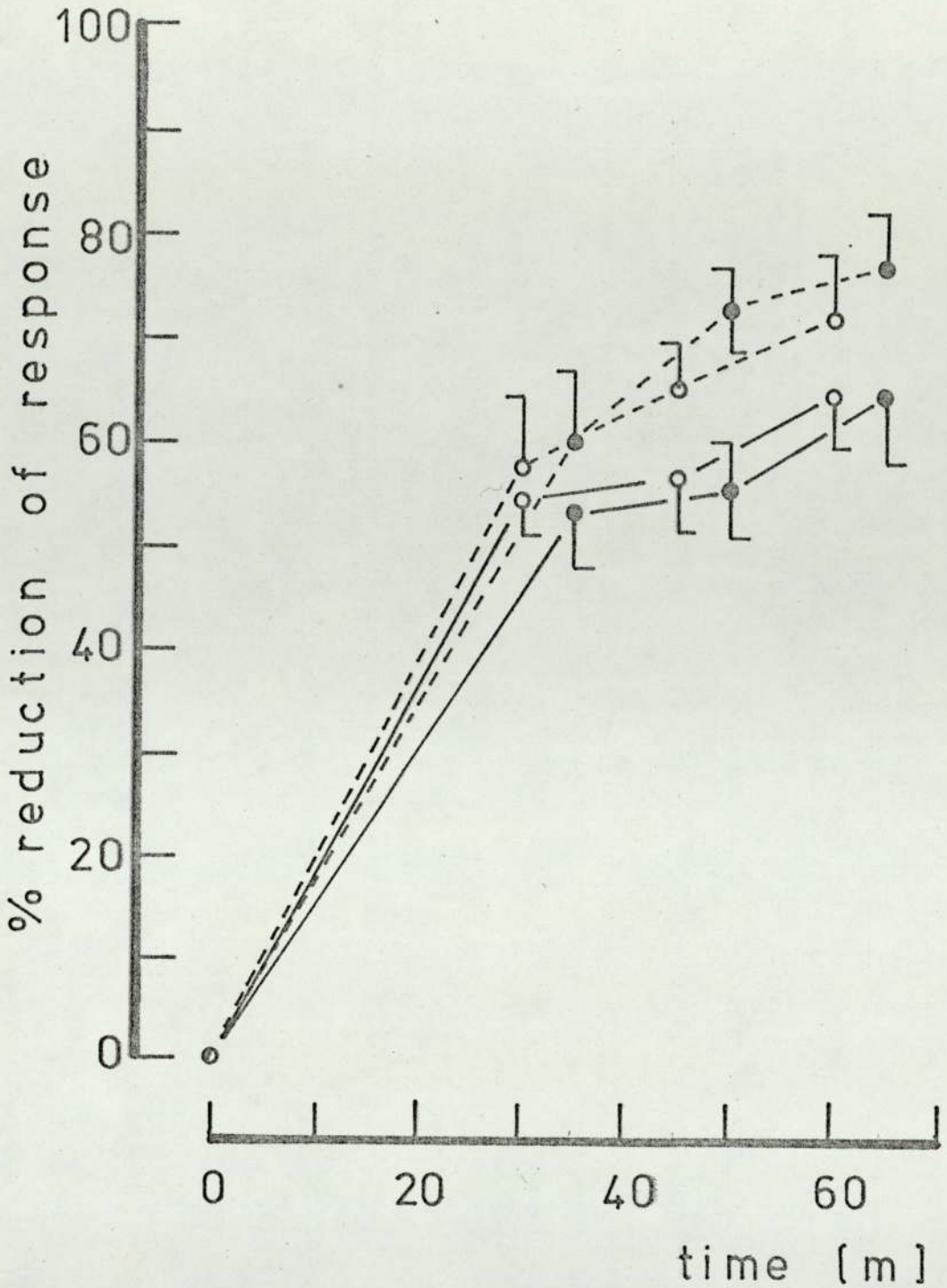

FIGURE 29. The alteration in the response of dioestrous rat uterus to angiotensin and acetylcholine when the calcium concentration of the Tyrode solution was reduced from the normal concentration of 1.8 mmol. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean. Solid lines denote the inhibition pattern produced by a calcium concentration of 0.9 mmol and broken lines that produced by a calcium concentration of 0.45 mmol.

$p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

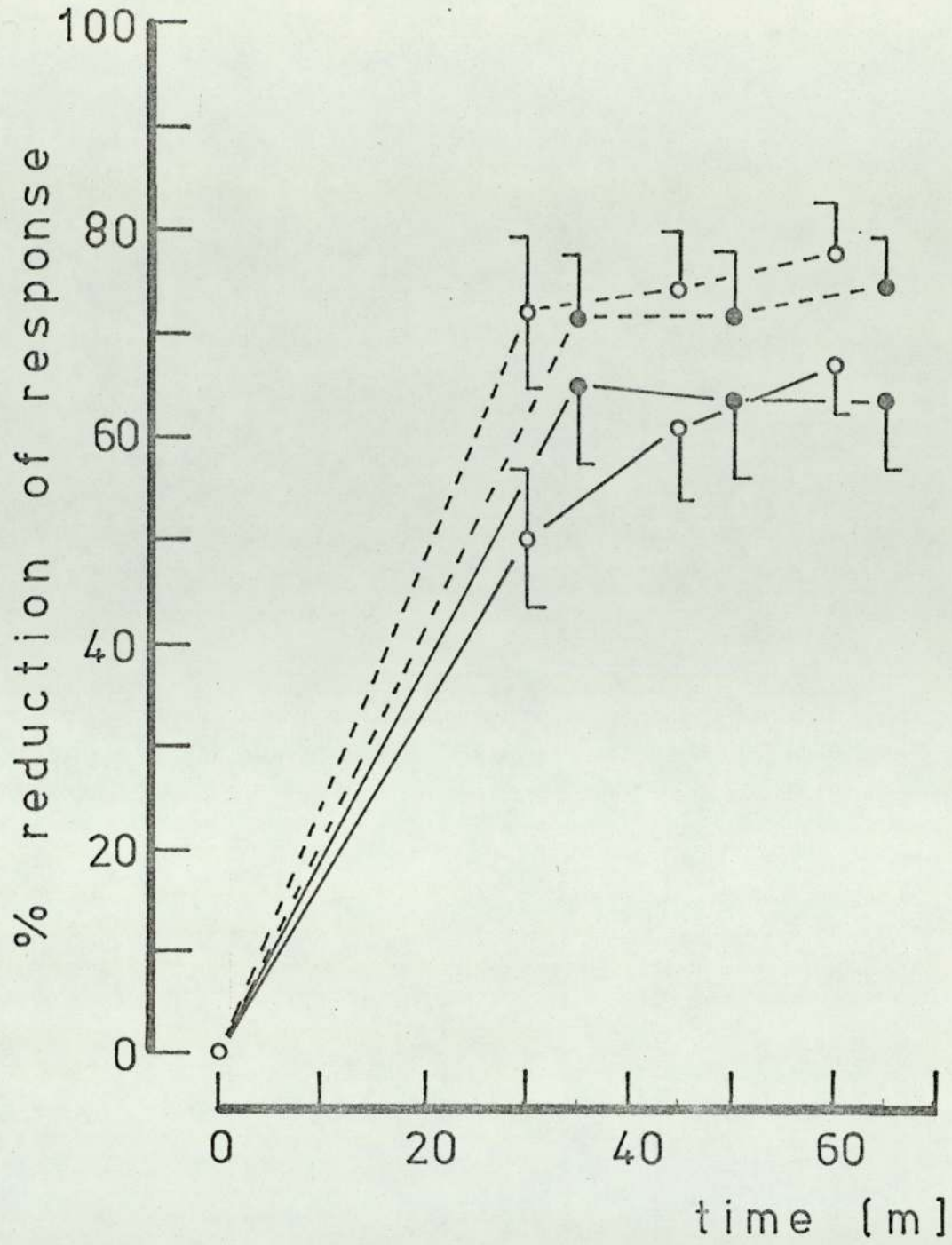

FIGURE 30. The alteration in the response of oestrous rat uterus to angiotensin and acetylcholine when the calcium concentration of the Tyrode solution was reduced from the normal concentration of 1.8 mmol. Mean % reduction of responses to angiotensin (●) and acetylcholine (○) with the standard error of the mean. Solid lines denote the inhibition pattern produced by a calcium concentration of 0.9 mmol and broken lines that produced by a calcium concentration of 0.45 mmol.

$p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the immediately succeeding acetylcholine response.

It appeared, therefore, that rat uterus was far more sensitive to reduced extracellular calcium concentration than rat descending colon.

(4) The Effect of Lanthanum Chloride upon Responses of Rat Descending Colon to Acetylcholine and Angiotensin

The trivalent lanthanum ion has been shown to have a high affinity for superficial membrane binding sites for calcium. By displacement of membrane bound calcium and occupation of calcium transporting sites, the lanthanum ion blocks transmembrane calcium movement so rendering the membrane impermeable to calcium (see reviews by Weiss, 1974; Van Breemen et al, 1973). To further investigate the role of extracellular calcium in the contraction of smooth muscle to angiotensin, preparations of rat descending colon were exposed to a modified Tyrode solution containing lanthanum chloride. The phosphate bicarbonate buffer in normal Tyrode solution was replaced with a tris buffer to prevent precipitation of the insoluble carbonate and phosphate salts of lanthanum. The pH of the solution was maintained in the normal range 7.0 - 7.5 and preliminary experiments were performed to ensure that the change in the composition of the solution had no effect upon responses to acetylcholine and angiotensin.

Several workers have used 10 mmol lanthanum chloride for investigations of isolated smooth muscle (Mayer, Van Breemen & Casteels, 1972; Van Breemen et al, 1973; Marshall & Kroeger, 1973). In the present experiments 10 mmol lanthanum caused precipitation of calcium from solution and completely abolished responses to both acetylcholine and angiotensin. Similar effects were observed with 1 mmol lanthanum, and 0.5 mmol was the highest concentration to allow measurement of induced responses.

Figure 31 shows the percentage reduction of contractile responses to acetylcholine and angiotensin which occurred when

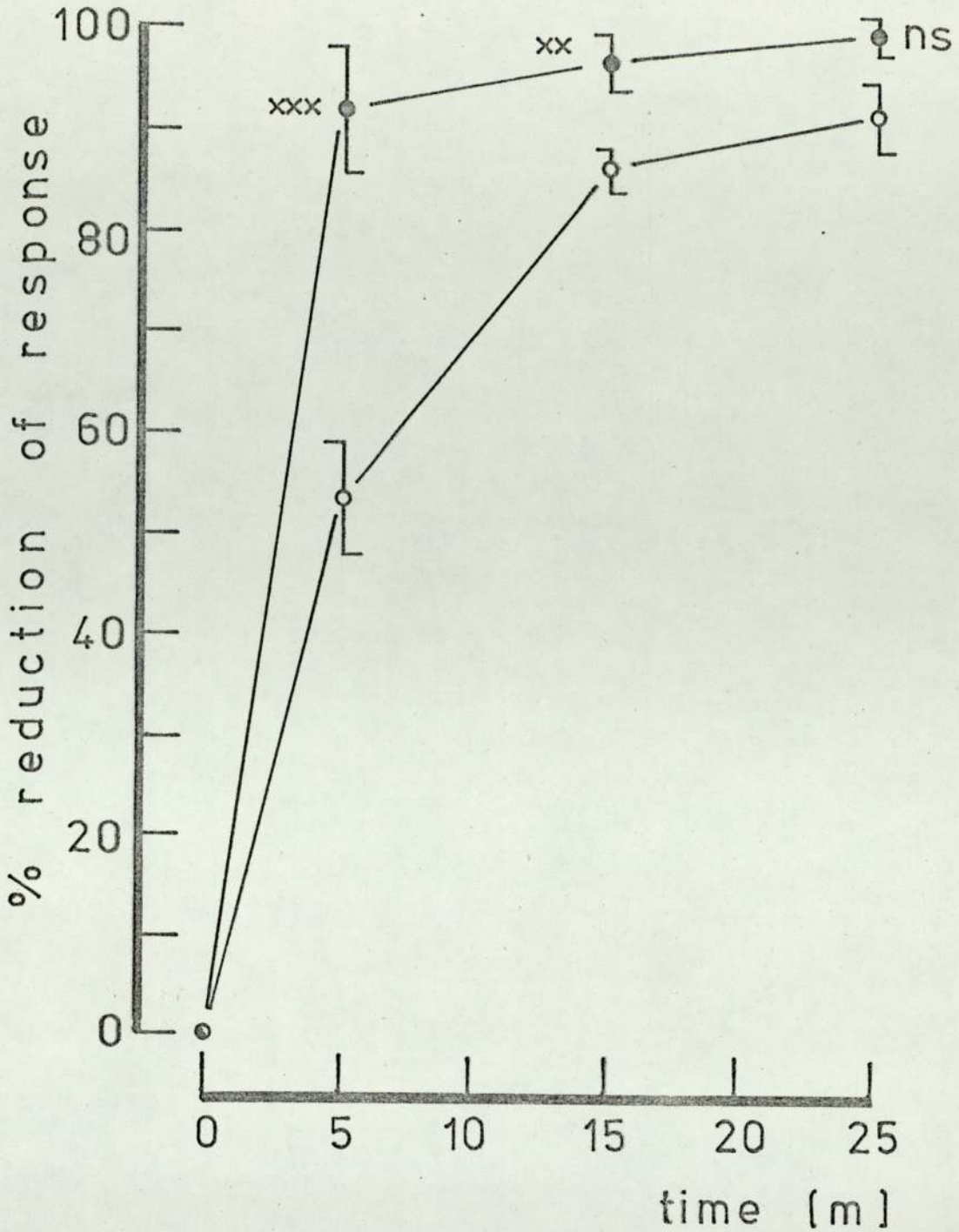

FIGURE 31. The effect of 0.5 mmol lanthanum chloride upon responses of male rat descending colon to angiotensin and acetylcholine. Mean % reduction of angiotensin (●) and acetylcholine (○) responses with the standard error of the mean.

xxx $p < 0.001$ xx $0.001 < p < 0.01$ ns $p > 0.05$ when comparing the percentage reduction of the angiotensin response with that of the corresponding acetylcholine response.

preparations of rat descending colon were exposed to a Tyrode solution containing 0.5 mmol lanthanum chloride. Responses to both agonists were severely impaired but during the first 15 minutes the percentage reduction of angiotensin responses was significantly greater than the percentage reduction of corresponding acetylcholine responses. ($p < 0.01$). After 5 minutes the angiotensin response was reduced by $91.9 \pm 2.5\%$ ($n=6$) and after 15 minutes by $96.7 \pm 2.6\%$ ($n=6$), while the corresponding reductions of the acetylcholine response were $53.6 \pm 6.0\%$ ($n=6$) and $86.5 \pm 2.1\%$ ($n=6$) respectively. After 25 minutes the angiotensin response was reduced by $98.9 \pm 1.1\%$ ($n=6$) and the acetylcholine response was reduced by $91.5 \pm 3.5\%$ ($n=6$).

This marked reduction of contractile responses during exposure to lanthanum suggested that the technique lacked specificity.

DISCUSSION

1. GENERAL INTRODUCTION

It is generally accepted that the angiotensin molecule like that of most other peptide hormones is too large to penetrate cellular membranes and therefore interacts with specific receptor sites on the surface of the cell (Rasmussen, 1969). An attempt has been made to separate this primary interaction of angiotensin with the smooth muscle cells from the resultant activation of the contractile process and to elucidate the mechanism involved. The effect of experimental treatments upon angiotensin responses has been compared with the effect upon acetylcholine responses since the mechanism of action of acetylcholine is well documented.

2. THE METABOLIC REQUIREMENTS FOR THE CONTRACTILE ACTION OF ANGIOTENSIN UPON SMOOTH MUSCLE

(a) Introduction

Energy is necessary both for the contractile process and to maintain the integrity of the cellular structure of the smooth muscle cell. However, it has been suggested that intracellularly produced energy may not be used indiscriminately for all processes and that it may be functionally compartmentalised (Webb, 1966). Prasad & Macleod (1969) and Bueding and co-workers (1967) have reported that the energy for membrane events may be separated from the energy for smooth muscle contraction and may be generated by a different process. Furthermore, since metabolic inhibition would be expected to affect superficial membrane sites before the more deeply located contractile proteins (Detar & Bohr, 1968) this may provide a means of separating the primary interaction of a spasmogen with the cell membrane from the resulting contraction. Since the early work of Rona & Neukirch (1912), there has been an extensive investigation of the energy pathways in isolated smooth muscle but little interest in the energy requirements of individual spasmogens. In the present study, the energy requirements for acetylcholine and angiotensin responses were investigated by exposing isolated smooth muscle preparations to conditions which interfered with normal energy production. The primary action of acetylcholine upon smooth muscle has been reported to involve an increase in the passive permeability to sodium ions (Bolton, 1972; Szurswęski & Bulbring, 1973; Bulbring & Szurswęski, 1974) and to potassium ions (Setekliev, 1970; Bulbring & Szurswęski, 1974), and therefore the energy requirements of an acetylcholine induced contraction are those of the muscle contraction process. In the present study, responses to acetylcholine were used as an indication of the ability of the muscle to contract under conditions where energy production was impaired and thus a greater reduction of the angiotensin response would indicate the involvement

of an energy dependent mechanism in the angiotensin contraction distinct from the contractile process.

(b) The Metabolic Requirements for the Contractile Action of Angiotensin upon Guinea-Pig Ileum

Isolated guinea-pig ileum has been used extensively for the investigation of the contractile action of angiotensin and the regular and repeatable responses have been shown to be dose dependent (Gross & Turian, 1960; Bisset & Lewis, 1962; Regoli & Vane, 1964). In the present study the energy dependence of the angiotensin response of guinea-pig ileum was compared with the energy dependence of the acetylcholine responses.

The contractile action of angiotensin upon guinea-pig ileum has been shown to consist of two components, a direct one and an indirect one via parasympathetic nervous elements (Khairallah & Page, 1961; Robertson & Rubin, 1962; Godfraind, Kaba & Polster, 1966). In the first series of experiments no attempt was made to separate these components, either by the use of chemical inhibitors (Khairallah & Page, 1961; Robertson & Rubin, 1962), or by the selection of recording techniques (Godfraind et al, 1966). Therefore, the angiotensin response that was recorded and measured, was the combination of the individual contributions of these two components and this was compared with the response to acetylcholine under the same experimental conditions. It has been shown that the contractile action of acetylcholine upon guinea-pig ileum is produced by direct stimulation of the smooth muscle cells with no significant contribution from the intramural nervous elements (Robertson & Rubin, 1962; Day & Vane, 1963).

The effect of withdrawing glucose (Prasad, 1935b; Feldberg & Solandt, 1942; Furchgott & Wales, 1951; Axelsson, Hogberg & Timms, 1965; Coe, Detar & Bohr, 1968) and oxygen (Gross & Clark, 1923; Garry, 1928; Prasad, 1935b; West, Hadden & Farah, 1951; Detar & Bohr,

1972) from in vitro smooth muscle preparations has been investigated extensively and the reduction in mechanical activity, both spontaneous and induced, has been attributed to an impairment of energy processes within the muscle. Therefore, the effect of these treatments upon responses of guinea-pig ileum to angiotensin and acetylcholine was investigated with respect to time.

Removal of glucose from the Tyrode solution was found to cause a preferential reduction of the angiotensin response compared with the acetylcholine response which indicated that glucose was involved in certain stages of the angiotensin response that have no parallel in the acetylcholine response. The possibility that glucose was required as a source of energy for part of the angiotensin interaction with smooth muscle cells was investigated using nitrogen. Although there was a marked reduction of the angiotensin response, the responses to acetylcholine were unaffected by anoxia which confirmed the report of Day and Vane (1963). Since glucose was present, anaerobic glycolysis would be expected to function but the reduction of the angiotensin response indicated that this was not an adequate energy source. It appeared that aerobic metabolism was necessary to support the angiotensin response.

These findings were confirmed by the use of carbon monoxide which is a very specific inhibitor of cytochrome oxidase (Dixon & Webb, 1960) and therefore blocks the production of ATP by oxidative phosphorylation. Exposure of tissues to carbon monoxide resulted in a large reduction of the response to angiotensin. However, this was not due to an impairment of the ability of the muscle to contract since responses to acetylcholine were unaffected, an observation which has been reported for rat uterus (Coceani & Wolfe, 1966). It was therefore concluded that ATP was involved in the angiotensin response at some stage prior to the contraction process.

In this and in all other experiments, the recovery of the responses to acetylcholine and angiotensin were investigated to ensure that there was no permanent damage to the tissue which might lead to a non-specific reduction of induced contractions. No treatment produced any indication of tissue damage during the exposure times used, although all forms of metabolic inhibition have been shown to do so when these exposures are extended (Feldberg & Solandt, 1942; Garry, 1928; Gross & Clark, 1923; Prasad, 1935b; West et al, 1951). The difference between the rates of recovery for acetylcholine and angiotensin appeared to be a reflection of the initial reduction in the responses produced by the experimental treatments.

All these experiments involved a study of both components of the angiotensin response, i.e. direct and indirect, although the final mediator of the indirect response is acetylcholine, which was little reduced by any of the treatments. However, the nervous transmission giving rise to its release could well be effected, and inactivation of nervous tissue has been reported both under conditions of anoxia (Gross & Clark, 1923; Garry, 1928; West et al, 1951; Day & Vane, 1963) and during cooling (Blair & Clark, 1956; Innes, Kosterlitz & Robinson, 1957). It was therefore necessary to ensure that the treatments were affecting the direct component of the angiotensin response.

Hyoscine and other muscarinic blocking agents have been shown to abolish the indirect cholinergic component of the contractile response of guinea-pig ileum to certain spasmogens (Garven, 1956; Kosterlitz & Robinson, 1958; Day & Vane, 1963). In the present experiments, a concentration of 2×10^{-7} mol hyoscine hydrobromide reduced control responses of guinea-pig ileum by 50-60%, which confirmed reports by other workers (Khairallah & Page, 1961; Godfraind et al, 1966), and abolished both control and maximal acetylcholine responses. The effect of glucose removal from a Tyrode solution

containing 2 x 10^{-7}mol hyoscine was therefore investigated upon responses of guinea-pig ileum to angiotensin and histamine. There was a greater reduction of the angiotensin responses than of the histamine responses which confirmed that the experimental treatments did affect the direct component of the angiotensin response. Hyoscine had little affect upon contractile responses to histamine which confirmed reports that histamine acts directly upon the smooth muscle cells of the guinea-pig ileum (Feldberg, 1951; Ambache & Lessin, 1955; Kosterlitz & Robinson, 1958; Innes et al, 1957; Day & Vane, 1963).

A series of experiments were also performed in which ileal segments were electrically stimulated through co-axial electrodes. Removal of glucose from the Tyrode solution for 30 minutes produced only a 17% reduction in the contractions. Since the contractions of co-axially stimulated guinea-pig ileum are largely due to activation of parasympathetic nervous elements (Paton, 1955; Day & Vane, 1963), these experiments provided further evidence that the reduction in the angiotensin responses produced by metabolic inhibition were not solely due to inactivation of the indirect cholinergic mechanism. In addition, it was shown that 2 x 10^{-7}mol hyoscine was effective in inhibiting electrically induced contractions and it may therefore be presumed that the same concentration would also be effective in inhibiting the indirect component of the angiotensin response.

These experiments suggested that the interaction of angiotensin with the smooth muscle cell is dependent upon an energy supply which is separate from that required for the contractile process. Although it appeared that the energy was required for the direct action of angiotensin upon guinea-pig ileum, it was difficult to exclude concomitant effects of the experimental treatments upon the indirect cholinergic component. Furthermore, it is unsatisfactory to rely upon anti-muscarinic agents for abolition of the indirect component since

these agents may have unspecific effects which are not related to their action at the acetylcholine receptor. The investigation was therefore extended to smooth muscle preparations where the action of angiotensin has been shown wholly direct.

(c) THE METABOLIC REQUIREMENTS FOR THE CONTRACTILE ACTION OF ANGIOTENSIN UPON RAT UTERUS AND RAT DESCENDING COLON

The contractile action of angiotensin has been shown to be wholly direct on rat descending colon (Regoli & Vane, 1964; Ellis & Reit, 1969) and rat uterus (Khairallah & Page, 1961; Regoli & Vane, 1964).

Anoxia and glucose lack separately failed to produce the differential reduction of the angiotensin response of rat colon that had been observed with guinea pig ileum. Similarly, responses rat dioestrous and oestrous uterus to angiotensin and acetylcholine were unaffected by anoxia or glucose lack separately. This failure to confirm the previous findings may have been due to differences in tissue metabolism which made it difficult to demonstrate the same effects. This is supported by many reports in the literature showing that tissues do vary in their susceptibility to metabolic inhibition. Other workers have also observed contractions of isolated smooth muscle preparations both under anaerobic conditions in the presence of glucose (Gross & Clark, 1923; Prasad, 1935b; West, Hadden & Farah, 1951; Day & Vane, 1963; Hughes & Coret, 1965; Detar & Bohr, 1972; Furchgott, 1966; Shibita & Briggs, 1967) and under aerobic conditions in the absence of glucose (Prasad, 1935b; Furchgott & Wales, 1951; Axelsson et al, 1965; Furchgott, 1966; Shibita & Briggs, 1967; Hughes & Coret, 1968; Coe, Detar & Bohr, 1968; Rangachari et al, 1972). Presumably in rat colon and rat uterus enough energy was generated by anaerobic glycolysis of exogenous glucose to support the contractile responses to both agonists under conditions of anoxia or by aerobic metabolism of endogenous glycogen and βoxidation of fatty acids after removal of glucose from the Tyrode solution.

When anoxia and glucose lack were combined differences were obtained between the responses of the four tissues to both agonists

but again no differential reduction of the angiotensin response was observed. The responses of dioestrous uterus and oestrous colon and dioestrous uterus to acetylcholine and angiotensin were reduced by the combined treatment whereas the responses of oestrous uterus were unaffected. Again these findings supported the idea that the problem of demonstrating a differential reduction of the angiotensin response might be related to differences in the metabolism of these tissues. It has been shown that isolated smooth muscle preparations derive energy from carbohydrate metabolism and β oxidation of fatty acids (Furchgott & Shorr, 1946; Coe, Detar & Bohr, 1968; Dhalla & Olson, 1967). It is also known that concentrations of ATP and creatine phosphate are low in both uterine (Walaas & Walaas, 1950; Menkes & Cspao, 1952; Cspao & Gergely, 1950) and intestinal smooth muscle (Born, 1956; Dwaraczek & Barenscheen, 1937). Therefore the ability of a preparation to respond under conditions of metabolic inhibition is a reflection of its ability to generate energy by the metabolic pathways operative under these conditions. It has been demonstrated that many isolated smooth muscle preparations are unable to maintain contractility under conditions of anaerobic substrate depletion (Gross & Clark, 1923; Garry, 1928; Prasad,1935b; West et al, 1951; Furchgott, 1966; Shibita & Briggs, 1967; Rangachari et al, 1972) when the tissues would be dependent upon anaerobic glycolysis of endogenous glycogen.

Therefore, in the present experiments, the marked effect of anaerobic substrate depletion on the responses of rat colon and dioestrous uterus compared with the lack of effect on the responses of oestrous uterus might be a reflection of differences in the energy produced by these tissues from anaerobic glycolysis of endogenous glycogen stores. Thus either oestrous uterus contained a larger amount of glycogen than dioestrous uterus or colon, or the glycolytic pathway was more active in the oestrous uterus. However, both descending colon and dioestrous

uterus were capable of generating sufficient energy to maintain contractions under anaerobic conditions in the presence of glucose. It seemed probable, therefore, that the activity of the anaerobic glycolytic pathway was not the limiting factor and that differences in energy production under conditions of anoxia and glucose lack could be attributed to differences in the available glycogen stores.

An analysis of the glycogen content of rat uterus revealed that the glycogen level of oestrous uterus was significantly higher than that of dioestrous uterus. This confirmed reports that uterine glycogen varies during the oestrous cycle (Boettiger, 1946; West, Jones & Loomis, 1953; West & Cervoni, 1955). The glycogen content of rat descending colon was found to be significantly lower than that of dioestrous uterus but was independent of either the stage of the oestrous cycle or the sex of the animal. Thus oestrous uterus which was unaffected by anaerobic substrate depletion, contained significantly more glycogen than either dioestrous uterus or descending colon, both of which were inhibited by anaerobic substrate depletion. Furthermore, descending colon contained significantly less glycogen than oestrous uterus and was more rapidly inhibited by anaerobic substrate depletion.

Thus the differences in the responses of rat colon and rat uterus after metabolic inhibition might be related to differences in their metabolism which in turn might account for the failure to confirm the findings on guinea-pig ileum. This is illustrated by the observation that rat descending colon was insensitive to conditions of metabolic inhibition which had reduced the responses of guinea-pig ileum and revealed the differential reduction of the angiotensin response. These findings with colon are supported by reports from several workers that there is a metabolic gradient down the length of the intestine (Alvarez, 1922; Evans, 1923; Prasad, 1935; Dickens & Weil-Malherbe, 1941; Farah, West & Angel, 1950; Sherrat, 1968), and that colon preparations are

far less dependent upon oxidative metabolism than preparations of the small intestine (Prasad, 1935b; Farah et al, 1950).

In the experiments where preparations of rat colon and uterus were exposed to anoxia, nitrogen was substituted for the normal air supply to the tissues so that the degree of anoxia produced was liable to variation and was probably never complete because of the difficulty of removing all dissolved oxygen from the Tyrode solution. There have been several reports which demonstrate that the effect of anoxia is markedly dependent upon the actual reduction in pO_2 achieved (Detar & Bohr, 1968a and 1968b; Detar & Bohr, 1972; Namm & Zucker, 1973). To overcome this problem, a chemical inhibitor of oxidative metabolism, 2,4-dinitrophenol, was used. 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 (West et al, 1951; Farah et al, 1950; Born & Bulbring, 1955; Daniel, 1964b; Rangachari et al, 1972; Greenberg, Wilson & Long, 1973). Preparations of rat descending colon and uterus were exposed to a Tyrode solution containing 2,4-dinitrophenol with glucose as a substrate for anaerobic glycolysis. There was a progressive reduction of the responses of oestrous and dioestrous colon and dioestrous uterus to acetylcholine and angiotensin, but at all times the percentage reduction of the angiotensin response was significantly greater than that of the corresponding acetylcholine response. With oestrous uterus, however, there was a slow reduction of responses to both acetylcholine and angiotensin with no preferential reduction of the angiotensin response. This difference between the effect of dinitrophenol upon oestrous uterus and that on dioestrous uterus and descending colon was consistent with the biochemical changes which occur in the uterus during the course of the oestrous cycle. Several workers have reported that anaerobic glycolysis is more

efficient in the oestrous uterus than in the dioestrous uterus (Kerly, 1937 and 1940; Walaas, Walaas & Löken, 1952). In addition, it is known that the relative efficiences of anaerobic and aerobic carbohydrate metabolism in uterine tissue varies during the oestrous cycle (Kerly, 1937). Anaerobic metabolism, the most important energy yielding process (West et al, 1953), is at maximum efficiency in the early oestrous uterus when aerobic metabolism is at minimum efficiency. Inhibition of aerobic metabolism would therefore be expected to have less affect upon the overall capacity of oestrous uterus to generate energy than it would on dioestrous uterus. The capacity of oestrous uterus to generate energy ATP anaerobically might then mask the differential reduction of the angiotensin response seen in dioestrous uterus and descending colon.

The reduction of the induced responses of oestrous uterus by 2,4-dinitrophenol contrasted with the lack of effect produced by combined substrate depletion and anoxia. Dinitrophenol was used in the presence of glucose and thus it would be expected that the reduction of responses would be less than the reduction produced by anaerobic substrate depletion if the dinitrophenol acted solely as an uncoupling agent. Other workers have reported an inhibitory action of 2,4-dinitrophenol upon anaerobic contractions of smooth muscle and this has been attributed to interference with the formation of energy rich phosphate bonds (West et al, 1951; Farah et al, 1950).

Exposure of preparations of rat dioestrous uterus and oestrous and dioestrous rat colon to 2,4-dinitrophenol produced a preferential reduction of the angiotensin response and therefore provided further evidence for energy dependence of the angiotensin contraction. Since the contractile action of angiotensin on both tissues is wholly direct, these experiments confirm the findings with guinea-pig ileum that the

energy dependent stage is involved in the direct interaction of the peptide with the smooth muscle cells. However, it is evident that the energy for this stage of the angiotensin response may be supplied by aerobic or anaerobic metabolism, depending upon the tissue. The contractile action of angiotensin upon guinea-pig ileum appears to be dependent upon energy from aerobic metabolism and this also appears to be the major source in the dioestrous uterus and oestrous and dioestrous colon. On oestrous uterus, however, the additional energy for the angiotensin response appears to be supplied by either aerobic metabolism or anaerobic metabolism.

(d) THE EFFECT OF METABOLIC INHIBITION UPON THE MAINTENANCE OF INDUCED CONTRACTIONS OF RAT DESCENDING COLON

It has been reported that removal of glucose or inhibition of oxidative metabolism reduces the ability of smooth muscle preparations to sustain induced contractions, even in the continued presence of the agonist (Feldburg & Solandt, 1942; Born & Bulbring, 1955; Born, 1956; West et al, 1951; Eckenfels & Vane, 1972). Born (1956) suggested that the development of tension by smooth muscle involves two mechanisms, one responsible for the immediate rise in tension which occurs when the muscle is stimulated and a second which is responsible for the sustained tension following stimulation. There is now considerable evidence that the initial rapid contraction is due to release of intracellular calcium and the sustained contraction is due to influx of extracellular calcium (Bohr, 1964; Godfraind & Kaba, 1969 and 1972; Sitrin & Bohr, 1971; Hurwitz & Suria, 1971; Van Breemen et al, 1973; Ohashi, Ohga & Saito, 1973; Deth & Van Breemen, 1974). However, considerable variation has been reported both between different spasmogens and between the action of an individual spasmogen on different tissues. Eckenfels and Vane (1972) have suggested that the second, sustained phase of contraction may be due to increased synthesis and release of prostaglandins within the smooth muscle preparation whereas the initial rapid phase is the result of direct interaction of the spasmogen with the smooth muscle cell.

In the present study contractions to acetylcholine and angiotensin were measured at the maximum maintained level during the time of contact with the tissue. During metabolic inhibition there was a small but variable difference in the maintained contraction to acetylcholine and the initial contraction. Since the contractile response to angiotensin was slow to develop, there was no initial rapid phase and consequently no detectable change in the maintenance of the response during metabolic inhibition. However, the contractile

response of isolated smooth muscle preparations to potassium (40 mmol) has been reported to consist of two phases, the second of which is dependent upon energy metabolism and is abolished during metabolic inhibition (Urakawa & Holland, 1964; Pfaffman et al, 1965; Nasu & Urakawa, 1973). Previous experiments had revealed that the angiotensin response was dependent upon energy and therefore the possible relationship between the angiotensin contraction and the second phase of the potassium contraction was investigated.

Contractions of rat descending colon to potassium (40 mmol) were measured as the initial tension on addition of the agonist (phasic component) and the final tension after 90 seconds exposure (tonic component). Glucose free Tyrode solution combined with anoxia or Tyrode containing 0.1 mmol 2,4-dinitrophenol, caused a progressive reduction of both components but at all times the reduction of the tonic component was significantly greater than that of the phasic component. This confirmed the reports that the tonic component is energy dependent (Urakawa & Holland, 1964 etc). Under conditions of glucose lack and anoxia, there was no significant difference between the percentage reduction of angiotensin responses and the corresponding tonic potassium responses but the percentage reduction of angiotensin responses was significantly greater than the percentage reduction of the corresponding phasic potassium responses. When angiotensin was added during a maintained potassium contraction in normal Tyrode, there was a further contraction which confirmed reports that the contractile action of angiotensin is unaffected by membrane depolarisation (Shibita & Briggs, 1966; Khairallah et al, 1965; Shibita et al, 1968; Sullivan & Briggs, 1968). After 15 minutes exposure to glucose free Tyrode and anoxia the tonic maintained potassium response was almost abolished and addition of angiotensin failed to elicit a further contraction.

These findings with anoxia and no glucose suggested that the energy dependence of the tonic phase of the potassium response on rat colon was similar to the energy dependence of the angiotensin response and one possibility is that both responses involve the same energy dependent mechanism. The tonic potassium contraction has been reported to involve an active influx of extracellular calcium (Urakawa & Holland, 1964) which may be linked to active sodium movement (Pfaffman et al, 1965) while there have been several reports that the contractile action of angiotensin involves an influx of extracellular calcium (Villamil, 1972; Godfraind, 1970; Shibita, Carrier & Frankenheim, 1968; Sullivan & Briggs, 1968; Deth & Van Breemen, 1974). However, during exposure to a more specific metabolic inhibitor, 2-4 dinitrophenol, there was a significantly greater reduction of angiotensin responses compared with the corresponding tonic potassium responses, which was observed both with normal responses of 90 second duration and with prolonged responses of three minutes duration. It therefore appears that even during extended contractions, more energy is required for the angiotensin response than for the maintenance of the potassium response. This suggests that although energy is required for both angiotensin and potassium responses of smooth muscle the actual energy dependent mechanisms may be different. This hypothesis is supported by the results of a further experiment in which potassium responses were elicited in the presence of sub-contractile concentrations of angiotensin. There was an equal potentiation of both phases of the potassium response but no preferential effect upon the tonic response.

Further investigations are required to establish the relationship between the energy dependent angiotensin and tonic potassium responses of smooth muscle preparations, but the present study demonstrates that, under certain conditions of energy limitation, the two responses may be differentiated

(e) A PRELIMINARY STUDY OF THE MOBILISATION OF TISSUE GLYCOGEN IN RAT DESCENDING COLON AND UTERUS, AND THE EFFECT OF ANGIOTENSIN AND ACETYLCHOLINE

It has been reported that the stores of high energy phosphate compounds in smooth muscle are inadequate to support even a single contraction (Lundholm & Mohme-Lundholm, 1962 and 1965; Furchgott, 1966) and therefore energy must be supplied by a simultaneous increase in cellular energy metabolism. Both a stimulation of glycogenolysis (Lundholm & Mohme-Lundholm, 1962 and 1963a and b) and an activation of phosphorylase (Diamond & Brody, 1966; Diamond, 1973) have been observed during aerobic and anaerobic contractions of smooth muscle. Since previous findings had suggested that the angiotensin contraction of smooth muscle required more energy than the acetylcholine contraction it was possible that addition of angiotensin to smooth muscle produced a greater stimulation of metabolism than the addition of acetylcholine. Preliminary experiments were therefore performed to investigate the effect of contractor concentrations of angiotensin and acetylcholine upon the glycogen content of rat descending colon and rat uterus maintained under aerobic conditions. Since this study is still incomplete and has to be extended to include preparations maintained under anaerobic conditions, it has not been included in the results section. However, it is interesting to consider the preliminary findings since they suggest that in rat uterus not only the amount of glycogen, but also its availability, varies during the oestrous cycle.

During a 2 hour exposure to normal Tyrode solution there was no change in the glycogen content of preparations of descending colon from either oestrous or dioestrous rats. Addition of angiotensin (10^{-8}mol) or acetylcholine (10^{-6}mol) had no consistent effect upon the glycogen content and even prolonged contractions were not accompanied by increased glycogenolysis. Angiotensin also failed to affect the glycogen content of preparations of rat uterus from

oestrous or dioestrous animals and so again contraction was not accompanied by increased glycogenolysis. These results confirm reports that in smooth muscle, unlike skeletal muscle, contraction is not consistently associated with enhanced glycogen breakdown (Lundholm & Mohme-Lundholm, 1963b). However, no evidence was obtained to support the hypothesis that angiotensin might cause a greater stimulation of energy metabolism than acetylcholine.

Although there was no change in the glycogen content of dioestrous uterus and oestrous and dioestrous colon during a 2 hour exposure to normal Tyrode solution, there was a significant reduction in the glycogen content of oestrous uterus. Thus after 60 minutes incubation in Tyrode the glycogen content of oestrous uterus was reduced by 20.4 ± 3.2% (n=13) which was significantly greater ($p < .001$) than the reduction in the glycogen content of dioestrous uterus, 4.4 ± 1.9% (n=10), dioestrous colon, 5.2 ± 3.4% (n=20), or oestrous colon, 8.2 ± 2.3% (n=21). In previous experiments metabolic inhibition was shown to have less effect upon induced contractions of oestrous uterus than upon the induced contractions of dioestrous uterus and descending colon and this was attributed to differences in the glycogen stores. These results suggest that a further reason for the resistance of oestrous uterus to metabolic inhibition may be the existence of a mobile store of glycogen readily available for glycogenolysis. This is consistent with a report by West and Cervoni (1955) who described a critical glycogen threshold for rat uterus at which the maintenance of contractility under anaerobic substrate free conditions became critical. They suggested that the glycogen content of oestrous uterus was above the threshold and thus this tissue was relatively unaffected by conditions of metabolic inhibition that rapidly inhibited responses

of the immature uterus, where glycogen content was only just above the threshold. Further experiments are required to determine the change in glycogen content under anaerobic conditions before definite conclusions can be drawn from this study.

3. THE ROLE OF PROSTAGLANDINS AND CYCLIC AMP IN THE CONTRACTILE ACTION OF ANGIOTENSIN UPON SMOOTH MUSCLE

The action of many large molecular weight peptide hormones has been shown to be mediated via changes in intracellular cyclic AMP (reviews: Birnbauer et al, 1970; Sutherland & Robison, 1966; Robison, Butcher & Sutherland, 1965 and 1968; Sutherland, Oye & Butcher, 1965; Rasmussen, 1970). More recently it has been suggested that prostaglandins may also be involved in the action of certain hormones although the relationship remains controversial (Butcher, 1970; Kuehl et al, 1972; Bergstrom, 1967; Horton, 1969). Since neither cyclic AMP nor prostaglandins are stored in tissues, their release by a hormone necessitates a stimulation of synthesis. Thus an involvement of cyclic AMP or prostaglandins in the action of angiotensin upon smooth muscle might be the reason for energy dependence of the angiotensin response observed in previous experiments. This possibility was investigated by examining the effect of theophylline, an inhibitor of cyclic Amp hydrolysis, and indomethacin, an inhibitor of prostaglandin synthesis, on the contractile responses to angiotensin and acetylcholine.

More recently there has been interest in the possible role of cyclic GMP in smooth muscle contraction (Dunham et al, 1974; Bar, 1974), but this has not been considered in the present study.

(a) PROSTAGLANDINS

It has been suggested that intramural prostaglandin synthesis is important in the maintenance of smooth muscle tone (Ferreira, Herman & Vane, 1972; Eckenfels & Vane, 1972; Bennet & Posner, 1971) and may contribute to the maintenance of induced contractions (Eckenfels & Vane, 1972). The synthesis of prostaglandins has been shown to be dependent upon molecular oxygen (Samuelsson, Granstrom & Hamberg, 1967; Nugteren, Beerthuis & Van Dorp, 1967) and furthermore, the contractile action of prostaglandins upon isolated smooth muscle is markedly dependent upon energy (Chandler & Strong, 1972; Greenberg et al, 1973). In previous experiments the contractile action of angiotensin upon rat descending colon and rat uterus was shown to involve an energy dependent stage not present in the acetylcholine response and therefore, distinct from the contractile process. A similar finding has also been reported for the contractile action of prostaglandin E1 on rat uterus (Paton & Daniel, 1967) and rat stomach strip (Coceani & Wolfe, 1966) and it was shown that metabolic inhibition caused a greater reduction of the contractile responses of these tissues to prostaglandin than to acetylcholine. Since angiotensin has been shown to stimulate the release of prostaglandin like substances from the kidney (McGiff et al, 1970; Aiken & Vane, 1971 and 1973; Gagnon et al, 1974) and the spleen (Ferreira, Moncada & Vane, 1973; Douglas et al, 1973) it was possible that the observed energy dependence of the angiotensin induced responses of smooth muscle might also be due to release of prostaglandins.

The non-steroidal anti-inflammatory agent indomethacin has been shown to be a potent inhibitor of prostaglandin synthesis (Vane, 1971; Ferreira, Moncada & Vane, 1971; Flower & Vane, 1974; Flower, 1974) and in concentrations of 3-6 μmol inhibits prostaglandin synthesis in isolated smooth muscle preparations (Ferreira et al, 1972;

Eckenfels & Vane, 1972; Flower, 1974). The effect of indomethacin was therefore investigated upon the contractile responses of rat descending colon and rat uterus to acetylcholine and angiotensin.

Experiments with rat colon confirmed the findings of other workers (Eckenfels & Vane, 1972) that low concentrations of indomethacin (3 μmol and 6 μmol) have no effect upon the tone or spontaneous activity of this preparation. Higher concentrations (30 μmol and 60 μmol) reduced spontaneous activity but had little effect upon either the maintenance of induced contractions or the tone. Again this confirmed the suggestion that in rat colon prostaglandin synthesis makes little contribution to tone or contractility (Eckenfels & Vane, 1972). At a concentration of 30 μmol, indomethacin caused an equal reduction of responses to acetylcholine and angiotensin but an increase in the concentration to 60 μmol indomethacin produced no further reduction in the responses to either agonist. These results suggest that the energy dependence of the angiotensin response observed in previous experiments on rat colon cannot be attributed to a mediation via prostaglandin synthesis. Furthermore, the high concentrations of indomethacin necessary to reduce induced contractions suggests that it is not acting by an inhibition of prostaglandin synthesis.

On dioestrous uterus low concentrations of indomethacin again had no effect upon contractile responses to acetylcholine or angiotensin and caused no reduction in tone or spontaneous activity. Higher concentrations of up to 0.3mmol indomethacin caused a reduction of responses to both agonists but there was a consistent and significant difference between the reduction of the angiotensin response and the reduction of the acetylcholine response. However, even at 0.3 mmol, indomethacin had no effect upon the maintenance of the induced contractions or the tone of the smooth muscle although there was a reduction of spontaneous activity. These findings do not support

the suggestion that prostaglandin release is important for the maintenance of smooth muscle tone and for the maintenance of induced contractions (Eckenfels & Vane, 1972). Furthermore, the concentration of indomethacin necessary to affect responses to angiotensin and acetylcholine was far higher than that reported to inhibit prostaglandin synthesis in isolated smooth muscle. Since the inhibitory action of indomethacin was also dose dependent, this suggested that the action of indomethacin was unlikely to be upon prostaglandin synthesis.

Inhibition of smooth muscle contraction by high concentrations of indomethacin (0.3-0.6 mmol) has also been reported by Northover (1967, 1971). The effect was observed on contractions to several agonists and during electrical stimulation and therefore appeared to be non-specific. In later experiments he demonstrated that these concentrations of indomethacin caused inhibition of calcium uptake by the smooth muscle and reduced calcium binding to microsomal membranes (Northover, 1972 and 1973). It is therefore possible that the reduction of responses to acetylcholine and angiotensin observed during exposure of rat colon and uterus to indomethacin was due to an effect upon calcium movement. Since Northover has reported considerable differences between the sensitivity of different smooth muscle preparations to indomethacin, an action upon calcium movement may explain why a differential reduction of the angiotensin and acetylcholine responses was only observed on rat uterus. This will be discussed more fully later in the discussion.

These findings with indomethacin do not support the hypothesis that the contractile action of angiotensin upon smooth muscle might be mediated via a stimulation of prostaglandin synthesis and release. This is supported by the report that angiotensin induced vasoconstriction in the hindlimb of the dog is not associated with prostaglandin release (Aiken & Vane, 1973). Furthermore the release of prostaglandins from

the spleen and kidney during infusion of angiotensin appears to be related to the vasoconstriction rather than to a specific action of the peptide. Thus it has been shown that noradrenaline and renal nerve stimulation also causes the release of prostaglandins from spleen (Ferreira, Moncada & Vane, 1973) and the kidney (Dunham & Zimmerman, 1970; McGiff et al, 1972) and it has been suggested that prostaglandins may be part of an auto-regulatory system (Hedqvist, 1969; Herbaczynska-Cedo & Vane, 1973).

(b) CYCLIC AMP

Investigations on the role of cyclic AMP in the action of angiotensin have yielded contradictory results and there is little convincing evidence for an involvement of the nucleotide. However, since many peptide hormones do act via cyclic AMP this mechanism must also be considered for angiotensin.

In addition to its role as a secondary messenger in hormone action, cyclic AMP has many other actions and appears to be intimately involved in smooth muscle contraction. Although the full significance of intracellular cyclic AMP in the control of smooth muscle tone has not yet been identified, there is considerable evidence that the nucleotide has an important regulatory function mediated via changes in calcium. Increased intracellular cyclic AMP has been shown to cause a relaxation of smooth muscle (review: Andersson, 1972) and this has been suggested as the mechanism for the action of catecholamines (Sutherland & Robison, 1966; Andersson, 1972; Marshall & Kroeger, 1973). This action of cyclic AMP has been attributed to a reduction in intracellular calcium concentration (Andersson, 1972; Rasmussen & Tenenhouse, 1968) and it has been shown to increase calcium binding to microsomal membranes (Andersson & Nilsson, 1972; Andersson, 1972; Baudouin-Legros & Meyer, 1973) and to stimulate active calcium efflux from the smooth muscle cell (Marshall & Kroeger, 1973). However, contraction of smooth muscle is not consistently associated with a decrease in intracellular cyclic AMP (Andersson, 1972) and there may be an increase in cyclic AMP during the early stages of induced contractions.

In the present experiments theophylline was used to investigate the effect of altered intracellular cyclic AMP concentration upon contractile responses of rat colon to acetylcholine and angiotensin. This methyl xanthine has been shown to be an effective inhibitor of

phosphodiesterase (Sutherland & Rall, 1960; Butcher & Sutherland, 1962) and potentiates many actions of drugs that are mediated via cyclic AMP (Rall & West, 1963; Robison, Butcher & Sutherland, 1971; Andersson & Mohme-Lundholm, 1969). It has been suggested that theophylline provides a better method of increasing intracellular cyclic AMP than addition of the nucleotide, which is very slow to penetrate cellular membranes and rapidly hydrolysed by phosphodiesterase (Robison, Butcher, Oye, Morgan & Sutherland, 1965; Robison, Butcher & Sutherland, 1970). Furthermore, high concentrations of extracellular cyclic AMP produce non-specific actions which have been attributed to the adenosine moiety (Iso, 1973).

In the present studies, low concentrations of theophylline (up to 1 mmol) had no consistent effect upon responses to either acetylcholine or angiotensin but higher concentrations (2-5 mmol) caused an equal reduction of responses to both agonists which was directly related to the concentration of theophylline. At 5mmol, responses to both acetylcholine and angiotensin were considerably reduced which indicated a severe impairment of the contractile process, but again there was no significant difference between the reduction of acetylcholine responses compared with the reduction of angiotensin responses. These results suggested that the increase in intracellular cyclic AMP was causing a non-specific inhibition of all contractile responses through a reduction in the intracellular calcium concentration. Other workers have also observed a general depression of smooth muscle contractility both during exposure to theophylline and dibutyryl cyclic AMP (McFarland, Guyton & Pfaffman, 1971; Pfaffman & McFarland, 1973).

Therefore, in further experiments the effect of 4 mmol theophylline was investigated upon responses of rat colon to potassium (40 mmol). The potassium contracture of smooth muscle has been

reported to consist of two phases, a rapid transient phase due to release of intracellular calcium and a slow sustained phase due to influx of extracellular calcium (Urakawa & Holland, 1964; Godfraind & Kaba, 1969; Hurwitz & Suria, 1971). Theophylline caused an equal reduction of both phases of the potassium response and furthermore, there was no significant difference between the reduction of the potassium response and the reduction of the acetylcholine and angiotensin responses. Inhibition of the second phase of the potassium response is consistent with an increase in intracellular cyclic AMP, since increased calcium binding to the plasma membrane would reduce calcium permeability (Burnstock, 1963; Bohr, 1964; Hurwitz, Joiner & Von Hagen, 1967; Somlyo & Somlyo, 1968; Rothstein, 1968), which together with the increased intracellular calcium binding and rate of active efflux would oppose the influx of calcium produced by potassium.

If the action of theophylline was due to a reduction of the intracellular calcium concentration, then an increase in extracellular calcium concentration would be expected to antagonise its effect. This was confirmed in preliminary experiments where the calcium concentration of the Tyrode solution was increased from 1.8 mmol to 7.2 mmol in the presence of theophylline (4 mmol). In the high calcium solution there was a marked recovery of responses to both acetylcholine and angiotensin but the recovery of the angiotensin response was significantly greater than the recovery of the acetylcholine response. This suggested that extracellular calcium was more important for the contractile action of angiotensin than for the contractile action of acetylcholine and this was confirmed in later experiments. However, further experiments are required to investigate the effect of high calcium solution upon acetylcholine and angiotensin responses in the absence of theophylline before definite conclusions can be drawn about the mechanism of action of this agent.

Since all concentrations of theophylline had an equal effect upon responses to acetylcholine and angiotensin it appeared that the contractile action of angiotensin was not specifically associated with a change in cyclic AMP concentration. This conclusion is supported by work on rat uterus where contractor concentrations of angiotensin have been shown to have no effect upon adenyl cyclase activity (Angles D'Auriac & Meyer, 1972), phosphodiesterase activity (Angles D'Auriac & Meyer, 1973a) or intracellular cyclic AMP concentration (Angles D'Auriac & Meyer, 1973b). Volicer and Hynie (1971) have reported that the contractile action of angiotensin upon isolated rat aorta is associated with a decrease in cyclic AMP concentration which they attributed to an inhibition of adenyl cyclase. However, these workers observed a similar effect with catecholomines which suggests that they were recording the general reduction of cyclic AMP concentration which is associated with smooth muscle contraction (Andersson, 1972; Triner, Nahas, Vulliemoz, Overweg, Verosky, Habif & Ngai, 1971; Bar, 1974). Furthermore, it has been shown that smooth muscle contraction is always accompanied by a reduction of adenyl cyclase activity due to the increased intracellular calcium concentration (see review by Sharma, 1973).

4. INORGANIC IONS AND THE CONTRACTILE ACTION OF ANGIOTENSIN

(a) SODIUM IONS

There is now considerable evidence that the main physiological role of angiotensin is concerned with the control of sodium and water homeostasis. Studies of the effect of angiotensin upon renal function in the intact animal (reviews: Gross, 1971; Gross & Mohring, 1973; Kover et al, 1971; Lockett, 1972) and upon sodium transport in isolated epithelia (Crocker & Munday, 1967 and 1970; Crocker, 1971; Davies et al, 1969 and 1970; Hornych et al, 1973) and kidney cortex slices (Munday et al, 1971 and 1972) have shown that effective concentrations of angiotensin are very much lower than those required for smooth muscle contraction. It is therefore possible that the primary action of angiotensin is upon active sodium transport and that in smooth muscle this triggers the contractile response through an ultimate increase in intracellular calcium concentration. Since the primary action of acetylcholine upon smooth muscle has been reported to involve an increase in passive membrane permeability to sodium and potassium (Bolton, 1972; Setekliev, 1970; Szurszewski & Bulbring, 1973; Bulbring & Szurszewski, 1974), an action of angiotensin upon active sodium movement might explain the previous observation that metabolic inhibition caused a greater reduction of contractile responses to angiotensin than to acetylcholine. It has been shown that active ion movement in smooth muscle is inhibited by exposure to glucose free solution, to anoxia or to chemical inhibitors of metabolism (Daniel, 1964b 1964c; Rangachari et al, 1972). The role of sodium in the contractile responses to acetylcholine and angiotensin was therefore investigated by exposing smooth muscle preparations to changes in extracellular sodium concentration and to inhibitors of active sodium movement.

When the sodium concentration of the Tyrode solution was reduced there was an equal reduction of the contractile responses of rat descending colon to acetylcholine and angiotensin. The reduction of responses to both agonists increased in direct relation to the reduction in sodium concentration but there was never any significant difference between the reduction of angiotensin responses compared with the reduction of corresponding acetylcholine responses. This effect was not due to a change in osmotic pressure since this was maintained by addition of sucrose. These findings are consistent with reports that a reduction in sodium concentration caused an equal reduction of responses to acetylcholine and angiotensin on guinea-pig ileum (Blair-West et al, 1967).

In the present study an increase in sodium concentration caused an equal reduction of responses to acetylcholine and angiotensin which was directly related to the change in concentration. However, when the osmotic pressure was increased by addition of equi-osmotic equivalents of sucrose there was no effect upon responses to either agonist. On guinea-pig ileum it has been reported that increased sodium concentration causes a potentiation of contractile responses to angiotensin but reduces responses to acetylcholine (Blair-West et al, 1967) and this has been attributed to an effect of sodium upon the direct interaction of angiotensin with the smooth muscle cells. The present findings do not support this suggestion but confirm that an increase in sodium concentration has the same effect upon contractile responses of smooth muscle preparations to both acetylcholine and angiotensin (Khairallah et al, 1965).

An involvement of sodium ions in the interaction of angiotensin with smooth muscle cells has also been reported in the rabbit ear artery (Blair-West et al, 1968) and on cat papillary muscle (Lefer, 1967). However, these investigations were controlled with adrenaline

and it has recently been reported that a reduction in sodium concentration potentiates the contractile action of adrenaline upon smooth muscle (Bulbring & Szurszerski, 1974). Furthermore, it has been reported that a reduction in sodium concentration only effects the responses of isolated rat portal vein under hypoosmolar conditions and has no effect under isosmolar conditions (Blair-West et al, 1971). There have been several reports that the pressor action of angiotensin is enhanced by increased plasma sodium but these experiments are difficult to interpret because of changes in the activity of the renin-angiotensin system and of circulating angiotensin. Thus although it has been claimed that changes in plasma sodium directly affect the interaction of angiotensin with the intact vasculature (Blair-West et al, 1972; Weinberger et al, 1972; Brunner et al, 1972), these reports cannot be considered conclusive.

In the present study changes in extracellular sodium concentration had the same effect upon responses to acetylcholine and angiotensin and this suggested that there was a nonspecific impairment of smooth muscle contraction and therefore provided no evidence for any specific involvement of sodium ions in the angiotensin response. Other reports on the effect of altered extracellular sodium concentration upon contractile responses of smooth muscle are very confusing (see reviews by Bohr, 1964; Sitrin & Bohr, 1971; Bohr, 1973; Somlyo & Somlyo, 1968). This is probably a reflection of the complex relationship between sodium ions and smooth muscle contraction which is not yet fully understood. There is considerable evidence that the transmembrane sodium concentration gradient affects the transmembrane calcium distribution (Tomita & Watanabe, 1973; Reuter et al, 1973; Burton & Godfraind, 1973; Brading, 1973; Bohr et al, 1969) while the membrane excitability, and hence permeability, is intimately related to the intracellular extracellular sodium concentration gradient (Bohr, 1964; Hofmann, 1969; Casteels, Droogmans & H endrick, 1973).

The distribution of sodium in smooth muscle is largely controlled by the sodium potassium exchange pump, enzymatically Na-K-Mg-activated ATPase (Reviews: Casteels, 1970; Daniel, Robison, Kidwai, Wolowyck, Taylor & Paton, 1969; Caldwell, 1968; Burnstock et al, 1963; Casteels et al, 1973) which is inhibited by the cardiac glycoside ouabain (reviews: Glynn, 1957 and 1964; Caldwell, 1968; Whittam & Wheeler, 1970; Lee & Klaus, 1971). Exposure of smooth muscle preparations to ouabain therefore results in a change in the transmembrane sodium gradient similar to that observed during exposure of smooth muscle to metabolic inhibition (Daniel, 1964b and 1964c; Rangachari et al, 1972). Thus if the preferential reduction of the angiotensin response observed in previous experiments, was due to inhibition of sodium movement by the sodium potassium pump, then ouabain would be expected to cause a similar differential reduction of contractile responses to acetylcholine and angiotensin. Furthermore, it has been reported that low concentrations of ouabain (10 ng/ml) specifically inhibit the contractile responses of rat uterus to angiotensin while having no effect upon responses to oxytocin and vasopressin and this led to the suggestion that the action of angiotensin upon smooth muscle might involve a stimulation of the sodium-potassium pump (Turker et al, 1967). The present study was therefore extended to investigate the effect of ouabain upon responses of rat colon and rat uterus to acetylcholine and angiotensin.

Concentrations of 1 μmol and 10 μmol ouabain had no effect upon the responses of rat descending colon to either acetylcholine or angiotensin and 0.1 mmol ouabain produced only a small reduction of responses to both agonists. At a concentration of 1 mmol, ouabain caused a progressive reduction of responses to both agonists but there was no significant difference between the reduction of the angiotensin responses and the reduction of the acetylcholine responses. Recovery

of responses after removal of ouabain was slow but was complete within two hours. This confirmed other reports that the effect of ouabain upon smooth muscle contractility is slowly reversible (Godfraind & Godfraind-De Becker, 1963; Griffin, Szaro & Weltman, 1972).

Rat uterus was also unaffected by concentration of ouabain less than 0.1 mmol. This insensitivity to ouabain is characteristic of rat tissue and has been reported by many workers (Daniel, 1964b; Bentley & Holland, 1961; Marshall & Kroeger, 1973; Rangachari et al, 1972). 0.1 mmol ouabain caused a progressive reduction of the contractile responses of rat uterus to acetylcholine and angiotensin but, as with rat colon, there was no significant difference between the reduction of the corresponding responses to the two agonists. The effect of this concentration of ouabain upon responses to acetylcholine and angiotensin was the same on dioestrous uterus as on oestrous uterus. These findings do not support the suggestion that the contractile action of angiotensin upon smooth muscle is due to a stimulation of the sodium-potassium pump (Turker et al, 1967). This is consistent with reports that angiotensin has no effect upon the sodium potassium ATPase isolated from rat kidney preparations (Bonting, Canady & Hawkins, 1964; Marc-Auriele & Bergeron, 1966) or rat colon (Munday et al, 1971). Furthermore, angiotensin stimulated sodium transport in rat kidney cells (Munday et al, 1971) and rat colon (Parsons & Munday, 1972) is unaffected by either high concentrations of ouabain or potassium free solutions, conditions which inhibit the sodium-potassium pump. The present findings confirm other reports that exposure of smooth muscle preparations to ouabain causes a general reduction of induced contractions (Godfraind & Godfraind-De Becker, 1963; Mathews & Sutter, 1967; Griffin et al, 1972) and this suggests that the sodium-potassium pump may be important for smooth muscle contractility.

It has been suggested that in addition to the sodium-potassium

pump, there may be a potassium independent active sodium extrusion mechanism responsible for volume regulation of the cell (Whittembury & Proverbio, 1970). This pump has been reported insensitive to ouabain but sensitive to the diuretic ethacrynic acid (Whittembury & Proverbio, 1970; Proverbio, Robinson & Whittembury, 1970; Robinson, 1972). Parsons and Munday have shown that the angiotensin stimulated sodium movement in rat kidney cortex slices and rat colon is inhibited by 7 mmol ethacrynic acid and they have suggested that angiotensin acts through a stimulation of the 'second' sodium pump (Parsons & Munday, 1972). However, these experiments were controlled by comparing the effect of ethacrynic acid upon angiotensin stimulated sodium movement with the effect upon basal sodium movement in normal physiological solution. Since the mechanism of basal sodium movement is unknown, it is difficult to estimate the specificity of ethacrynic acid in these experiments. Several workers have suggested that the action of ethacrynic acid upon active ion movement is due to an inhibition of cellular energy production (Macknight, 1969; Epstein, 1972; Landon & Fitzpatrick, 1972; Case, Gunther & Cannon, 1973; Daniel, Kidwai, Robinson, Freeman & Fair, 1971) and this could therefore explain the inhibition of angiotensin stimulated transport observed by Parsons and Munday.

To overcome these problems, the action of ethacrynic acid was investigated upon the contractile responses of angiotensin upon smooth muscle and the experiments were controlled by recording contractile responses to acetylcholine.

Exposure of rat descending colon to 0.1 mmol ethacrynic acid caused a progressive reduction of responses to both agonists but at all times there was a significantly greater reduction of the angiotensin responses than of the corresponding acetylcholine responses. However, on rat uterus although responses to both agonists were progressively reduced during exposure to 0.1 mmol ethacrynic acid, there was no

preferential reduction of the angiotensin response. In these experiments contractions of rat colon were recorded isometrically against a 1 g resting tension but with rat uterus this was impracticable because of rapid deterioration of the tissue and so responses were recorded isotonically. In previous experiments, however, it has been shown that angiotensin contractions of smooth muscle require more energy than acetylcholine contractions and it was therefore possible that the failure to observe a preferential reduction of the angiotensin response on rat uterus was due to differences in the energy demands imposed by the recording system. This explanation is compatible with the use of different recording methods for the two tissues since it has been shown that, for a constant resting load, the energy demand during an isometric contraction is appreciably greater than during an isotonic contraction (Axelsson et al, 1965; Lundholm & Mohme-Lundholm, 1965). However, an increase in isotonic load has been shown to increase the energy expenditure during contraction (Axelsson et al, 1965) and so the effect of ethacrynic acid was investigated upon contractions of rat uterus measured against an increased isotonic load of 3 g.

Contractile responses to acetylcholine were progressively reduced but at no time was the effect of ethacrynic acid significantly different from that observed under 1 g loading. In contrast, the reduction of angiotensin responses was significantly greater when measured under 3 g load than had been observed under 1 g load, and furthermore under 3 g loading there was a consistent and significant difference between the reduction of angiotensin responses and the corresponding acetylcholine responses. Thus in these experiments ethacrynic acid only produced a preferential reduction of the angiotensin response in circumstances where muscle energy expenditure would be high and this suggested that the action of the diuretic was upon cellular energy production.

To investigate this further the concentration of ethacrynic acid was increased to 0.5 mmol. There was an almost complete abolition of isometric responses of rat colon and of isotonic responses of rat uterus to both acetylcholine and angiotensin and no significant difference between the reduction of responses to the two agonists on either tissue. This indicated that ethacrynic acid was producing a non-specific impairment of the muscles' ability to contract which cannot be explained on the basis of an inhibition of the second sodium pump.

These findings suggest that the action of ethacrynic acid upon the contractile responses of isolated smooth muscle preparations to angiotensin and acetylcholine was due to an inhibition of cellular energy production. This is consistent with several reports that ethacrynic acid, in similar concentrations to those used in the present study, causes a non-specific depression of aerobic and anaerobic metabolism in isolated tissues (Macknight, 1969; Daniel et al, 1971; Epstein, 1972b; Landon & Fitzpatrick, 1972; Case, Gunther & Cannon, 1973).

The present experiments with ethacrynic acid provide further evidence that angiotensin requires more energy for its action upon smooth muscle than acetylcholine. Furthermore, it has been reported that the low concentrations of ethacrynic acid used in the present study cause a greater inhibition of aerobic metabolism than of anaerobic metabolism (Jones & Landon, 1967; Landon & Fitzpatrick, 1972; Daniel et al, 1971). This supports the hypothesis that in rat colon and rat uterus, the energy dependent step in the angiotensin response is primarily linked to aerobic metabolism.

(b) CALCIUM IONS

(i) The Effect of Changes in Extracellular Calcium Concentration

The final common pathway for all spasmogens is an increase in the free calcium concentration in the region of the contractile filaments (Daniel, 1964a; Bohr, 1964; Hurwitz & Suria, 1971; Axelsson, 1970; Somlyo & Somlyo, 1968). However, this single event may be produced by a variety of mechanisms and the activator calcium may be mobilised from intracellular binding sites, plasma membrane sites or the extracellular space (see; Somlyo & Somlyo, 1968; Hurwitz & Suria, 1971; Ruegg, 1971; Devine et al, 1973). The primary action of acetylcholine upon smooth muscle has been reported to involve an increase in plasma membrane permeability to sodium and potassium (Bolton, 1972; Bulbring & Szurszewski, 1974; Szurszewski & Bulbring, 1973) which triggers calcium release from intracellular binding sites. There may also be an increase in the passive plasma membrane permeability to calcium resulting in influx of extracellular calcium (Bulbring & Kuriyama, 1963; Durbin & Jenkinson, 1961; Szurszewski & Bulbring, 1973). In the present study it appeared that the angiotensin contraction of smooth muscle was fundamentally different from that of acetylcholine and this could be due to differences in the source or mobilisation of activator calcium. This possibility was investigated by comparing the effect of reduced extracellular calcium concentration upon the contractile responses of rat colon and rat uterus to acetylcholine and angiotensin.

When the calcium concentration of the Tyrode solution was progressively reduced from 1.8 mmol to 0.45 mmol there was a marked reduction of the angiotensin responses of rat colon but no consistent effect upon acetylcholine responses. It therefore appeared that extracellular calcium was more important for the angiotensin contraction and may constitute the source of activator calcium for the contraction process.

When the calcium concentration was reduced to 0.18 mmol, there was a reduction of contractile responses to both agonists but the reduction of the angiotensin responses was significantly greater than the reduction of the acetylcholine responses. In calcium free solution responses to acetylcholine and angiotensin were rapidly reduced but there was no difference in the percentage reduction of the two agonists which indicated a non-specific impairment of the muscles' contractile ability. Thus as the extracellular calcium concentration was reduced towards the concentration required for activation of the contractile process all induced responses were inhibited. Calcium free solution did not completely abolish responses to either agonist and this is consistent with reports that a chelating agent is required to ensure removal of all calcium from the extracellular tissue (Keatinge, 1972a and b).

In previous experiments with theophylline there was also an equal reduction of responses to acetylcholine and angiotensin which was attributed to an increase in intracellular cyclic AMP concentration. Increased extracellular calcium caused a recovery of induced responses which appeared to be consistent with the known action of cyclic AMP upon calcium metabolism. However, there was a significantly greater recovery of angiotensin responses compared with acetylcholine responses, which is consistent with the present observation that the angiotensin response of rat colon is more sensitive to changes in extracellular calcium concentration.

When oestrous or dioestrous uterus was exposed to Tyrode solution containing 0.9 mmol or 0.45 mmol calcium, there was an equal reduction of angiotensin and acetylcholine responses. However, the reduction of induced responses was far greater than had been observed on rat colon and 0.9 mmol calcium caused a reduction similar to that observed with calcium free solution on rat colon. This suggested that

the failure to observe a preferential reduction of the angiotensin response on this tissue was not due to a different mechanism of action but to a difference in the tissue's sensitivity to calcium. This is consistent with a report that rat uterus is far more sensitive to reduced extracellular calcium concentration than guinea-pig ileum (Khairallah et al, 1965). Furthermore, the difference in sensitivity of rat colon and rat uterus to extracellular calcium may explain the previous observations with indomethacin which caused a preferential reduction of acetylcholine and angiotensin responses on rat uterus. It has been shown that indomethacin causes an inhibition of smooth muscle contractility (Northover, 1967 and 1971) and this has been correlated with an inhibition of calcium uptake (Northover, 1971 and 1972) and plasma membrane binding (Northover, 1973). Thus the change in calcium movement caused by indomethacin may be sufficient to reveal the differential reduction of the angiotensin response on rat uterus, due to the sensitivity of the tissue to changes in extracellular calcium concentration, but insufficient to reveal a differential on rat colon.

The marked reduction of the angiotensin response observed during exposure of rat colon to low calcium Tyrode solution suggested that the main source of activator calcium was superficial and was probably the extracellular space. Since responses to acetylcholine were less affected, this confirmed that the main source of activator calcium was probably the intracellular binding sites (see reviews: Daniel, 1964a; Bohr, 1964; Hurwitz & Suria, 1971). To investigate this further, preparations of rat descending colon were exposed to a Tyrode solution containing 0.5 mmol lanthanum chloride and responses to acetylcholine and angiotensin were recorded with respect to time.

The trivalent lanthanum ion has been shown to displace calcium from anionic sites on the plasma membrane and so render the membrane

impermeable to calcium ions (reviews: Van Breemen et al, 1973; Weiss, 1974). Thus movement of calcium into and out of the smooth muscle cell is prevented and agonists which mobilise extracellular calcium can no longer cause contraction. In the present experiments there was a marked reduction of responses to both acetylcholine and angiotensin but during the first ten minutes of exposure the reduction of the angiotensin response was significantly greater than the reduction of the corresponding acetylcholine response. It therefore appeared that acetylcholine was less dependent upon extracellular calcium than angiotensin and could mobilise activator calcium from the intracellular stores. This is consistent both with the previous findings that reduced extracellular calcium concentration had little effect upon the acetylcholine response, and with other studies of the effect of lanthanum upon acetylcholine induced contractions of isolated smooth muscle (Van Breemen & Daniel, 1966; Goodman & Weiss, 1971; Szurszewski & Bulbring, 1973). After removal of acetylcholine or angiotensin there was a normal relaxation of the smooth muscle during exposure to lanthanum and this confirmed other reports that activator calcium can be absorbed by intracellular binding sites (Van Breemen et al, 1973; Deth & Van Breemen, 1974). Furthermore, since the acetylcholine responses were progressively reduced this supports the suggestion that the intracellular binding sites responsible for the release of activator calcium may be distinct from those responsible for its re-uptake (Van Breemen et al, 1973; Deth & Van Breemen, 1974).

These findings with lanthanum supported the hypothesis that the activator calcium for the angiotensin response is derived from a superficial site. This is also consistent with several reports that angiotensin induced contractions of smooth muscle are associated with an increased uptake of calcium (Shibita et al, 1968; Shibita & Carrier, 1967; Sullivan & Briggs, 1968; Deth & Van Breemen, 1974).

However, it is not possible to distinguish the exact source of activator calcium since lanthanum not only prevents calcium influx, but in addition displaces calcium from the anionic binding sites on the plasma membrane. Further, although the results of these experiments appeared useful, the marked reduction of responses to both acetylcholine and angiotensin observed during exposure to lanthanum was further evidence that this method is not entirely specific. It has been suggested that lanthanum effects the mobility of calcium at deeply located calcium binding sites through an effect upon the superficial sites (Weiss & Goodman, 1969). However, Daniel and co-workers have suggested that lanthanum may penetrate the plasma membrane and bind directly to intracellular sites (Hodgson, Kidwai & Daniel, 1972).

(ii) Preliminary Studies of the Role of Calcium Ions in the Energy Dependent Mechanism of the Angiotensin Response

In previous experiments it appeared that angiotensin required more energy for its contractile action upon smooth muscle than acetylcholine. Since the increase in intracellular calcium concentration produced by acetylcholine is a passive process, it is possible that the contractile action of angiotensin might involve an active increase in intracellular calcium. This hypothesis is supported by two workers who have suggested that angiotensin might stimulate an active inwardly directly calcium pump which may be coupled to sodium efflux (Villamil, 1972; Godfraind, 1970). To investigate this, preparations of rat descending colon were exposed to low calcium Tyrode solution under conditions where energy production would be limited. An energy dependent influx of calcium would then be expected to be reduced to a greater extent than would occur under the same conditions of extracellular calcium concentration or metabolic inhibition applied separately. At present only a few preliminary experiments have been performed but it is interesting to consider briefly the results and the experimental

problems so far encountered.

Initial experiments were performed with a glucose-free Tyrode solution containing 0.18 mmol calcium, the minimum concentration at which it is possible to observe a differential reduction of angiotensin and acetylcholine responses. Exposure of rat colon to this solution caused a reduction of contractile responses to both agonists but again there was a significant difference between the percentage reduction of angiotensin responses compared with the percentage reduction of corresponding acetylcholine responses. The reduction of acetylcholine responses was similar to that observed in low calcium (0.18 mmol) Tyrode and although there was a greater reduction of angiotensin responses in glucose free low calcium Tyrode than had been observed in low calcium Tyrode, this difference was not significant. This failure to demonstrate any difference in the reduction of the angiotensin response during exposure to low extracellular calcium in a glucose free solution compared with a normal solution may have been due to adequate energy production under substrate free conditions. Since severe conditions of metabolic inhibition have been shown to cause a rapid reduction of responses to angiotensin and acetylcholine on this tissue, an alternative and more sensitive method of investigation was necessary.

It has been reported that isometric contractions of smooth muscle require more energy than isotonic contractions (Lundholm & Mohme-Lundholm, 1965; Axelsson et al, 1965). Since the previous experiments on rat colon had been recorded isometrically, the effect of reduced calcium concentration was investigated upon isotonic contractions of this tissue to acetylcholine and angiotensin. A significant difference in the effect of low calcium Tyrode upon angiotensin responses measured by isometric conditions compared with isotonic conditions, would then indicate that the energy dependence

of the angiotensin response is related to active calcium movement. Tyrode solution containing 0.18 mmol calcium caused a marked reduction of isotonic responses to acetylcholine and angiotensin with a significantly greater reduction of the angiotensin responses compared with the corresponding acetylcholine responses. Although this confirmed the observations made with isometric recording, there was no significant difference between the reduction of the induced responses under isotonic conditions compared with isometric conditions. Similarly, calcium free Tyrode solution caused an equal reduction of isotonic responses to acetylcholine and angiotensin which was not significantly different from the reduction obtained under isometric conditions. When a glucose and calcium free solution was used there was again an equal reduction of isotonic contractions to acetylcholine and angiotensin but the effect was not significantly different from that observed in calcium free solutions containing glucose.

Thus reduction of extracellular calcium had the same effect upon isotonic responses of rat colon to angiotensin as upon isometric responses. However, since the reduction of the acetylcholine responses were also the same under isotonic conditions as under isometric conditions, this indicated that again the tissue energy supply was adequate for both types of contraction. Again it appeared that the experimental technique was insufficiently sensitive to detect whether calcium is involved in the energy dependent stage of the angiotensin response.

Although these preliminary experiments have failed to indicate any connection between the energy required for the angiotensin contraction and mobilisation of extracellular calcium, this study is continuing.

5. CONCLUSIONS

The contractile action of angiotensin has been recognised for many years and has been extensively investigated but there is still considerable doubt as to the mechanism involved. The present study represents a new approach to this problem and has involved a comparative study of the contractile action of angiotensin and acetylcholine. The mode of action of acetylcholine is well established and has been shown to involve a primary increase in passive sodium permeability (Bolton, 1972). Thus it has been possible to separate the effect of experimental treatments upon the smooth muscle contraction process from the effect upon the primary interaction of angiotensin with the smooth muscle membrane.

It has been shown that the contractile action of angiotensin is more sensitive to conditions where tissue energy production is reduced than the contractile action of acetylcholine. This is the first demonstration that angiotensin requires energy for its interaction with the smooth muscle cell in addition to the energy required for the contractile process. Metabolic inhibition has been shown to cause different effects upon the angiotensin response of several isolated smooth muscle preparations and this appears to be due to differences in the metabolism of these tissues. In most tissues the energy for the primary interaction of angiotensin appears to be supplied by aerobic metabolism but in oestrous rat uterus anaerobic glycolysis was sufficiently active to supply energy both for the initial interaction and for the contractile process. This raises the interesting possibility that the reported differences in the angiotensin response of different smooth muscle preparations (Papadimitrio & Worcel, 1974) may be related to differences in the energy metabolism of these tissues. This is consistent with the suggestion that apparent differences in the angiotensin receptor may in fact be the result of differences in

the excitation-contraction coupling mechanism.

Conditions of reduced tissue energy production have been shown to affect the maintenance of induced contractions to potassium (40 mmol). This has been confirmed in the present study but a comparative investigation of the angiotensin response revealed no change in the maintenance of contractions. It therefore appears that the angiotensin response involves a single mechanism and that a reduction in available energy impairs the initiation of the response without affecting its duration.

Angiotensin like most other peptide hormones, is generally considered to act at the surface of the cell (Rasmussen, 1969) and it is therefore probable that the energy is required to translate the action of the hormone to the intracellular environment. Many peptide hormones act via a mediator and both cyclic AMP and prostaglandins have been implicated in this role. However, in the present study conditions which altered intracellular cyclic AMP or prostaglandin concentrations, had the same effect upon responses to acetylcholine and angiotensin which suggested that there is no specific involvement of either substance in the angiotensin response. The one exception was with the prostaglandin synthesis inhibitor, indomethacin, which caused a preferential reduction of angiotensin responses on rat uterus. However, this was only observed with concentrations of indomethacin far higher than those required to affect prostaglandin synthesis and it is suggested that the only action may in fact be upon transmembrane calcium movement.

The role of inorganic ions in the contractile action of angiotensin is of particular interest since the hormone has been shown to affect active sodium movement at concentrations far below those necessary for the contractile action. However, in the present study changes in extracellular sodium concentration had the same effect upon responses to acetylcholine and angiotensin and no evidence has been

found to support a specific involvement of the sodium ion in the cellular action of angiotensin. Reports that angiotensin may stimulate the sodium-potassium pump (Turker et al, 1967) or the potassium-independent sodium pump (Parsons & Munday, 1972; Davies et al, 1972) in smooth muscle have been investigated but have not been substantiated. Ethacrynic acid which is claimed to specifically inhibit the potassium independent 'second' sodium pump only caused a preferential reduction of the angiotensin response under conditions where tissue energy expenditure was high. This has been demonstrated by the use of selective recording techniques and provides further evidence for the energy dependence of the angiotensin response. At the same time, however, it throws doubt upon the specificity of this inhibitor and therefore upon previous conclusions which have been based on its use.

Calcium ions are intimately involved in muscle contraction and the final common pathway for all spasmogens is an increase in the intracellular calcium concentration. In the present study it has been shown that the contractile action of angiotensin is far more sensitive to changes in the extracellular calcium concentration than the contractile action of acetylcholine. This suggests that the activator calcium for the angiotensin response is mobilised from a superficial site which may be the extracellular space. The lanthanum technique has been used to confirm these observations but the method appears to lack specificity and the results must therefore be interpreted with care.

In view of the energy dependence of the angiotensin response it is interesting to consider the possible relationship between energy and increased intracellular calcium concentration. Although preliminary experiments have been inconclusive, two mechanisms are tentatively suggested whereby angiotensin might stimulate an active increase in intracellular calcium concentration and thereby cause smooth muscle contraction.

1. Several workers have suggested the existence of an inwardly directed calcium pump which is coupled with outward movement of sodium ions (Bohr et al, 1969; Brading, 1973; Reuter, 1973). Stimulation of this mechanism by angiotensin would be energy dependent and would result in contraction. A decrease in the extracellular calcium concentration would reduce the number of calcium ions available for combination at the superficial anionic sites of the pump and in addition would increase the competition from sodium ions (Bohr et al, 1969; Bohr, 1973; Brading, 1973; Caldwell, 1968). This mechanism could account for the increased calcium influx which has been observed during angiotensin induced contractions of smooth muscle (Shibita & Carrier, 1967; Shibita et al, 1968; Deth & Van Breemen, 1974). It is also interesting that the effect of angiotensin upon sodium movement in everted sacs of rat colon has been shown to be dependent upon calcium at the mucosal surface (Munday, Parsons, Poat & Smith, 1973). Thus it is tempting to speculate that the mechanism for contraction and ion-movement might be common and involve a sodium-calcium exchange pump.
2. Alternatively angiotensin may release membrane-bound calcium as has been suggested by Meyer and co-workers (Devynck et al, 1973 and 1974). This is compatible with the observed dependence of the angiotensin response upon extracellular calcium since the superficial location of the plasma membrane bound calcium makes it vulnerable to changes in the extracellular environment. Since uptake of calcium by the cell membrane binding sites is an active process (Sparrow & Simonds, 1965; Rothstein, 1968; Devynck et al, 1973), this mechanism could also account for the energy dependence of the angiotensin response.

It is hoped to continue these investigations of the role of calcium ions in the contractile action of angiotensin. The observation that the angiotensin interaction is energy dependent provides the first conclusive evidence that the angiotensin response involves an initial step prior to the contraction process which may be linked to the known action of the hormone upon inorganic ion movement.

BIBLIOGRAPHY

1. Aiken, J.W. & Vane, J.R. (1971). Blockade of Angiotensin-Induced Prostaglandin Release from Dog Kidney by Indomethacin. Pharmacologist, 13, 293.

2. Aiken, J.W. & Vane, J.R. (1973). Intra-renal Prostaglandin Release Attenuates the Renal Vasoconstrictor Activity of Angiotensin. J. Pharmac. exp. Ther., 184, 678-687.

3. Altura, B.M. & Altura, B.T. (1970). Differential Effects of Substrate Depletion on Drug-Induced Contractions of Rabbit Aortae. Am. J. Physiol., 219, 1698-1705.

4. Altura, B.M. & Altura, B.T. (1974). Magnesium and Contraction of Arterial Smooth Muscle. Microvasc. Res., 7, 145-155.

5. Alvarez, W.C.A. (1922). The Mechanism of the Digestive Tract. 1st Edn. New York. Paul B. Hoeber.

6. Ambache, N. & Lessin, A.W. (1955). Classification of Intestinomotor Drugs by Means of Type D Botulinium Toxin. J. Physiol., Lond., 127, 449-478.

7. Ames, R.P., Borkowski, A.J., Sicinski, A.M. & Laragh, J.H. (1965). Prolonged Infusions of Angiotensin II and Norepinephrine and Blood Pressure, Electrolyte Balance, and Aldosterone and Cortisol Secretion in Normal Man and in Cirrhosis with Ascites. J. Clin. Invest., 44, 1171-1186.

8. Andersson, R. (1972). Cyclic AMP and Calcium Ions in Mechanical and Metabolic Responses of Smooth Muscles; Influence of Some Hormones and Drugs. Acta Physiol. Scand., Supp. 382,

9. Andersson, R. & Mohme-Lundholm, E. (1969). Studies on the Relaxing Actions Mediated by Stimulation of Adrenergic and Receptors in Taenia Coli of the Rabbit and Guinea-Pig. Acta Physiol. Scand., 77, 372-384.

10. Andersson, R., Lundholm, L. & Mohme-Lundholm, E. (1972). Role of Cyclic AMP and Ca^{2+} in Mechanical and Metabolic Events of Smooth Muscle. In: Vascular Smooth Muscle, ed. Betz, E. pp. 14-15. Springer-Verlag: Berlin.

11. Andersson, R. and Nilsson, K. (1972). Cyclic AMP and Calcium in Relaxation in Intestinal Smooth Muscle. Nature, New Biol., 238, 119-120.

12. Angles D'Auriac, G. & Meyer, P. (1972). Effects of Angiotensin II on Cyclic AMP in Rat Uterus. Life Sci., 11, 631-641.

13. Angles D'Auriac, G. & Meyer, P. (1973a). 3',5'-Cyclic AMP Phosphodiesterases in Cytosol of Uterine Smooth Muscle; Effects of Angiotensin. Life Sci., 12, 233-239.

14. Angles D'Auriac, G. & Meyer, P. (1973b). Effects of Angiotensin II on Adenyl Cyclose Activity, Phosphodiesterase Activity and on the Level of Cyclic AMP in Rat Uterus. Clin. Sci. Molic. Med., 45, 247-250.

15. Axelsson, J. (1970). Mechanical Properties of Smooth Muscle, and the Relationship between Mechanical and Electrical activity. In: Smooth Muscle, ed. Bulbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 289-315. Arnold: London.

16. Axelsson, J., Hogberg, S.G.R. & Timms, A.R. (1965). The effect of removing and readmitting glucose on the electrical and mechanical activity and glucose and glycogen content of intestinal smooth muscle from the taenia coli of the guinea-pig. Acta Physiol. Scand., 64, 28-42.

17. Bar, H.P. (1974). Cyclic Nucleotides and smooth muscle. In: Advances in Cyclic Nucleotide Research, Vol. 4, ed. Greengard, P. and Robison, G.A. pp. 195-237. Raven Press: New York.

18. Barbour, B.H., Gill, J.R. & Bartter, F.C. (1964). Effects of Angiotensin on the toad skin. Proc. Soc. exp. Biol., N.Y., 116, 806-808.

19. Barraclough, M.A. (1965). Dose-dependent opposite effects of angiotensin on renal sodium excretion. Lancet, ii, 987-988.

20. Barraclough, M.A., Jones, N.F. & Marsden, C.D. (1967). Effect of angiotensin on renal function in the rat. Am. J. Physiol., 212, 1153-1157.

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

22. Baudouin, M., Meyer, P. & Worcel, M. (1971). Specific binding of 3H-angiotensin II in rabbit aortae. Biochim. Biophys. Acta, 42, 434-440.

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

24. 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.

25. Beaulness, A., Nantel, A. & Cardinal, J. (1965). Union. Med. Canada, 94, 88.

26. Bennet, M.R. (1967). The effect of cations on the electrical properties of the smooth muscle cells of guinea-pig vas deferons. J. Physiol., Lond., 197, 30-31P.

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

28. Bentley, P.J. & Holland, A. (1961). Some in vitro observations on water, sodium and potassium metabolism in the oestrous rat uterus. J. Physiol., Lond., 159, 58-67.

29. Bergstrom, S. (1967). Prostaglandins: members of a new hormonal system. Science, 157, 382-391.

30. Birnbauer, L., Pohl, S.L., Krans, M.J. & Rodbell, M. (1970). The actions of hormones on the adenyl cyclase system. In: Advances in Psychopharmacology, Volume 3. Role of Cyclic AMP in Cell Function, ed. Greengard, P. & Costa, E. pp. 185-208. Raven Press: New York.

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

32. Blaine, E.H., Davis, J.O. & Harris, P.D. (1972). A steady-state control analysis of the Renin-Angiotensin-Aldosterone system. Circulation. Res., 30, 713-730.

33. Blair, M.R. & Clark, B.B. (1956). An evaluation of the action of substance P on the jejenum of the rabbit. J. Pharmac. exp. Ther., 117, 467-477.

34. Blair-West, J.R., Coghlan, J.P., Denton, D.A., Goding, J.R., Munrow, J.A. & Wright, R.D. (1963). The reduction of the pressor response of angiotensin II in sodium-deficient conscious sheep. Aust. J. Exp. Biol. Med. Sci., 41, 369-376.

35. 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.

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

37. Blair-West, J.R., Coghlan, J.P., Denton, D.A., Goding, J.R., Wintour, M. & Wright, R.D. (1968). The effect of nephrectomy on aldosterone secretion in the conscious sodium-depleted hypophysectomised sheep.
Aust. J. Exp. Biol. Med. Sci., 46, 295-318.

38. Blair-West, J.R., McKenzie, J.S. & McKinley, M.J. (1971). The actions of angiotensin II on the isolated portal vein of the rat. Eur. J. Pharmac., 15, 221-230.

39. Blair-West, J.R., Coghlan, J.P., Denton, D.A., Scoggins, B.A. & Wintour, E.M. (1972). The pressor effect of angiotensin II in pregnant sheep. Aust. J. Exp. Biol. Med. Sci., 50, 739-744.

40. Boettiger, E.G. (1946). Changes in the glycogen and water content of rat uterus. J. Cell. Comp. Physiol., 27, 9-14.

41. Bohr, D.F. (1963). Vascular smooth muscle: dual effects of calcium. Science, 139, 597-599.

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

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

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

45. Bolton, T.B. (1972). The depolarising action of acetylcholine or carbachol in intestinal smooth muscle. J. Physiol., Lond., 220, 647-671.

46. Bonting, S.L., Canady, M.R. & Hawkins, N.M. (1964). Angiotensin and renal Na-K activated adenosine triphosphatase. Biochim. Biophys. Acta., 82, 427-429.

47. Borle, A. (1973). Calcium metabolism at the cellular level. Fedn. Proc., 32, 1944-1950.

48. Born, G.V.R. (1956). The relation between the tension and the high energy phosphate content of smooth muscle. J. Physiol., Lond., 131, 704-711.

49. 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.

50. Bostrom, S-L., Hogberg, G. & Johansson, R.G. (1973). Changes in enzyme activities in smooth muscle after contractile activity in vitro. Int. J. Biochem., 4, 415-420.

51. Bozler, E. (1948). Conduction, automaticity and tonus of visceral muscles. Experientia, 4, 213-218.

52. Boyd, G.W. & Peart, W.S. (1971). The relationship between angiotensin and aldosterone. Adv. in Metabolic Disorders, 5, 77-119.

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

54. Brading, A.F. & Tomita, T. (1968). The action potential of the guinea-pig taenia coli in low sodium solution. J. Physiol., Lond., 197, 30-31P.

55. Braun-Menendez, E., Fasciolo, J.C., Leloir, L.F. & Munoz, J.M.(1940). The substance causing renal hypertension. J. Physiol., Lond., 98, 283-298.

56. Braun-Menendez, E., Fasciolo, J.C., Leloir, L.F., Munoz, J.M. & Taquini, A.C. (1946). Renal Hypertension. Translated by Dexter, L., Charles C. Thomas: Springfield, Illinois.

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

58. Brunner, H.R., Chang, P., Wallach, R., Sealley, 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, 58-68.

59. Buckley, J.P. (1972). Actions of angiotensin on the central nervous system. Fedn. Proc., 31, 1332-1337.

60. Bueding, E., Bulbring, E., Gercken, G. Hawkins, J.T. & Kuriyama, H. (1967). The effect of adrenaline on the adenosine triphosphate and creative phosphate content of intestinal smooth muscle. J. Physiol., Lond., 193, 187-212.

61. Bulbring, E. (1953). Measurement of oxygen consumption in smooth muscle. J. Physiol., Lond., 122, 111-134.

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

63. Bulbring, E. & Tomita, T. (1970). The effects of calcium removal on the smooth muscle of the guinea-pig taenia coli. J. Physiol., Lond., 210, 217-232.

64. Bulbring, E. & Szurszweski, J.H. (1974). The stimulant action of noradrenaline (α action) on guinea-pig myometrium compared with that of acetylcholine. Proc. R. Soc. Lond. B., 185, 225-262.

65. Bumpus, F.M. (1971). Angiotensin - structure - activity relationships of protein and polypeptide hormones. Margoulies, M. & Greenwood, F.C. pp. 18-22. Exerpta Medica: Amsterdam.

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

67. Burton, J. & Godfraind, T. (1973). Na-Ca sites in the longitudinal smooth muscle of the guinea-pig ileum and the action of drugs. Arch. int. Pharmacodyn., 204, 184-186.

68. Busselin, P. & Carmeliat, E. (1973). Protagonistic effects of sodium and calcium on tension development in cardiac muscle at low extracellular sodium concentrations. Nature, New Biol., 243, 57-59.

69. Butcher, R.W. (1970). Prostaglandins and cyclic AMP. In: Advances in Biochemical Psychopharmacology Volume 3. The Role of Cyclic AMP in Cell Function, ed. Greengard, P. & Costa, E. pp. 173-183. Raven Press: New York.

70. 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 characterise adenosine 3', 5' - phosphate in human urine. J. biol. Chem., 237, 1244.

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

72. Carriere, S. & Friborg, J. (1969). Intrarenal blood flow and PAH extraction during angiotensin infusion. Am. J. Physiol., 217, 1708 - 1715.

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

74. Case, D.B., Gunther, S.J. & Cannon, P.J. (1973). Ethacrynate-induced depression of respiration in transport systems and kidney mitochondria. Am. J. Physiol., 224, 769-780.

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

76. 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.

77. Chandler, J.T. & Strong, C.G. (1972). The actions of prostaglandin E on isolated rabbit aortae. Arch. int. Pharmacodyn., 197, 123-131.

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

79. Coe, J., Detar, R. & Bohr, D.F. (1968). Substrates and vascular smooth muscle contraction. Am. J. Physiol., 214, 245-250.

80. Cosby, R.H., Sartorelli, A.C. & Roth, R.H. (1971). Pharmacologist, 13, 126.

81. Coviello, A. (1972). Hydrosmotic effect of angiotensin II in isolated toad bladder. Acta. physiol. latinoam., 22, 218-226.

82. Coviello, A. (1973). Hydrosmotic effect of angiotensin II in the toad bladder: role of cyclic AMP. Acta. physiol. latinoam., 23, 350-357.

83. Coviello, A. & Crabbe, J. (1965). Effect of angiotensin II on active transport of sodium by toad bladder and skin. Biochem. Pharmac., 14, 1739-1744.

84. Coviello, A. & Brauckmann, E.S. (1973). Hydrosmotic effect of angiotensin II: isolated toad skin. Acta. physiol. latinoam., 23, 18-23.

85. Crocker, A.D. (1971). Variations in mucosal water and sodium transfer associated with the rat oestrous cycle. J. Physiol., Lond., 214, 257-264.

86. Crocker, A.D. & Munday, K.A. (1967). Aldosterone and angiotensin action on water absorption in the rat jejenum. J. Physiol., Lond., 192, 36-38P.

87. Crocker, A.D. & Munday, K.A. (1970). The effect of the renin-angiotensin system on mucosal water and sodium transfer in everted sacs of rat jejenum. J. Physiol., Lond., 206, 323-333.

88. Csapo, A. & Gergely, J. (1950). Energetics of uterine muscle contraction. Nature, Lond., 166, 1078.

89. Daemers-Lambert, C. (1958). Les comprés phosphorés du muscle lisse vasculaine. Symp. int. Biochemie de la pavoi vasculaire, Friborg. 1968. Part III pp. 185. Karger: Basel.

90. Daniel, E.E. (1964a). Effect of drugs on contractions of vertebrate smooth muscle. Ann. Rev. Pharmac., 4, 189-221.

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

92. Daniel, E.E. (1964c). Effects of cocaine and adrenaline on contractures and downhill ion movements induced by inhibition of membrane ATPase in rat uterus. Can. J. Physiol. Pharmac., 42, 497-526.

93. Daniel, E.E. (1965). Effect of sympathomimetic amines on active ion transport in smooth muscles. Arch. int. Pharmacodyn., 158, 131-138.

94. Daniel, E.E., Robinson, K., Kidwai, A.M., Wolowyck, M.W., Taylor, G.S. & Paton, D.M. (1969). The sodium pump in smooth muscle. Proc. Symp. Physiol. Pharmac. Vasc. Neuroeffector Systems, Interlaken 1969. pp. 229-246. Karger: Basel (1971).

95. Daniel, E.E., Kidwai, A.M., Robinson, K. Freeman, D. & Fair, S. (1971). The mechanisms by which ethacrynic acid affects ion content, ion fluxes, volume and energy supply in the rat uterus. J. Pharmac. exp. Ther., 176, 563-579.

96. Davies, N.T., Munday, K.A. & Parsons, B.J. (1969). Dose dependent biphasic effects of angiotensin on fluid transport by rat colon. J. Physiol., Lond., 204, 142-143P.

97. Davies, N.T., Munday, K.A. & Parsons, B.J. (1970). The effect of angiotensin on rat intestinal fluid transfer. J. Endocrinology, 48, 39-46.

98. Davies, N.T., Munday, K.A. & Parsons, B.J. (1972). Studies on the mechanism of action of angiotensin on fluid transport by the mucosa of rat distal colon. J. Endocr., 54, 483-492.

99. Davis, J.O. (1960). "Discussion in Symposium on Aldosterone" at First International Congress of Endocrinology, Copenhagen.

100. Davis, J.O. (1967). The Regulation of Aldosterone Secretion. In: The Adrenal Cortex, ed. Eisentein, A.B. pp. 203-247. Little Brown and Co. : Boston, Massachusetts.

101. Davis, J.O. (1971). The Renin-Angiotensin-Aldosterone System. In: Kidney Hormones, ed. Fisher, J.W. pp. 173-205. Academic Press: New York and London.

102. Davis, J.O., Carpenter, C.C.J., Ayers, C.R., Holmann, J.E. & Bahn, R.C. (1961). Evidence for secretion of an aldosterone-stimulating hormone by the kidney. J. clin. Invest., 40, 684-696.

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

104. Day, M. & Vane, J.R. (1963). An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum. Br. J. Pharmac. Chemother., 20, 150-170.

105. Denton, D.A., Goding, J.R. & Wright, R.D. (1959). Control of adrenal secretion of electrolyte-active steroids. Br. Med. J. ii; 447, 522.

106. Detar, R. & Bohr, D.F. (1968a). Adaption to hypoxia in vascular smooth muscle. Fedn. Proc., 27, 1416-1419.

107. Detar, R. & Bohr, D.F. (1968b). Oxygen and vascular smooth muscle contraction. Am. J. Physiol., 214, 241-244.

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

109. 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. B., 265, 17-23.

110. Devynck, M-A., Pernollet, M-G., Meyer, P., Fermandjian, S. & Fromageat, P. (1973). Angiotensin receptors in smooth muscle membranes. Nature, New Biol., 245, 55-58.

111. Devynck, M-A., Pernollet, M-G., Meyer, P., Fermandjian, S., Fromageot, P. & Bumpus, F.M. (1974). Solubilisation of angiotensin II receptors in rabbit aortae membranes. Nature, Lond., 249, 67-69.

112. Dhalla, N.S. & Olson, R.E. (1967). Correlation of the endogenous fuel supply and contractile force in the isolated perfused rat heart. Fedn. Proc. 26, 352.

113. Diamond, J. (1973). Phosphorylase, calcium and cyclic AMP in smooth muscle contraction. Am. J. Physiol., 225, 930-937.

114. Diamond, J. & Brody, T.M. (1966). Relationship between smooth muscle contraction and phosphorylase activation. J. Pharmac. exp. Ther., 152, 212-220.

115. Dickens, F. & Weil-Malherbe, H. (1941). Metabolism of normal and tumour tissue: the metabolism of intestinal mucous membrane. Biochem. J., 35, 7-15.

116. Dixon, M. & Webb, E.C. (1960). Enzymes. Longmans, Green and Co.: London.

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

118. Dunham, E.W. & Zimmerman, B.G. (1970). Release of prostaglandin like material from dog kidney during nerve stimulation. Am. J. Physiol., 219, 1279-1285.

119. Dunham, E.W., Haddox, M.K. & Goldberg, N.D. (1974). Alteration of vein cyclic 3', 5' nucleotide concentrations during changes in contractility. Proc. Nat. Acad. Sci. U.S.A., 71, 815-819.

120. Durbin, R.P. & Jenkinson, D.H. (1961). The calcium dependence of tension development in depolarised smooth muscle. J. Physiol., Lond., 157, 90-96.

121. Dworaczek, E. & Barrenscheen, H.K. (1937). Uber die chemische Urnsetzung der glotten musculatur. Biochem. Z., 292, 388.

122. Ebashi, E. (1969). Comparative aspects of structural proteins of muscle with particular reference to regulatory proteins. Proc. Symp. Physiol. Pharmacol. (Interlaken, 1969) pp. 190-201. Karger: Basel. 1971.

123. Ebashi, S. (1972). Calcium ions and muscle contraction. Nature, Lond., 240, 217-218.

124. Ebashi, S., Endo, M. & Ohtsuki, I. (1969). Control of muscle contraction. Q. Rev. Biophys., 2, 351-384.

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

126. Elliot, D.F. & Peart, W.S. (1956). Amino-acid sequence in a hypertensin. Nature, Lond., 177, 527-528.

127. Ellis, D.E. & Reit, E. (1969). Inhibition by lidoflazine of the contractile response of the rat isolated colon to angiotensin. Br. J. Pharmac., 35, 132-140.

128. Epstein, R.W. (1972). The effects of ethacrynic acid on active transport of sugars and ions and on other metabolic processes in rabbit kidney cortex. Biochim. biophys. Acta., 274, 128-139.

129. Evans, C. Lovatt (1923). The oxygen usage of smooth muscle and its relation to tonus. J. Physiol., Lond., 58, 22-32.

130. Evans, C. Lovatt (1926). The physiology of plain muscle. Physiol. Rev., 6, 358-398.

131. Fanestil, D.D. (1969). Mechanism of action of aldosterone. Ann. Rev. Med., 20, 223-232.

132. Farah, A., West, T.C. & Angel, R. (1950). The action of sodium flouroacetate on intestinal smooth muscle. J. Pharmac. exp. Ther., 98, 234-244.

133. Feldberg, W. (1951). Effects of ganglion blocking substances on the small intestine. J. Physiol, Lond.,113, 483-505.

134. Feldberg, W. & Solandt, O.M. (1942). The effects of drugs, sugars and allied substances on the isolated small intestine of the rabbit. J. Physiol., Lond., 101, 137-171.

135. Ferreira, S.H., Moncada, S. & Vane, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature, New Biol., 231, 237-239.

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

137. 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.

138. Finberg, J.P.M. & Peart, W.S. (1970). Renal tubular flow dynamics during angiotensin diuresis in the rat. Br. J. Pharmac., 39, 357-372.

139. Finberg, J.P.M. & Peart, W.S. (1972). The effect of angiotensin, noradrenaline and vasopressin on blood flow distribution in the rat kidney. J. Physiol., Lond., 220, 229-242.

140. Fitzpatrick, D.F., Landon, E.J., Debbas, G. & Hurwitz, L. (1972). A calcium pump in vascular smooth muscle. Science, 176, 305-306.

141. Fitzsimons, J.T. (1972). Thirst. Physiol. Rev., 52, 468-561.

142. Flower, R.J. & Vane, J.R. (1974). Inhibition of prostaglandin biosynthesis. Biochem. Pharmac., 23, 1439-1450.

143. Flower, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis. Pharmac. Rev., 26, 33-90.

144. Freer, R. J. (1974). Evidence for two different mechanisms for angiotensin II induced contractions of rat uterus. Fedn. Proc. 32, 547.

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

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

147. Friedman, S.M. & Friedman, C.L. (1967). The Ionic Matrix of Vasoconstriction. Circulation Res., 20 and 21 Suppl II, 147-155.

148. Furchgott, R.F. (1966). Metabolic factors that influence the contractility of vascular smooth muscle. Bull. N.Y. Acad. Med., 42, 996-1006.

149. Furchgott, R.F. & Shorr, E. (1946). Sources of energy for intestinal smooth muscle contraction. Proc. Soc. exp. Biol. Med., 61, 280-286.

150. Furchgott, R.F. & Wales, M. (1951). Effect of pH on contractile activity of rabbit intestinal smooth muscle with and without added substrates. Am. J. Physiol. 167, 386-398.

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

152. Gabella, G. (1973). Fine structure of smooth muscle. Phil. Trans. R. Soc. B., 265, 7-16.

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

154. Gagnon, D.J. & Hessler, S. (1974). Accumulation of cyclic AMP in the neurohypophysis in response to angiotensin. Biochim. Biophys. Acta., 338, 394-397.

155. Gagnon, D.J., Gauthier, R. & Regali, D. (1974). Release of prostaglandins from the rabbit perfused kidney: effects of vaso-constrictors. Br. J. Pharmac., 60, 553-558.

156. Garry, R.C. (1928). The effect of oxygen lack on surviving smooth muscle. J. Physiol., Lond., 66, 235-248.

157. Garven, J.D. (1956). Pharmacological blocking actions of atropine and certain atropine like compounds. J. Pharm. Pharmac., 8, 256-263.

158. Genest, J., Koiw, E., Nowacynski, W. & Leboef, C. (1958). Further studies on urinary aldosterone in human arterial hypertension. Proc. Soc. exp. Biol. Med., 97, 676-679.

159. Gillespie, J.S. (1966). Summary of discussion and commentary on Section V: "Adrenergic Transmission" in Second Symposium on Catecholamines, Milan, July 4-9, 1965. Pharmac. Rev., 18, 537-540.

160. Glynn, I.M. (1957). The action of cardiac glycosides on sodium and potassium movements in human red cells. J. Physiol., Lond., 136, 148-173.

161. Glynn, I.M. (1964). The action of cardiac glycosides on ion movements. Pharmac. Rev., 16, 381-407.

162. Godfraind, T. (1968). The pharmacology of angiotensin antagonists. In: The pharmacology of hormonal peptides and proteins pp. 497-506. Plenum Press: New York.

163. Godfraind, T. (1970). Angiotensin autopotentiation. Br. J. Pharmac., 40, 542-543P.

164. Godfraind, T. & Godfraind-De Becker, A. (1963). Actions des glycosides cardiotoniques sur le muscle lisse 1. Leur, antagonisme vis-á-vis de l'histamine et de l'acetylcholine sur l'itéon isolé de cobaye. Arch. int. Pharmacodyn., 144, 226-240.

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

166. Godfraind, T. & Kaba, A. (1969). Actions phasique et tonique de l'adrénaline sur un muscle lisse vasculaire et leur inhibition par des agents pharmacologiques. Arch. int. Pharmacodyn. 178, 488-491.

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

168. Godfraind, T. & Verbecke, N. (1973). The action of adrenaline on calcium pump and adenosine triphosphatases activities of intestinal smooth muscle microsomes. Arch. int. Pharmacodyn., 204, 187-190.

169. Goldberg, N.D., Haddox, M.K., Hartle, D.K. & Hadden, J.W. (1973). Proc. V. International Congress Pharmacology. V5. pp. 146-169. Karger: Basel.

170. Goldenberg, M.M. (1967). Action of angiotensin on isolated gerbil, mouse and rat intestines. Fedn. Proc., 26, 465.

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

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

173. Goormaghtigh, N. (1944). La fonction endocrine des arterioles renales. Louvain: Librarie Fonteyn.

174. Gordon, R.D., Küchel, O., Liddle, G.W. & Island, D.P. (1967). Role of the sympathetic nervous system in regulating renin and aldosterone in man. J. clin. Invest., 46, 599-605.

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

176. Gross, F. (1958). Renin und Hypertensin, physiologische oder pathologische Wirkstoffe. Klin. Wschr., 36, 693-706.

177. Gross, F. (1963). Angiotensin. Arch. Exp. Path. Pharmak., 245, 196-229.

178. Gross, F. (1971). Angiotensin. In: International encyclopaedia of pharmacology and therapeutics, Section 72, Volume 1, Pharmacology of naturally occurring polypeptides and lipid-soluble acids, pp. 73-286. Pergamon: Oxford.

179. Gross, F. & Lichtlen, P. (1958). Verstärkung der pressorischen Wirkung von Renin drucksteigernden Peptiden und adrenergischen Wirkstoffen nach Nephrektomie und bei Veberdasiering von Cortexon und Kochsalz an der Ratte. Arch. Exp. Path. Pharmak., 233, 323-337.

180. 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. Pergamon: Oxford.

181. Gross, F. & Mohring, J. (1973). Renal pharmacology with special emphasis on aldosterone and angiotensin. Ann Rev. Pharmac., 13, 57-90.

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

183. Gross, S.R. & Mayer, S.E. (1974). Regulation of phosphorylase b to a conversion in muscle. Life. Sci. Oxford., 14, 401-414.

184. Griffin, J.D., Szaro, R.P. & Weltman, J.K. (1972). Ouabain antagonism of smooth muscle contraction. J. Pharmac. exp. Ther., 182, 378-387.

185. Gyang, E.A., Deuben, R.R. & Buckley, J.P. (1973). Interaction of prostaglandin E_1 and angiotensin II on centrally mediated pressor activities in the cat. Proc. Soc. exp. Biol. Med., 142, 532-537.

186. Hadden, J.W., Hadden, E.M., Haddox, M.K. & Goldberg, N.D. (1972). Guanosine 3', 5'-cyclic monophosphate: a possible intracellular mediator of mitogenic influences in lymphocytes. Proc. natn. Acad. Sci. U.S.A., 69, 3024-3027.

187. Hall, M.M., Khosla, M.C., Khairallah, P.A. & Bumpus, F.M. (1974). Angiotensin analogues the influence of sarcosine substituted in position 1. J. Pharmac. exp. Ther., 188, 222-228.

188. Hamoir, J. (1973). Excitability and properties of the contractile proteins of vertebrate smooth muscles. Phil. Trans. R. Soc. B., 265, 169-181.

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

190. Handler, J.S., Butcher, R.W., Sutherland, E.W. & Orloff, J. (1965). The effect of vasopressin and theophylline on the concentration of adenosine 3', 5' - phosphate in the urinary bladder of the toad. J. biol. Chem., 240, 4524-4526.

191. Heally, J.K., Barcena, C., O'Connell, J.M.B. & Schreiner, G.E. (1965). Renal and pressor actions of angiotensin in the normal dog. Am. J. Physiol., 208, 1093-1099.

192. Heally, J.K., Suszkiw, J.B., Dennis, V.W. & Schreiner, G.E. (1966). Effect of aldosterone and salt intake on renal and pressor actions of angiotensin. Nephron, 3, 229-243.

193. Hedqvist, P. (1969). Modulating effect of prostaglandin F_2 on noradrenaline release from isolated cat spleen. Acta physiol. Scand., 75, 511-512.

194. Herbaczynska-Cedro, K. & Vane, J.R. (1973). An intra-renal role for prostaglandin production. Abstracts of Seventh Annual Meeting of European Society for Clinical Investigation, Rotterdam.

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

196. Hofmann, W.W. (1969). Membrane potential and muscle function. Ann. Rev. Med., 20, 101-130.

197. Hogan, B. & Shields, R. (1974). Yin-Yan hypothesis of growth control. New Scientist, 6 2, 323-325.

198. Honoré, L.H. & Gardner, D.L. (1966). Cardiovascular reactivity of rats during the development of salt hypertension. I. The response to polypeptides. Arch. int. Pharmacodyn., 164, 173-182.

199. Hornych, A., Meyer, P. & Milliez, P. (1973). Angiotensin, vasopressin and cyclic AMP effects on sodium and water fluxes in rat colon. Am. J. Physiol. 224, 1223-1229.

200. Horton, E.W. (1969). Hypothesis on physiological role of prostaglandins. Physiol. Rev., 49, 122-169.

201. Hughes, M.J. & Coret, I.A. (1968). Metabolic transients: hypoxic and oxygenated rabbit aortae with and without substrate. Am. J. Physiol., 215, 318-323.

202. Hurwitz, L., Joiner, P.D. & Hagen, S. (1967). Mechanical responses of intestinal smooth muscle in a calcium free medium. Proc. Soc. exp. Biol. Med., 125, 518-522.

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

204. Hurwitz, L., Hubbard, W. & Little, S. (1972). The relationship between the drug receptor interaction and calcium transport in smooth muscle. J. Pharmac. exp. Ther., 183, 117-126.

205. Innes, I.R., Kosterlitz, H.W. & Robinson, J.A. (1957). The effects of lowering the bath temperature on the responses of the isolated guinea-pig ileum. J. Physiol., Lond., 137, 396-409.

206. Iso, T. (1973). Effects of cyclic 3', 5'-AMP and related compounds on catecholamine-induced contraction of isolated guinea-pig vas deferons. Jap. J. Pharmac., 23, 717-721.

207. Itskovitz, H.D., Murphy, J.J. & Schoenberg, H.W. (1967). Differential renal function studies of the effects of angiotensin in normotensive and hypertensive patients. Am. J. med. Sci., 253, 163-173.

208. Jones, V.D. & Landon, E.J. (1967). The effect of ouabain, meralluride and ethacrynic acid on respiration and glycolysis in kidney slices. Biochem. Pharmac., 16, 2163-2169.

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

210. Keatinge, W.R. (1972a). Mechanical responses with reversed electrical response to noradrenaline by calcium-deprived arterial smooth muscle. J. Physiol., Lond., 224, 489-500.

211. Keatinge, W.R. (1972b). Calcium concentration and flux in calcium-deprived arteries. J. Physiol., Lond., 224, 21-34.

212. Kerly, M. (1937). The effect of the oestrous cycle on the metabolism of isolated rat uterus. Biochem. J., 31, 1544-1552.

213. Kerly, M. (1940). The effect of oestrone on the metabolism of the rat uterus. Biochem. J., 34, 814-819.

214. Khairallah, P.A. (1971). The Pharmacology of Angiotensin. In: Kidney Hormones, ed. Fisher, J.W. pp. 129-171. Academic Press: New York.

215. Khairallah, P.A. (1972). Action of angiotensin on adrenergic nerve endings: inhibition of norepinephrine uptake. Fedn. Proc., 31, 1351-1357.

216. Khairallah, P.A. & Page, I.H. (1961). The mechanism of action of angiotensin and bradykinnin on smooth muscle in situ. Am. J. Physiol., 200, 51-57.

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

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

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

220. Khairallah, P.A., Toth, A. & Bumpus, F.M. (1970). Analogs of angiotensin II. II. Mechanism of receptor interaction. J. med. Chem., 13, 181-184.

221. Kinson, G.A. & Singer, B. (1968). Sensitivity to angiotensin and adrenocorticotrophic hormone in the sodium deficient rat. Endocrinology., 83, 1108-1116.

222. Kosterlitz, H.W. & Robinson, J.A. (1958). The inhibitory action of morphine on the contraction of the longitudinal muscle coat of the isolated guinea-pig ileum. Br. J. Pharmac., 13, 296-303.

223. Kővér, Gy., Bálint, T. & Tost, H. (1971). Angiotensin and renal function. Int. Urol and Nephrol., 3, 271-282.

224. Kuehl, F.A., Humes, J.L., Cirillo, V.J. & Ham, E.A. (1972). Cyclic AMP and prostaglandins in Hormone Action. In: Advances in Cyclic Nucleotide Research I ed. Greengard, P. and Robison, G.A. pp. 493-507. Raven Press: New York.

225. Kuriyama, H. (1970). Effects of Ions and drugs on the electrical activity of smooth muscle. In: Smooth Muscle, ed. Bulbring, E., Brading, A., Jones, A. & Tomita, T. pp. 366-395. Arnold: London.

226. Landon, E.J. & Fitzpatrick, D.F. (1972). Ethacrynic acid and kidney cell metabolism. Biochem. Pharmac., 21, 1561-1568.

227. Laragh, J.H., Angers, M., Kelly, W.G. & Lieberman, S. (1960). Hypotensive agents and pressor substances. J. Am. med. Ass., 174, 234-240.

228. Laragh, J.H., Cannon, P.J., Bentzel, C.J., Sicinski, A.M. & Meltzer, J.I. (1963). Angiotensin II, norepinephrine and renal transport of electrolytes and water in normal man and in cirrhosis with ascites. J. clin. Invest., 42, 1375-1376.

229. Laragh, J.H., Boer, L., Brunner, H.R., Buhler, F.R., Sealey, J.E. & Vaughan, E.D. (1972). Renin, angiotensin and aldosterone system in pathogenesis and management of hypertensive vascular disease. Am. J. Med. 52, 633-652.

230. Lee, K.S. & Klaus, W. (1971). The subcellular basis for the mechanism of the inotropic action of cardiac glycosides. Pharmac. Rev., 23, 193-261.

231. Lee, M.R. (1969). Renin and Hypertension. A modern synthesis. Lloyd-Luke: London.

232. Lefer, A.M. (1967). Influence of mineralocorticoids and cations on the inotropic effect of angiotensin and norepinephrine in isolated cardiac muscle. Am. Heart. J., 73, 674-680.

233. Leysacc, P. (1965). The in vivo effect of angiotensin and noradrenaline on the proximal tubular reabsorption of salt in mammalian kidneys. Acta physiol. scand., 64, 167-175.

234. Leysacc, P., Lassen, U.V. & Hess Thaysen, J. (1961). Inhibition of sodium transport in isolated renal tissue by angiotensin. Biochim. Biophys. Acta., 48, 602-603.

235. Limas, C.J. (1974). Stimulation by angiotensin of myocardial prostaglandin synthesis. Biochim. Biophys. Acta., 337, 417-420.

236. Limas, C.J. & Cohn, J.N. (1973). Alkaline phosphatase in vascular smooth muscle. Nature, New Biol., 245, 53-55.

237. Lipmann, E.A. (1941). Metabolic generation and utilisation of phosphate bond energy.
Adv. Enzymol., 1, 99-162.

238. Lo, S., Russel, J.C. & Taylor, A.W. (1970). Determination of glycogen in small tissue samples. J. appl. Physiol., 28, 234-236.

239. Lockett, M.F. (1972). Actions of adrenal, hypophysial and renal hormones on the renal excretion of water and electrolytes. Prog. Biochem. Pharmac. 7, 94-145.

240. Lowitz, H.D., Stumpe, K.O. & Ochwadt, B. (1969). Micropuncture study of the action of angiotensin II on tubular sodium and water reabsorption in the rat. Nephron., 6, 173-187.

241. Lullman, 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. Arnold: London.

242. Lundholm, L. & Mohme-Lundholm, E. (1960). The carbohydrate metabolism and tone of smooth muscle. Acta pharmac. tox., 16, 374-388.

243. Lundholm, L. & Mohme-Lundholm, E. (1962). Studies on the effect of drugs upon the lactic acid metabolism and contraction of vascular smooth muscle. Acta physiol. scand., 55, 45-63.

244. Lundholm, L. & Mohme-Lundholm, E. (1963a). Dissociation of contraction and stimulation of lactic acid production in experiments on smooth muscle under anaerobic conditions. Acta physiol. scand., 57, 111-124.

245. Lundholm, L. & Mohme-Lundholm, E. (1963b). Contraction and glycogenolysis of smooth muscle. Acta physiol. scand., 57, 125-129.

246. Lundholm, L. & Mohme-Lundholm, E. (1965). Energetics of isometric and isotonic contraction in isolated vascular smooth muscle under anaerobic conditions. Acta physiol. scand., 64, 275-282.

247. Macknight, A.D.C. (1969). The effects of ethacrynic acid on the electrolyte and water contents of rat renal cortical slices. Biochim. Biophys. Acta., 173, 223-233.

248. Malvin, R.L. & Vander, A.J. (1967). Effects of angiotensin infusion on renal function in the unanesthetized rat. Am. J. Physiol., 213, 1205-1208.

249. Marc-Aurièle, J. & Bergeron, M. (1966). Lack of evidence for an inhibitory effect of angiotensin on sodium potassium adenosine triphosphatase. Revue can. Biol., 25, 107-109.

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

251. Mathews, E.K. & Sutter, M.C. (1967). Ouabain-induced changes in the contractile and electrical activity, potassium content, and response to drugs, of smooth muscle cells. Can. J. Physiol. Pharmac., 45, 509-520.

252. 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 cali. Pflügers Arch. ges. Physiol., 337, 333-350.

253. McAfee, R-D. & Locke, W. (1967). Effect of angiotensin amide on sodium isotope flux and short circuit current of isolated frog skin. Endocrinology, 81, 1301-1305.

254. McFarland, S.A., Guyton, C.A. & Pfaffman, M.A. (1971). Effects of theophylline on the contractile activity of taenia cali. Arch. int. Pharmacodyn. 192, 179-187.

255. McGiff, J.C., Crowshaw, K., Terragno, N.A. & Lonigro, A.J. (1970). Release of prostaglandin-like substance into renal venous blood in response to angiotensin II. Circulation Res., 26-27, suppl. I, 121-130.

256. McGiff, J.C., Crowshaw, K.,Terragno, N.A., Malik, K.V. & Lonigro, A.J. (1972). Differential effect of noradrenaline and renal nerve stimulation on vascular resistance in the dog kidney and the release of a prostaglandin E-like substance. Clin. Sci., 42, 223-233.

257. Menkes, J.H. & Csapo, A. (1952). Changes in the adenosinetriphosphate and creatine phosphate content of the rabbit uterus throughout sexual maturation and after ovulation. Endocrinology, 50, 37-49.

258. Mimran, A., Hinrichs, K.J. & Hollenberg, N.K. (1974). Characterisation of smooth muscle receptors for angiotensin with an antagonist. Am. J. Physiol., 226, 185-190.

259. Munday, K.A., Parsons, B.J. & Poat, J.A. (1971). The effect of angiotensin on cation transport by rat kidney cortex slices. J. Physiol., Lond., 215, 269-282.

260. Munday, K.A., Parsons, B.J. & Poat, J.A. (1972). Studies on the mechanism of action of angiotensin on ion transport by kidney cortex slices. J. Physiol., Lond., 224, 195-206.

261. Munday, K.A., Parsons, B.J., Poat, J.A. & Smith, D.J. (1973). The effect of divalent cations on angiotensin stimulation of fluid transport by rat colon. J. Physiol., Lond., 232, 89P-91P.

262. Mulrow, P.J. & Goffnet, J.A. (1969). The Renin-Angiotensin System. In: The Physiology of the kidney, ed. Wesson, L.G. pp. 465-520. Crane and Stratton: New York and London.

263. Namm, D.H. & Zucker, J.L. (1973). Biochemical alterations caused by hypoxia in the isolated rabbit aortae. Circulation Res., 32, 1464-1470.

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

265. Nasu, T. & Urakawa, N. (1973). Effect of ch linergic drugs on calcium movement in guinea-pig taenia cali. Jap. J. Pharmac., 23, 553-561.

266. Naylor, W.G. (1966). Influx and efflux of calcium in the physiology of muscle contraction. Clin. Orthop., 46, 157-182.

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

268. Ng, K.K.F. & Vane, J.R. (1968). Fate of angiotensin I in the circulation. Nature, Lond., 218, 144-150.

269. Northover, B.J. (1967). The effect of anti imflammatory drugs on vascular smooth muscle. Br. J. Pharmac. Chemother., 31, 483-493.

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

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

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

273. Nugteren, D.H., Beerthuis, R.V. & Van Dorp, D.A. (1967). Biosynthesis of prostaglandins. In: Proc. II Nobel Symp. ed. Bergström, S. & Samuelsson, B. pp. 45-50. Interscience Publishers: Stockholm.

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

275. Ohashi, H., Ohga, A. & Saito, K. (1973). Enhancement of Ca-contractions by catecholamines and temperature dependency in the depolarised taenia cali of the guinea-pig. Jap. J. Pharmac., 23, 467-477.

276. Oliveira, M.M. & Holzhacker, S. (1974). Isolation and characterisation of smooth muscle cell membranes. Biochim. Biophys. Acta. 332, 221-232.

277. Page, I.H. & Helmer, O.M. (1940). A crystalline pressor substance (angiotonin) resulting from the reaction between renin and renin-activator. J. exp. Med., 71, 29-42.

278. Page, I.H. & Bumpus, F.M. (1961). Angiotensin. Physiol. Rev. 41, 331-390.

279. Page, I.H. & McCubbin, J.W. (1968). Renal Hypertension Year Book Medical Publishers Inc.: Illinois.

280. 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.

281. Panisset, J-C. (1967). Effect of angiotensin on the release of acetylcholine from pre-ganglionic and post-ganglionic nerve endings. Can. J. Physiol. Pharmac., 45, 313-317.

282. Papadimitriou, A. & Worcel, M. (1974). Dose-response curves for angiotensin II and synthetic analogues in three types of smooth muscle: existence of different forms of receptor sites for angiotensin II. Br. J. Pharmac., 50, 291-297.

283. Parsons, B.J. & Munday, K.A. (1972). The action of angiotensin on sodium and fluid transport. In: Transport across the intestine, A Glaxo Symposium, ed. Burband, W.L. & Samuel, P.D. pp. 59-70. Churchill-Livingstone: London.

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

285. Paton, W.D.M. (1955). The response of the guinea-pig ileum to electrical stimulation by co-axial electrodes. J. Physiol., Lond., 127, 40-41P.

286. Paton, W.D.M. (1961). A theory of drug action based on the rate of drug receptor combination. Proc. R. Soc. Lond. B., 154, 21-61.

287. Paton, W.D.M. & Rothschild, A.M. (1965). The changes in response and in ionic content of smooth muscle produced by acetylcholine action and by calcium deficiency. Br. J. Pharmac. Chemother., 24, 437-448.

288. Peach, M.J. (1971). Adrenal medullary stimulation induced by angiotensin II and analogues. Circulation Res., 28 and 29 Suppl. II, 107-117.

289. Peach, M.J., Bumpus, F.M. & Khairallah, P.A. (1969). Inhibition of norepinephrine uptake in hearts by angiotensin II and analogs. J. Pharmac. exp. Ther., 167, 291-299.

290. Peart, W.S. (1965). The functions for renin and angiotensin. Recent Prog. Horm. Res., 21, 73-101.

291. Peart, W.S. (1971). Renin-angiotensin system in hypertensive disease. In: Kidney Hormones, ed. Fisher, J.W. pp. 217-242. Academic Press: New York and London.

292. Peytremann, A., Nicholson, W.E., Brown, R.D., Liddle, G.W. & Hardmann, J.G. (1973). Comparative effects of angiotensin and ACTH on cyclic AMP and steroidogenesis in isolated bovine adrenal cells. J. clin. Invest., 52, 835-842.

293. Pfaffman, M., Urakawa, N. & Holland, W.C. (1965). Role of metabolism in potassium-induced tension changes in guinea-pig taenia cali. Am. J. Physiol., 208, 1203-1205.

294. Pfaffman, M.A. & McFarland, S.A. (1973). The effects of theophylline and cyclic AMP on intestinal smooth muscle contractions. Eur. J. Pharmac., 23, 147-152.

295. Prasad, B.N. (1935a). The carbohydrate metabolism of gut muscle. J. Physiol., Lond., 101, 239-248.

296. Prasad, B.N. (1935b). The mechanical activity of gut muscle under anaerobic conditions. J. Physiol., Lond., 85, 249-266.

297. Prasad, K. & MacLeod, D.P. (1969). Influence of glucose on the transmembrane action potential of guinea-pig papillary muscle. Circulation Res., 24, 939-950.

298. Proverbio, F., Robinson, J.W.L. & Whittembury, G. (1970). Sensitivities of ($Na^+ K^+$) - ATPase and Na^+ -extrusion mechanisms to ouabain and ethacrynic acid in the cortex of the guinea-pig kidney. Biochim. Biophys. Acta., 211, 327-336.

299. Raeymaekers, L., Wuytack, F. & Casteels, R. (1974). Na-Ca exchange in taenia cali of guinea-pig. Pflügers Arch. ges Physiol., 347, 329-340.

300. Rall, T.W. & Sutherland, E.W. (1962). Adenyl cyclase II. The enzymatically catalysed formation of adenosine 3', 5'-phosphate and inorganic pyrophosphate from adenosine triphosphate. J. biol. Chem., 237, 1228-1232.

301. Rall, T.W. & West, T.C. (1963). The potentiation of cardiac inotropic responses to norepinephrine by theophylline. J. Pharmac. exp. Ther., 139, 269-274.

302. Ramwell, P.W. & Shaw, J.E. (1967). Prostaglandin release from tissues by drug nerve and hormone stimulation. In: Prostaglandins, ed. Bergstrom, S. & Samuelsson, B . Almqvist & Weksell: Stockholm.

303. Ramwell, P.W. & Rabinowitz, I. (1971). Interaction of prostaglandins and cyclic AMP. In: Effects of Drugs on Cellular Control Mechanisms, ed. Rabin, B.R. & Friedman, R.B. pp. 207-235. Macmillan: London.

304. Rasmussen, H. (1969). Influence of polypeptide hormones upon ion transport and ion binding. In: Protein and Polypeptide Hormones, ed. Margoulies, M. & Greenwood, F.C., pp. 247-256. Exerpta Medica: Amsterdam.

305. Rasmussen, H. (1970). Cell communication, calcium Ion, and cyclic adenosine monophosphate. Science, 170, 404-412.

306. Rasmussen, H. & Tehenhouse, A. (1968). Cyclic adenosine monophosphate, calcium and membranes. Proc. Natn. Acad. Sci. U.S.A., 59, 1364-1370.

307. Regoli, D. & Vane, J.R. (1964). A sensitive method for the assay of angiotensin. Br. J. Pharmac. Chemother., 23, 351-359.

308. 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.

309. Renson, J., Barac, G. & Bacq, Z.M. (1959). Angiotensines, calcium et effet oxytocique. C.R. Soc. Biol., Paris, 153, 706-708.

310. Reit, E. (1972). Actions of angiotensin upon the adrenal medulla and autonomic ganglia. Fedn. Proc., 31, 1338-1343.

311. Reuter, H. (1974). Exchange of calcium ions in the mammalian myocardium. Mechanisms and physiological significance. Circulation Res., 34, 599-605.

312. Reuter, H., Blaustein, M.P. & Haesler, G. (1973). Na-Ca exchange and tension development in smooth muscle. Phil. Trans. R. Soc. Lond. B., 265, 87-94.

313. Robertson, A.L. & Khairallah, P.A. (1971). Angiotensin II: Rapid localisation in nuclei of smooth and cardiac muscle. Science, 172, 1138-1139.

314. Robinson, J.W.L. (1972). The inhibition of glycine and β-methylglucoside transport in dog kidney cortex slices by ouabain and ethacrynic acid: contribution to the understanding of sodium-pumping mechanisms. Comp. gen. Pharmac., 3, 145-159.

315. Robison, G.A., Butcher, R.W. & Sutherland, E.W. (1965). On the relation of hormone receptors to adenyl cyclase. In: Fundamental Concepts in Drug Receptor Interaction, ed. Danielli, J.F., Moran, J.F. & Triggle, D.J. pp. 55-91. Academic Press: New York and London.

316. Robison, G.A., Butcher, R.W., Oye, I., Morgan, H.E. & Sutherland, E.W. (1965). The effect of epinephrine on adenosine 3', 5'-phosphate levels in the isolated perfused rat heart. Mol. Pharmac., 1, 168-177.

317. Robison, G.A., Butcher, R.W. & Sutherland, E.W. (1968). Cyclic AMP. A. Rev. Biochem., 37, 149-174.

318. Robison, G.A., Butcher, R.W. & Sutherland, E.W. (1970). Fundamental concepts in drug receptor interactions., ed. Danielli, J.F., Moran, J.F. & Triggle, D.J., p.59. Academic Press: New York and London.

319. Robison, G.A., Butcher, R.W. & Sutherland, E.W. (1971). Cyclic AMP. Academic Press: New York and London.

320. Rona, P. & Neukirch, P. (1912). Experimentelle Beiträge zur Physiologie des Darmes. I. Pflügers Ach. ges. Physiol., 144, 555-568.

321. Rona, P. & Neukirch, P. (1912). Experimentelle Beiträge zur Physiologie des Darmes. II. Pflügers Arch. ges. Physiol., 146, 371-384.

322. Rona, P. & Neukirch, P. (1912). Experimentelle Beiträge zur Physiologie des Darmes. III. Pflügers Arch. ges. Physiol., 148, 273-284.

323. Rorive, G. & Hagemeyer, F. (1966). Influence de la noradrénaline et de l'angiotensine sur la composition ionique de la fibre musculaire de l'aortae de rat. Ann. Endocr., 27, 521-523.

324. Ross, C.A., Ludden, C.T. & Stone, C.A. (1960). Action of angiotensin on isolated guinea-pig ileum. Proc. Soc. exp. Biol. Med., 105, 558-559.

325. Roth, R.H. & Hughes, J. (1972). Acceleration of protein synthesis by angiotensin-correlation with angiotensin's effect on catecholamine biosynthesis. Biochem. Pharmac., 21, 3182-3187.

326. Rothstein, A. (1968). Membrane phenomina. A. Rev. Physiol., 30, 15-72.

327. Ruegg, J.C. (1971). Smooth Muscle Tone. Physiol. Rev., 51, 201-248.

328. Samuelsson, B., Granström, E. & Hamberg, M. (1967). On the mechanism of biosynthesis of prostaglandins. In: Proceedings 2nd Nobel Symposium, ed. Bergström, S. & Samuelsson, B. pp. 31-34. Interscience: Stockholm.

329. Schmidt, F.O. & Nicholl, P.A. (1933). Heavy metal catalysts in smooth muscle contracture. Am. J. Physiol., 106, 225-237.

330. Schwarz, H., Bumpus, F.M. & Page, I.H. (1957). Synthesis of a biologically active octapeptide similar to natural isoleucine angiotensin octapeptide. J. Am. chem. Soc., 79, 5697-5703.

331. Schwyzer, R., Iselin, B., Kappeler, H., Riniker, B., Rittel, W. & Zuber, H. (1957). Synthese von Hypertensin-Peptiden. Veber die partielle Hydrolyse von Hypertensin - Asp - β amiden zu den entsprechenden Dicarbonsäuren. Hypertensin - II - Analogue. Chimia, 11, 335-336.

332. Setekliev, J. (1970). Effects of drugs on ion distribution and flux in smooth muscle. In: Smooth Muscle, ed. Bulbring, E., Brading, A., Jones, A. & Tomita, T. pp. 343-365. Arnold: London.

333. Severs, W.B. & Daniel-Severs, A.E. (1973). Effects of angiotensin on the central nervous system. Pharmac. Rev., 25, 415-450.

334. Severs, W.B., Summy-Long, J. & Daniels-Severs, A. (1974). Angiotensin interaction with thirst mechanisms. Am. J. Physiol., 226, 340-344.

335. 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.

336. Sharmo, S.K. (1973). Inhibitors and activators of enzymes regulating the cellular concentration of cyclic AMP. In: Metabolic Inhibitors: A Comprehensive Treatise, ed. Hochster, R.M., Kates, M. & Quastel, J.H. pp. 389-434. Academic Press: New York and London.

337. Sharp, G.W.G. & Leaf, A. (1966). Mechanism of action of aldosterone. Physiol.Rev., 46, 593-632.

338. Sherratt, H.S.A. (1968). The metabolism of the small intestine. Oxygen uptake and l-lactate production along the length of the small intestine of the rat and guinea-pig. Comp. Biochem. Physiol., 24, 745-761.

339. Shibita, S. & Briggs, A.H. (1966). The relationships between electrical and mechanical events in rabbit aortic strips. J. Pharmac. exp. Ther., 153, 466-470.

340. Shibita, S. & Briggs, A.H. (1967). Mechanical activity of vascular smooth muscle under anoxia. Am. J. Physiol., 212, 981-984.

341. Shibita, S. & Carrier, O. (1967). Antagonising action of chlorpromazine, dibenamine and phenoxybenzamine on potassium induced contraction. Can. J. Physiol. Pharmac., 45, 587-596.

342. Shibita, S., Carrier, O. & Frankenheim, J. (1968). Effect of chlorpromazine, dibenamine and phenoxybenzamine on the contractile responses of taenia coli to potassium, acetylcholine, angiotensin and barium. J. Pharmac. exp. Ther., 160, 106-111.

343. Singh, J. (1964). The relation between ion fluxes and the action of drugs on smooth muscle. Experientia, 20, 33-35.

344. Singhai, R.L. (1973). Cyclic adenosine 3,5-monophosphate and estrogenic stimulation of uterine metabolism. Adv. Pharmac. Chemother., 11, 100-152.

345. Sitrin, M.D. & Bohr, D.F. (1971). Ca^{2+} and Na^{+} interaction in vascular smooth muscle contraction. Am. J. Physiol., 220, 1124-1128.

346. Skeggs, L.T., Lentz, K.E., Kahn, J.R., Shumway, N.P. & Woods, K.R. (1956). The aminoacid sequence of hypertensin II. J. exp. Med., 104, 192-197.

347. Small, J.V. (1974). Contractile units in vertebrate smooth muscle. Nature, Lond., 249, 324-327.

348. Somlyo, A.P. & Somlyo, A.V. (1968). Vascular smooth muscle I. Normal structure, pathology, biochemistry and biophysics. Pharmac. Rev., 20, 197-272.

349. Somlyo, A.P., Devine, C.E., Somlyo, A.V. & North, S.R. (1971). Sarcoplasmic reticulum and the temperature-dependent contraction of smooth muscle in calcium free solution. J. cell, Biol., 51, 722-741.

350. Sparrow, M.P. & Simmonds, W.J. (1965). The relationship of the calcium content of smooth muscle to its contractility in response to different modes of stimulation. Biochim. Biophys. Acta., 109, 503-511.

351. Stewart, J.M. & Freer, D.J. (1972). Properties of angiotensin receptors in smooth muscle: a proposed model. In: Chemistry an d Biology of Peptides, pp. 521-524. Ann Arbor Science Publishers: Ann Arbor, U.S.A.

352. 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.

353. Sutherland, E.W. & Rall, T.W. (1960). The relation of adenosine 3', 5'-phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharmac. Rev., 12, 265-299.

354. Sutherland, E.W., Rall, T.W. & Mennon, T. (1962). Adenyl cyclase I. Distribution, preparation and properties. J. biol. Chem., 237, 1220-1227.

355. Sutherland, E.W., Oye, I. & Butcher, R.W. (1965). The action of epinephrine and the role of the adenyl cyclase system in hormone actions. Recent Prog. Horm. Res., 21, 623-646.

356. Sutherland, E.W. & Robison, G.A. (1966). The role of cyclic 3', 5'-AMP in responses to catecholamines and other hormones. Pharmac. Rev., 18, 145-

357. Suzuki, A. & Matsumoto, H. (1965). The mechanism of the contracting action of angiotensin on the exised small intestine of guinea-pigs analysed by the concentration action curve. Kobe. J. Med. Sci., 11, 111-130.

358. Szursweski, J.H. & Bulbring, E. (1973). The stimulant action of acetylcholine and catecholamines on the uterus. Phil. Trans. R. Soc. Lond. B., 265, 149-156.

359. Tigerstadt, R. & Bergman, P.G. (1898). Nieve und Kreislauf. Skand. Arch. Physiol., 8, 223-271.

360. Tobian, L. (1960). Interrelationships of electrolytes, juxtaglomerular cells and hypertension. Physiol. Rev., 40, 280-312.

361. Tomita, T. (1970). Electrical properties of mammalian smooth muscle. In: Smooth Muscle, ed. Bulbring, E., Brading, A., Jones, A. & Tomita, T. pp. 366-395. Arnold: London.

362. Tomita, T. & Watanabe, H. (1973). Factors controlling myogenic activity in smooth muscle. Phil. Trans. R. Soc. Lond. B., 265, 73-85.

363. Türker, R.K., Page, I.H. & Khairallah, P.A. (1967). Angiotensin alteration of sodium fluxes in smooth muscle. Arch. int. Pharmacodyn., 165, 394-404.

364. Triner, L., Nahass, 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.

365. Urakawa, N. & Holland, W.C. (1964). Ca^{45} uptake and tissue calcium in K-induced phasic and tonic contraction in taenia-cali. Am. J. Physiol., 207, 873-876.

366. 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.

367. 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.

368. Vander, A.J. (1963). Inhibition of distal tubular sodium reabsorption by angiotensin II. Am. J. Physiol., 205, 133-138.

369. Vane, J.R. (1971). Inhibition of prostaglandin synthesis as the mechanism of action for aspirin-like drugs. Nature, New Biol., 231, 232-235.

370. Villamil, M.F. (1972). Angiotensin, hypertension and vascular ionic composition. Medicina (B. Aires), 32 Suppl. 1, 57-62.

371. Villamil, M.F., Rettori, V. & Yegatti, N. (1973). Calcium exchange and distribution in the arterial wall. Am. J. Physiol., 224, 1314-1319.

372. Volicer , L. & Hynie, S. (1971). Effect of catecholamines and angiotensin on cyclic AMP in rat aortae and tail artery. Eur. J. Pharmac., 15, 214-220.

373. Walaas, O. & Walaas, E. (1950). The content of adenosine triphosphate in uterine smooth muscle of rats and rabbits. Acta. physiol. scand., 21, 1-17.

374. Walaas, O., Walaas, E. & Loken, F. (1952). Effect of oestradiol monobenzoate on the metabolism of rat uterine muscle. Acta endocr., Copenh., 10, 201-211.

375. Waugh, W.H. (1972). Angiotensin II: Local renal effects of physiological increments in concentration. Can. J. Physiol. Pharmac., 50, 711-716.

376. Webb, J.L. (1966). Iodoacetate and Iodoacetamide. In: Enzymes and Metabolic Inhibitors, Vol. II pp. 168-172. Academic Press: New York and London.

377. Weinberger, M.H., Ramsdell, J.W., Rosner, D.R. & Geddes, J.J.L. (1972). Effect of chlorthiazide and sodium on vascular responsiveness to angiotensin II. Am. J. Physiol., 223, 1049-1052.

378. Weiss, G.B. (1974). The cellular pharmacology of lanthanum. Ann. Rev. Pharmac., 14, 343-354.

379. Weiss, G.B. & Goodman, F.R. (1969). Effect of lanthanum on contraction, calcium distribution and Ca^{45} movements in intestinal smooth muscle. J. Pharmac. exp. Ther., 169, 46-55.

380. 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.

381. West, T.C., Jones, D.M. & Loomis, T.A. (1953). Effect of ovarian hormones on contractile energy of rat uterus. Am. J. Physiol., 172, 541-546.

382. West, T.C. & Cervoni, P. (1955). Influence of ovarian hormones on contractile energy of rat uterus. Am. J. Physiol., 172, 541-546.

383. Whelan, R.F., Scroop, G.C. & Walsh, J.A. (1969). Cardiovascular actions of angiotensin in man. Am. Heart. J., 77, 546-565.

384. Whittam, R. & Wheeler, K.P. (1970). Transport across cell membranes. Ann. Rev. Physiol., 32, 21-60.

385. Whittembury, G. & Proverbio, F. (1970). Two modes of sodium extrusion in cells from guinea-pig kidney cortex slices. Pflugers Arch. ges. Physiol., 316, 1-25.

386. Wilkie, D.R. (1962). In: Principles of Human Physiology, ed. Davison, H. & Eggleton, M.G. p. 836. Churchill: London.

387. Wolowyck, M.W. (1971). Counter-cation interaction at the smooth muscle cell membrane in guinea-pig taenia cali. J. Physiol., Lond., 218, 36P-37P.

388. Worcel, M. & Meyer, P. (1970). Inhibitory effect of angiotensin on renal sodium reabsorption. Pflügers Arch. ges. Physiol., 317, 124-140.

389. Yankopoulos, N.A., Davis, J.O., Kliman, B. & Peterson, R.E. (1959). Evidence that a humoral agent stimulates the adrenal cortex to secrete aldosterone in experimental hyperaldosteronism. J. clin. Invest., 38, 1278-1289.

390. Zimmerman, B.G., Gomer, S.K. & Liao, J.C. (1972). Action of angiotensin on vascular adrenergic nerve endings: facilitation of norepinephrine release. Fedn. Proc., 31, 1344-1351.