A STUDY ON THE MODES OF ACTION OF ANGIOTENSIN ON ISOLATED SMOOTH MUSCLE PREPARATIONS

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ABSTRACT

Isolated preparations of ilea from guinea-pigs, rats and rabbits responded to angiotensin with a biphasic contraction. No biphasic contractions were observed in guinea-pig taenia coli, rat stomach fundus strip, rat colon, rabbit aortic strip or rat portal vein preparations.

The biphasic actions of angiotensin were examined using known pharmacological blocking agents in selective concentrations. The results obtained suggest that the receptors for angiotensin are probably different in the same tissues from different species and in different tissues of the same species.

Using a modified sucrose-gap bath, the effect of angiotensin on the electrical and mechanical activity of the guinea-pig taenia coli was studied. The main action of angiotensin appeared to be an increase in permeability to sodium ions. Although the permeability to K^+, Ca^{++} and Cl^- ions also appeared to be affected. Hyperpolarization was observed following angiotensin stimulation. The possible significance of this observation was discussed.

The effect of prostaglandin biosynthesis inhibitors, aspirin and indomethacin, on angiotensin induced contraction was examined in several smooth muscle preparations. Indomethacin selectively reduced angiotensin-induced contraction in all

preparations used with the exception of the rat colon. Attempts were made to determine the relationship between prostaglandin and angiotensin action in the guinea-pig ileum. The results suggest that at least part of the contractile response to angiotensin in the guinea-pig ileum involves the release of or requires the presence of prostaglandin. Indomethacin selectively reduced the pressor response to angiotensin in the pithed rat. These results are discussed in the light of recent evidence that angiotensin can release prostaglandins from several isolated organs. It is concluded that angiotensin besides being able to release acetylcholine, may also release prostaglandins which in turn can modulate the contractile actions of angiotensin.

No single mode of action can be used to explain the action of angiotensin in causing contraction of a relatively small number of preparations of smooth muscle taken from a few similar species.

TO MY PARENTS

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CONTENTS

SECTION ONE

INTRODUCTION

POLYPEPTIDES - GENERAL CONSIDERATION

Many hormones are either proteins or large polypeptides, for example, insulin and the adrenocorticotropic hormone (ACTH), however, in addition, there exists a group of polypeptides of low molecular weight which have potent pharmacological activities. Some of these peptides are true hormones, such as the neurohypophyseal hormones oxtycin and vasopressin, others like the kinins (e.g. bradykinin) function more as local tissue hormones, while angiotensin may act as both.

In addition to oxytocin which elicits the ejection of milk in lactating females, and vasopressin, the anti-diuretic hormone, the neurohypophysis also liberates two polypeptide hormones that stimulate the pigment-forming activity of the melanocytes, these are the alpha and beta melanocyte-stimulating hormones.

The angiotensins and kinins differ from the above peptide hormones in that they are derived from proteins, normally present in the plasma, that are themselves biologically inert. The angiotensins consist of a decapeptide (angiotensin I) and an octapeptide (angiotensin II) which contract smooth muscle, particularly vascular smooth muscle, while the kinins which also contract smooth muscle [illegible] generally relax vascular smooth muscle.

The physiological roles of the angiotensins and kinins are not yet clearly defined. It has been suggested that the kinins may mediate responses to injury and be involved in inflammation (Lewis 1964, 1970; Schachter 1970). One of the proposed physiological

roles of angiotensin is in the control of sodium and water reabsorption across renal tubules (see review by Thurau, Valtin and Schnermann, 1968) and in fluid transfer in intestines (Davies, Munday and Parsons, 1969, 1970).

Recent evidence (see reviews by Davis, 1971, 1974) suggests that the angiotensins may be of physiological significance in the control of aldosterone secretion from the zona glomerulosa of the adrenal cortex, which in turn leads to sodium retention and water reabsorption from the renal tubule cells. This renin-angiotensin-aldosterone system, operates in homeostasis and disease and is present throughout the vertebrates (see Davis, 1971). The intense vaso-constrictor and pressor properties of angiotensin have played a key role in its historical association with hypertensive disease. Johnson and Davis (1973) have suggested that the angiotensin-aldosterone system may play a role in the maintenance of arterial blood pressure. More recently, an angiotensin-prostaglandin interaction has also been implicated in the regulation of blood pressure (see McGiff, 1973).

The actions of angiotensin which are of particular interest in this thesis are those at a cellular level leading to contraction of intestinal and vascular smooth muscle.

The nomenclature of peptides used in this thesis is that suggested by du Vigneaud (1963). The noun form of the amino acid is used to indicate the replacement of one amino acid by another and a number is added to indicate the position. The adjectival form is reserved for peptide derivatives. For example, asparagine[1]-

angiotensin means that the terminal aspartic acid at the amino acid end of angiotensin is replaced by asparagine and cysteine^{8}-angiotensin means that terminal phenylalanine at the carboxyl end is replaced by cysteine. Bradykinyl-lysine means that lysine has been added to the carboxyl end of bradykinin. The amino acids should be designated by three-letter symbols; a capital followed by two lower-case letters, as in Val^{5}-angiotensin II.

I ANGIOTENSIN

Historical Introduction

A pressor substance present in saline extract of rabbit kidney was described by Tigerstedt and Bergman in 1898, which they named renin. However, this important finding did not receive much attention until Goldblatt, Lynch, Hanzal and Summerville (1934) demonstrated in the dog that a persistent hypertension resulted from mechanical constriction of the renal artery. It was later shown that renin was an enzyme which acted upon a plasma substrate to produce a biologically active substance called angiotonin (Page and Helmer, 1940) or hypertensin (Braun-Menendez, Fasciolo, Leloir and Munoz , 1940). The name angiotensin is now used to describe this pharmacologically active substance following the revised nomenclature by Braun-Menendez and Page (1958). The relationship between renin and the formation of angiotensin is summarized in Fig. 1.

Following the discovery of angiotensin by Page and Helmer (1940) and Braun-Menendez et al (1940) efforts were made to isolate pure angiotensin. However, it was not until 1956 that the amino acid sequences for both angiotensin I and II were determined (Skeggs, Lentz, Kahn, Woods and Shumway, 1956; Skeggs, Kahn and Shumway, 1956). The amino acid sequences of the angiotensin peptides have been confirmed by synthesis : Val^5-angiotensin I and II by Schwyzer, Iselin, Kappeler, Riniker, Rittel and Zuber (1958) and Ile^5-angiotensin II by Arawaka, Smeby and Bumpus (1962). Among the numerous analogs of angiotensin that have since been synthesized (see Bumpus, Smeby and Khairallah, 1970; Chaturvedi, Park, Smeby and Bumpus, 1970), it is

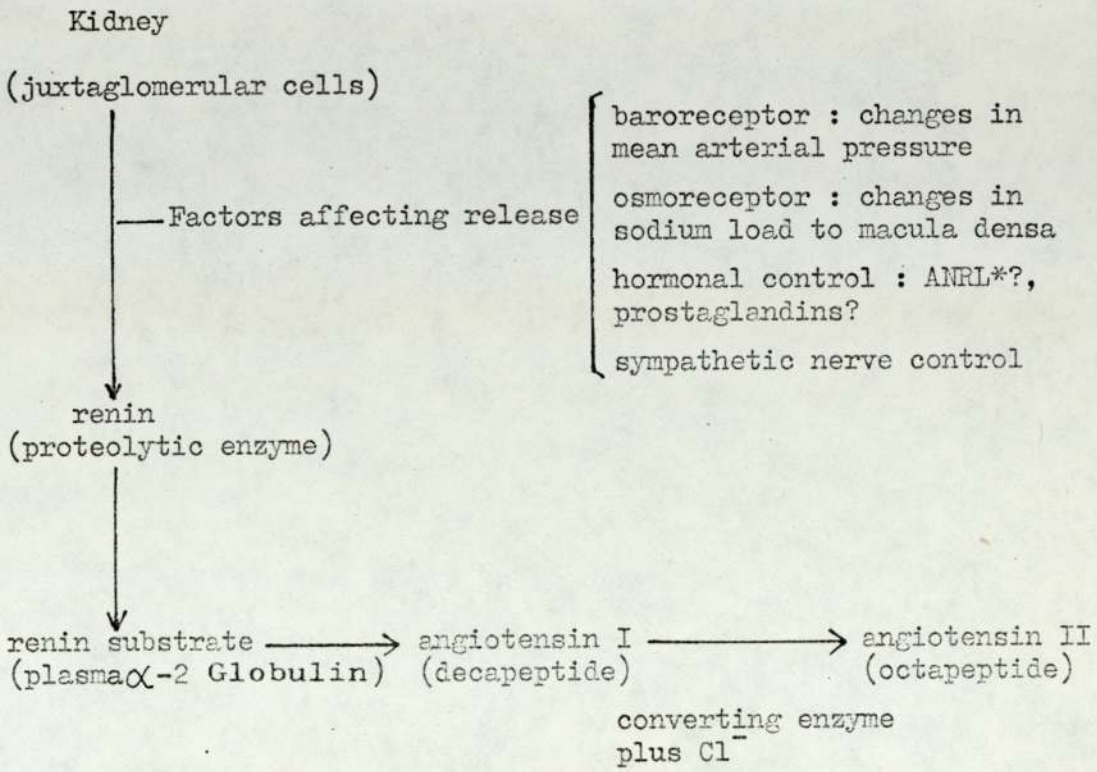

Fig. 1. The renin-angiotensin system.

ANRL* antihypertensive neutral renomedullary lipids

only Val^5-angiotensin II and Ile^5-angiotensin II which possess strong smooth muscle stimulating activities. The commercially available angiotensin II or Hypertensin CIBA, is $Asp(NH_2)^1$-Val^5-angiotensin II. Most of the recent work on the pharmacology of angiotensin that has been reported has been done with this synthetic angiotensin II, and the results obtained do not differ qualitatively from those seen with Asp^1-Val^5-angiotensin II or the corresponding Ile^5-angiotensin II (see review by Gross, 1971). However, the synthetic angiotensin II is degraded at a faster rate than the natural Asp^1-angiotensin II, and the peptidase involved in its hydrolysis is different from that which hydrolyzed the natural peptide (Nagatsu, Gillespie and Glenner, 1965). Because of the differences in enzymic characteristics of the natural and synthetic angiotensins, it has been suggested that only the natural Asp^1-angiotensin II should be used in the investigation of angiotensin II inactivation (Helmer, 1961). Table I summarizes the amino acid sequences of the natural derivatives of angiotensin.

The term 'angiotensin' in this thesis shall refer to the more potent octapeptide, angiotensin II, unless otherwise indicated.

Renin release and possible physiological roles of the renin-angiotensin system.

Following the early experiments of Goldblatt *et al* (1934) on the production of renal hypertension by partial constriction of the renal arteries, it had been assumed that the stimulus for renin release was ischaemia and that the resultant increase in the concentration of circulating renin in turn caused an increase in angiotensin concentration which was responsible for the elevated

Name	Amino Acid Sequence														Occurrence
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Angiotensin II	Asp.	Arg.	Val.	Tyr.	Ile.	His.	Pro.	Phe.							Horse and hog renin substrate
Angiotensin I	Asp.	Arg.	Val.	Tyr.	Ile.	His.	Pro.	Phe.	His.	Leu.					ditto
	Asp.	Arg.	Val.	Tyr.	Ile.	His.	Pro.	Phe.	His.	Leu.	Leu.	Val.	Tyr.	Ser.	ditto
Val^5-Angiotensin	Asp.	Arg.	Val.	Tyr.	Val.	His.	Pro.	Phe.							Bovine renin substrate

TABLE 1. Natural derivatives of Angiotensin

blood pressure (Braun-Menendez et al, 1940; Houssay and Braun-Menendez, 1942; Page, 1940). In recent years there has been evidence against the concept of renal ischaemia as the stimulus for renin release, for example, Skinner, McCubbin and Page (1964a,b) demonstrated that renin-release could be stimulated by a reduction of renal perfusion pressure too small to cause any measurable decrease in renal blood flow and that constriction of the renal vein so as to reduce blood flow by 50% did not cause renin release. These authors expressed the view that renin secretion was controlled by a renal baroreceptor rather than by ischaemia. They further suggested that a small amount of renin was secreted continuously and that the rate responded to physiological changes in blood pressure.

There is an inverse relationship between the renin content of the kidney or the renin concentration in plasma and sodium balance (Brown, Davies, Lever and Robertson, 1964, 1965). Sodium retention is followed by a reduction and sodium loss by an increase in the production and secretion of renin by the kidney (Gross, Brunner, and Ziegler, 1965). The two parts of the nephron that are involved in the renin-angiotensin system are the juxtaglomerular cells and the macula densa. The macula densa cells, which are in contact with fluid at the begining of the distal tubule, may have an osmoreceptor function that enables them to transmit information on the composition of the tubular fluid to the juxtaglomerular cells located at the entrance to the nephron (Vander and Miller, 1964a, 1964b; Gross, Schaechtelin, Brunner and Peters, 1964). From these observations, Gross (see review, 1971) suggested that renin release could be regulated by the osmolarity of the urine at the begining

of the distal tubule, changes being detected by the macula densa.

Infusion of adrenaline or noradrenaline or stimulation of the renal nerves causes a release of renin irrespective of whether pressure in the renal artery is maintained at a normal level or whether both glomerular filtration rate and renal blood flow are reduced (Bunag, Page and McCubbin, 1965; Vander, 1965). In contrast to noradrenaline, angiotensin infused intravenously into the renal artery does not stimulate renin release (Wathen, Kingsbury, Stouder, Schneider and Rostorfer, 1965). On the basis of this observation it has been suggested that there is a negative feedback mechanism between the level of circulating angiotensin and the release of renin (see Vander, 1967). There is also evidence to suggest that renin release can be inhibited by certain lipid compounds including prostaglandins arising from the kidney medulla (see Muirhead, Leach, Byers, Brooks, Daniels and Hinman, 1971 and Fig. 1).

Despite these observations the physiological role and pathophysiological significance of the renin-angiotensin system is not yet firmly established. It does not seem to regulate normal blood pressure, nor is it primarily responsible for the maintenance of the elevated blood pressure of renal hypertension, although it may be involved in the pathogenesis of renal hypertension in another way (see reviews by Gross, Brunner and Ziegler, 1965; Gross, 1971).

Some of the possible physiological actions of angiotensin are summarized in Table II.

1. Participation in the regulation of blood pressure
2. Effect on renal haemodynamics
3. Participation in the regulation of sodium balance
    a) effect on renal tubular function
    b) stimulation of aldosterone secretion
4. Participation in the regulation of intravascular volume
5. Increase in sympathetic tone
6. Effect on intestinal water transport

Table II

Possible physiological actions of angiotensin

Actions of Angiotensin

Angiotensin is one of the most active biological substances known and many of its pharmacological actions can be attributed to its potent vaso-constrictor and smooth muscle stimulant activities. When synthetic angiotensin became available, it prompted a comprehensive pharmacological investigation and several other sites of action of the peptide have been reported (see reviews, McCubbin and Page, 1968; Khairallah, 1971; Gross, 1971). The more prominent ones are the kidneys, the sympathetic nervous system, the adrenal cortex and medulla, and the central nervous system. The stimulant effect of angiotensin on various types of smooth muscles will now be considered in detail.

Effects on smooth muscle

A) Isolated uterus

Uteri from rats in oestrous or pretreated with stilboestrol respond by dose-dependent contractions to angiotensin. Responses are linear on a log scale between 0.2 and 10 ng/ml and are so regular that the rat uterus is one of the preparations used for assay of angiotensin (Gross and Turrian, 1960; Khairallah and Page, 1962 and Paiva and Paiva, 1960). The contractions become smaller with repeated exposure to the peptide, but can be restored by the administration of synthetic oxytocin (Gross and Turrian, 1960; Paiva and Paiva, 1960). High concentrations of angiotensin (10-15 µg/ml muscle bath) given every five minutes lead to tachyphylaxis after the 3rd or 4th contractile response (Khairallah and Page, 1962). The oxytocic action of angiotensin is not yet established, although it has been asserted

that calcium ions are necessary (Renson, Barac and Bacq, 1959).

B) Isolated intestines

Angiotensin I and II are strong stimulants of the isolated intestine of the guinea pig. The contractile action is slightly delayed, begining about 20 to 30 seconds after the addition of the peptides to the bath and reaching a maximum within 90 to 120 seconds. There is a linear dose-response relationship in the range 10^{-9} to 10^{-8}g/ml, the decapeptide (angiotensin I) being less active (Gross and Turrian, 1960; Ross, Ludden and Stone, 1960; Bisset and Lewis, 1962; Khairallah and Page, 1961, 1962).

The rabbit ileum is less sensitive to angiotensin and reacts more slowly than the guinea-pig ileum. Slow contractions are provoked, reaching their maximum within 3 minutes of the addition of angiotensin to the bath fluid (Robertson and Rubin, 1962). The rat colon contracts to a concentration of angiotensin as low as 10^{-10}g/ml, and has been suggested for use as a bioassay for angiotensin circulating in the blood (Regoli and Vane, 1964a,b, 1966). The hen's rectal caecum also contracts to angiotensin but at much higher concentrations (Bisset and Lewis, 1962). The chicken rectum and cat jejunum do not respond to angiotensin with levels of up to 10^{-8}g/ml, while the rat's stomach strip and the rabbit rectum respond only very weakly (Regoli and Vane, 1964a)

Recently, the response of the guinea-pig ileum to angiotensin was described as biphasic by Godfraind, Kaba and Polster (1966a) It consists of an initial fast spike-like contraction which declines

and is followed by a secondary slow increase in tension. Similar biphasic contractile responses to angiotensin have also been reported by Goldenberg (1967) in the ileum of rat, mouse and the Mongolian gerbil.

C) Isolated arteries

The spirally cut rabbit aorta (Furchgott and Bhadrakom, 1953) has been extensively used to study the effect of angiotensin on vascular smooth muscle. Angiotensin produces a slow developing contraction, which is followed by slow relaxation after washing out (Helmer, 1957, 1964). The onset of action is delayed from about 2 to 4 minutes. However, other authors have reported that the response started within 30 seconds and reached a maximum within 1 to 2 minutes (Khairallah, Page, Bumpus and Turker, 1966; Bohr and Uchida, 1967).

Spirally cut arteries from different animals vary in their sensitivity (Khairallah et al, 1966), cat carotids respond to 10^{-9}g/ml of angiotensin, while dog carotid and rat aorta both require 10^{-7}g/ml or more. Responses of rabbit and guinea-pig aortae and sheep carotid are intermediate in sensitivity (see review Gross, 1971). All of the spirally cut arteries lose sensitivity upon repeated application of angiotensin (Bohr and Uchida, 1967; Khairallah et al, 1966) with the exception of the rabbit and guinea-pig aortae. Angiotensin I also contracted rabbit aorta (Ng and Vane, 1968) at a concentration ten times greater than angiotensin II. The contractile action of angiotensin I on the rabbit aorta may be related to the presence of the converting enzyme and angiotensinases

which are found in rat and rabbit aorta (Bumpus, Smeby and Page, 1961; Bumpus, Smeby, Page and Khairallah, 1964).

In the isolated perfused renal artery of the rat, angiotensin has a small effect which is much less than that of adrenaline, noradrenaline or 5-hydroxytryptamine (Hrdina, Bonaccorsi and Garattini (1967). Renal arteries from dogs do not respond to angiotensin (Bohr and Uchida, 1967). The isolated pulmonary artery of the rabbit is constricted by angiotensin in concentrations of 10^{-9} g/ml or more, the maximum response being about one-third of that obtained with noradrenaline (Su, 1965).

Angiotensin has only a slight effect on umbilical arteries (Somlyo, Woo and Somlyo, 1965; Gokhale, Gulati, Kelkar and Kelkar, 1966) although it constricts placental arteries (Klinge, Mattila, Penttila and Jukarainen, 1966).

D) Isolated veins

Isolated strips of hepatic portal, mesenteric and lobar pulmonary veins of dog respond to concentrations of angiotensin of 5×10^{-9} g/ml with small contractions (Somlyo and Somlyo, 1964, 1966). In contrast, saphenous, femoral or axillary veins and venae cavae are not contracted by angiotensin at concentrations one hundred times higher (Somlyo and Somlyo, 1966). In the venous segments that react to angiotensin, the threshold concentration is no higher than in arterial segments, but the size of the response is smaller. Angiotensin has much less activity on isolated venous strips when compared with either noradrenaline or histamine (Somlyo and Somlyo, 1966) and in addition shows marked tachyphylaxis (Bohr and Uchida, 1967).

An exception to this rule is the action of angiotensin on the rat isolated portal vein, where spontaneous rhythmic contractions are enhanced by concentrations as low as 10^{-11} g/ml (Bohr and Uchida, 1967).

Modification of Angiotensin Responses

A) Tachyphylaxis

The development of a refractory state towards a drug following the administration of repeated doses at short intervals and the failure to reproduce the initial response even with increasing doses are the essential characteristics of tachyphylaxis. (see Gross, 1971). Page and Helmer (1940) first described tachyphylaxis to crude natural angiotensin, and Bock and Gross (1961) using synthetic Asp^1-angiotensin, found a gradual decrease in pressor responses after repeated injections of the peptide. Onset of tachyphylaxis is much more rapid with larger doses of angiotensin and cross tachyphylaxis exists between angiotensin and renin (Page, McCubbin, Schwarz and Bumpus, 1967; Bock and Gross, 1961). Tachyphylaxis to angiotensin has been demonstrated in most tissues that respond to the peptide including isolated rabbit heart, adrenal medulla, superior cervical ganglion and the isolated rat lung (see reviews by Gross 1971; Khairallah, 1971).

The spirally cut rabbit and guinea-pig aortae (Khairallah, et al 1966) and the rat stomach strip (Rioux, Park and Regoli, 1973) do not show tachyphylaxis.

Two main theories have been proposed to explain tachyphylaxis to angiotensin. Distler, Liebau and Wolf (1965) and Liebau, Distler and Wolf (1966) found that angiotensin reduced the noradrenaline content of isolated rat aortic strips and that onset of tachyphylaxis

corresponded with the total depletion of noradrenaline, furthermore, responsiveness could be restored by re-equilibrating the aortic strips with exogenous noradrenaline. These authors also reported that cross tachyphylaxis existed between angiotensin and tyramine and therefore attributed angiotensin tachyphylaxis to noradrenaline depletion.

The other theory is based on the assumption of the occupation theory for drug action, in that tachyphylaxis follows the filling of all the available receptor sites so that none remains accessible (Khairallah et al, 1966; Bohr and Uchida, 1967). These investigators demonstrated a reversal of tachyphylaxis after incubation of arterial strips with a plasma fraction or kidney extract rich in angiotensinase A or by Dowex 50, a resin that binds strongly to angiotensin (Scornik and Paladini, 1961).

Recently, angiotensin tachyphylaxis in the isolated guinea-pig ileum and rat uterus has been studied with the aid of synthetic peptide analogs of angiotensin (Freer and Stewart, 1972; Paiva, Juliano, Nouailhetas and Paiva, 1973) and was shown to be dependent on the pH of the bath fluid. Tachyphylaxis was absent at pH 8 and above, was slight at pH 7.4 and became pronounced below pH 6.8 (Freer and Stewart, 1972). This observation was interpreted as evidence that the protonated imidazole ring of the histidine residue of angiotensin played an important role in the phenomenon of tachyphylaxis (Freer and Stewart, 1972). However, evidence was also presented which showed a good correlation of tachyphylaxis with amino group protonation rather than imidazole protonation (Paiva, et al, 1973).

B) Effect of ions

A large number of studies have been carried out to establish the role of sodium ion on the contraction produced by angiotensin (Khairallah, Vadaparampil and Page, 1965; Blair-West, Harding and McKenzie, 1967, 1968; Lefer, 1967), because of the preponderant contribution of this ion in the electrophysiological events (see later). Reduction of the sodium ion concentration in the bath fluid reduces the action of angiotensin in the perfused rat tail arteries (Hinke and Wilson, 1962), guinea-pig ileum (Khairallah et al, 1965; Blair-West et al, 1967) and cat papillary muscle (Lefer, 1967), while increasing the sodium concentration potentiates the stimulating effect of angiotensin on these preparations. These observations led several workers to suggest that the direct effect of angiotensin on smooth muscle or heart muscle cells is at least partly sodium dependent.

On the other hand, a reduction of sodium ion concentration in the medium has been shown to increase the effect of angiotensin on rabbit aorta (Napodano, Caliva, Lyons, DeSimone and Lyons, 1962) and rat uterus (Khairallah et al, 1965). These results are highly variable and do not allow any definite conclusion.

The response to angiotensin is intimately related to the external calcium ion concentration. Decreasing the calcium ion concentration inhibits response of the guinea-pig ileum, rat uterus (Khairallah et al, 1965), dog mesenteric arteries (Burks, Whitacre and Long, 1967) and perfused adrenal glands (Robinson, 1967) to angiotensin. A similar depression of the response to 5-hydroxy-tryptamine, noradrenaline and adrenaline has been observed in some

of these experiments (Burks et al, 1967) suggesting that calcium ion is probably a common essential co-factor for the myotropic action of several agonists.

Alteration in the concentration of potassium ion has no appreciable effect on the response to angiotensin in smooth muscle, for example, potassium-deplorized smooth muscle still responds to angiotensin (Khairallah et al, 1965; Shibata and Briggs, 1966; Sullivan and Briggs, 1968), an action which seems to be linked to calcium ions.

C) Prostaglandins

Khairallah, Page and Türker (1967) reported that prostaglandin-E_1 (PGE_1) potentiated responses of isolated rabbit aortae to angiotensin. This effect was independent of an adrenergic nerve supply or the presence of catecholamines. These authors postulated that PGE_1 enhances response of isolated smooth muscle to angiotensin by causing a deplorization of the cell membrane.

The pressor response to angiotensin in the dog and rat can be diminished by PGE_1 (Holmes, Horton and Main, 1963; Weeks and Wingerson, 1964). This inhibition of angiotensin pressor response does not appear as a result of pharmacological antagonism (see Alpert and Hickler, 1971). The underlying mechanism is not known.

Modes of Action of Angiotensin

The mechanism underlying the contractile effects of angiotensin on various types of smooth muscle is still obscure. Pharmacological analysis of this action on isolated organ preparations such as arterial wall, intestine and uterus with the aid of drugs which could be shown to exert selective action, (either blockade or potentiation), indicates that angiotensin may have both a direct and an indirect action. The interaction of angiotensin with receptor sites on target organs producing a physiological response, has been referred to direct effects, in contradistinction to effects caused indirectly by other hormones or mediators liberated by angiotensin (see review Khairallah, 1971).

The contractile response of the guinea-pig or rabbit ileum to angiotensin is potentiated by anti-cholinesterases such as BW 284C51 or neostigmine and reduced by atropine (Ross et al, 1960; Khairallah and Page, 1961; Robertson and Rubin, 1962; Suzuki and Matsumoto, 1965; Blair-West, Harding and McKenzie, 1967). The contraction of the rabbit ileum to angiotensin is abolished by the prior addition of botulinum toxin to the bath (Robertson and Rubin, 1958, 1962). Botulinum toxin is known to prevent the release of acetylcholine from cholinergic nerve endings (Burgen, Dickens and Zatman, 1949). Morphine and high concentrations of nicotine also reduce angiotensin-induced contractile responses of the isolated intestine and uterus (Khairallah and Page, 1961). These results led to Khairallah and Page (1961) and Robertson and Rubin (1962) to propose that angiotensin has mainly an indirect action on smooth muscle.

Detailed analysis of the contractile response to angiotensin of the guinea-pig, mouse or gerbil ileum shows that it is biphasic and that the fast phase can be abolished by atropine (Godfraind, Kaba and Polster, 1966a; Goldenberg, 1967). These authors suggested that the fast component was due to the release of acetylcholine from cholinergic nerve endings. This indirect component was ascribed by Khairallah and Page (1961) to stimulation of the ganglion cells in Auerbach's plexus. Other investigators assumed the site of action to be on postganglionic fibres (Panisset, 1967). A non-nicotinic stimulant action of angiotensin on sympathetic ganglia has been demonstrated by Trendelenberg (1966).

The slow component of the angiotensin-induced contractile response of the guinea-pig ileum (Godfraind, Kaba and Polster, 1966a) or the rat uterus (Khairallah and Page, 1961, 1963) is not inhibited by atropine. These authors therefore suggested that angiotensin must also have a direct effect on these preparations, and this was further demonstrated in the isolated rat colon (Regoli and Vane, 1964a) and rat ileum (Goldenberg, 1967).

There are drugs which can inhibit the direct component of action of angiotensin, these include cinnarizine (van Nueten, Dresse and Dony, 1964), lidoflazine (Godfraind, Kaba and Polster, 1966b) and osajin (Gascon and Walaczek, 1966). All of these compounds may possibly act by preventing the binding of angiotensin to its receptor sites (see review by Khairallah, 1971). However, these drugs have also been shown to antagonize a number of other agonists on smooth muscle preparations (Ellis and Reit, 1969; Rioux et al, 1973).

It has not yet been established whether an indirect action of angiotensin comparable to that exerted in the isolated intestine is responsible for its effect on vascular smooth muscle. Feldberg and Lewis (1963, 1964, 1965) showed in the cat that angiotensin releases adrenaline and noradrenaline from the adrenal medulla when it is injected into the coeliac artery close to the origin of the adrenal arteries. Although there is no direct evidence that angiotensin causes a release of noradrenaline from sympathetic nerve endings, recent studies on various isolated and perfused vascular smooth muscle preparations suggest that at least part of the constrictor effect of angiotensin is due to an interaction with the sympathetic nervous system to cause the release of noradrenaline (Schumann and Güther, 1967; Liebau, Distler and Wolf, 1965, 1966; Zimmerman and Whitmore, 1966, 1967). For example, in isolated pig and rat arteries exposed for several hours to angiotensin a decrease in noradrenaline content was found, which was accompanied by a reduced contractile reaction to both angiotensin and tyramine (Distler, Liebau and Wolf, 1965; Liebau *et al*, 1965, 1966), furthermore, the contractile response to angiotensin in aortic strips from several animal species can be reduced by alpha adrenoceptor blocking agents (Schumann and Güther, 1967). Human umbilical vessels which have been described as non-innervated vascular smooth muscle free of chemically demonstrable stored catecholamines (Davignon and Shepherd, 1964) respond only with a weak and inconsistent vaso-constriction to angiotensin (Somlyo *et al*, 1965).

Angiotensin is known to enhance sympathetically mediated vascular responses in the rat (McGregor, 1965), the rabbit (Sakuri and Hashimoto, 1965; Su, 1965; Hughes and Roth, 1971) and the dog (Zimmerman and Gisslen, 1968; Kadowitz, Sweet and Brody, 1971). Angiotensin also enhances the effect of sympathetic nerve stimulation on the isolated rabbit heart (Thompson, 1970) and the effect of transmural stimulation on the isolated rabbit aortic strip (Toda, 1973). It has been suggested that this angiotensin induced potentiation is due to a facilitation of the release of noradrenaline from sympathetic nerve endings (Day and Owen, 1968; Zimmerman and Gisslen, 1968; Hughes and Roth, 1971; Toda, 1973) or to an inhibition of the re-uptake of noradrenaline by adrenergic nerves (Panisset and Bourdois, 1967; Peach, Bumpus and Khairallah, 1969). Suzuki and Matsumoto (1966) consider that the catecholamines released from sympathetic nerve endings are of minor importance for the vaso-constrictor mechanism of angiotensin, which they considered to be due to a direct stimulation of vascular muscle cells.

Like acetylcholine, angiotensin depolarizes isolated arteries, an effect that is still demonstrable in the presence of nicotine or other blocking agents. Even when complete depolarization was achieved by potassium, drugs such as angiotensin, bradykinin and acetylcholine were still able to contract them (Keatinge, 1966). In other studies, angiotensin was found to have a weak depolarizing action, which could be blocked by bretylium (Kiran and Khairallah, 1969). From these observations, Kiran and Khairallah (1969) concluded that angiotensin and other vaso-constrictor substances do not interfere with active ion transport, or act only in part by changing the membrane potential.

Angiotensin like other vaso-constrictors shifts sodium into and potassium out of vascular smooth-muscle cells and shows no specificity in this respect (Friedman and Friedman, 1964, 1965). Although Rorive and Hagemeijer (1966) showed that in contrast to noradrenaline, which increases the potassium efflux from aortic strips of the rat, angiotensin in much higher concentrations caused only a rapid, transient rise in potassium efflux. In isolated artery strips and uterine muscle, an increase in ^{22}Na efflux was demonstrated under the influence of angiotensin (Turker, Page and Khairallah, 1967). Recently, angiotensin was shown to increase ^{24}Na influx and sometimes ^{24}Na efflux even in depolarized muscles by Hamon and Worcel (1973). It was suggested that angiotensin may act through an increase of sodium permeability. These authors also observed an increase in ^{42}K and ^{36}Cl efflux in polarized muscles, an effect which could be suppressed by depolarization, and concluded that this phenomenon is a consequence of the depolarization induced by angiotensin.

On the other hand, a stimulating effect of angiotensin on the sodium pump has been suggested as a common pathway for the effect of the drug on smooth muscle (Turker et al, 1967). However, an inhibitory action of angiotensin on the sodium pump has also been suggested in the isolated rabbit aorta (Day and Moore, 1973).

The currently accepted hypothesis of the mechanism of action of peptide hormones affecting smooth muscle includes an initial obligatory step of binding to specific receptors on or in the cell membranes (see Margoulies and Greenwood, 1972 and references therein). The recent development of structural analogues of angiotensin which act as competitive antagonists (Marshall, Vine and Needleman, 1970; Pals, Masucci, Sipos and Denning, 1971; Regoli and Park, 1972) has facilitated the characterization of receptor sites for angiotensin in various different smooth-muscle preparations (Baudouin, Meyer, Worcel, Fermandjian and Morgat, 1972; Mimran, Hinrichs and Hollenberg, 1974). Very recently, a membrane fraction derived from the plasma membrane possessing specific binding sites for angiotensin has been isolated (Devynik, Pernollet, Meyer, Fermandjian and Fromageot, 1973; Devynik, Fernollet, Meyer, Fermandjian, Fromageot and Bumpus, 1974). Using structural analogues, these workers were able to define the structural requirements of the angiotensin molecule for binding to such a membrane fraction.

II SMOOTH MUSCLE

Smooth muscle is present in the walls of the hollow organs of the abdominal visceral (the gastro-intestinal and the urino-genital systems), the walls of vascular structures (blood vessels other than capillaries, and in the spleen), in the walls of the bronchi, in the capsules and ducts of exocrine glands, in structures associated with the eye (intrinsic muscles) and in the skin (piloerector muscles). There are marked species differences among smooth muscles and their properties in any one species depend on the organ in which they occur (see Prosser, 1962; Holman, 1968).

Bozler (1948) divided vertebrate smooth muscle into two categories:

1. 'Unitary' muscles, which include gut, ureter and uterus. These muscles behave like single units and conduction is from fibre to muscle fibre. They are usually spontaneously active.

2. 'Multiunit' muscles, which include nictitating membrane, iris sphincter, ciliary muscle, pilomotors, urinary bladder and most vascular smooth muscles. These muscles are normally activated by nerves and consist of numerous independent units. They are usually not spontaneously active.

This classification has been useful, but it is clear that it cannot be regarded as rigid; many muscles, for example, guinea-pig vas deferens and bladder show features of both types (Burnstock and Holman, 1961, 1963; Ursillo, 1961). Furthermore, recent work indicates that many vascular smooth muscles do not belong to the multiunit type (Bohr and Uchida, 1967).

Most smooth muscle cells are spindle shaped structures with an approximately centrally-placed nucleus (Prosser, Burnstock & Kahn, 1960). Occasionally, the cells have irregularly-shaped branched processes (Keech, 1960; Pease and Paule, 1960). Serial section sampling from electron microscopy studies of various smooth muscles indicate a wide range of cell size, for example, in the intestine, they are 5 - 6 μ in diameter and 30 - 40 μ long, whereas in the uterus they may be as long as 0.5 mm. The smallest smooth muscle cells are in the walls of blood vessels where they are 2 - 3 μ in diameter and 15 - 20 μ long. (Rhodin, 1967; Taxi, 1965; Yamauchi, 1964; Merrillees, 1968). For further details on morphology of smooth muscles see reviews by Burnstock, (1968, 1970).

In most hollow organs, there is an outer longitudinal muscle coat and an inner circular coat. In blood vessels, the muscles are usually confined to the media and are arranged in spiral or helical fashion with the dominant orientation being circular (Strong, Pease and Paule, 1960; Rhodin, 1962; Verity, 1967). In the muscle coats of most visceral tissues, the muscle cells are arranged in branching bundles or sheets enveloped by connective tissue (Prosser et al, 1960; Bennett and Rogers, 1967). The muscle cells in the media of many arteries have also been shown to be arranged in bundles (Boucek, Takashita and Fojaco, 1963). The longitudinal muscle coat of the large intestine of some animals for example, the guinea-pig, is gathered mainly into distinctive bands or taenia coli, and these are composed of smooth muscle fibres aggregated in bundles that are irregularly shaped in cross section (see Schofield, 1968).

Bennett and Burnstock (1968) have suggested that muscle bundles rather than individual muscle cells are the effector units in smooth muscle systems, and the exact relationships of the muscle cells to each other within a bundle is of great functional significance.

Comprehensive accounts of the morphological aspects of smooth muscles have recently been reviewed (see Schofield, 1968; Dewey and Barr,1968; Burnstock, 1970; Verity, 1971).

Innervation of Smooth Muscle

Intestinal

The smooth muscle of the gastrointestinal tract is innervated by the parasympathetic and sympathetic divisions of the autonomic nervous system. Parasympathetic fibres to the small intestine are in the vagus nerves. The post-ganglionic sympathetic fibres arise in the coeliac and mesenteric ganglia. They run to the intestinal wall in the mesentery, usually accompanying the blood vessels. These nerves together comprise the extrinsic innervation. Extrinsic nerves penetrate the longitudinal muscle coat from the mesentery to fuse with nerve bundles in the Auerbach's plexus lying between circular and longitudinal muscle coats (Auerbach, 1864; Dupont and Sprinz, 1964). The ganglion cells of the post-ganglionic vagal fibres are believed to be included in the Auerbach's plexus (see Burnstock, 1970). Fine bundles of nerves from this myenteric plexus pass inwards, mainly together with blood vessels to Meissner's plexus lying in the sub-mucosa (Meissner, 1857, cited Burnstock, 1968). The nerves in Auerbach's and Meissner's plexuses are the intrinsic nerves (Hillarp, 1960).

At least 3 different functional nerve types have been demonstrated in the intestine, these are : cholinergic excitatory, noradrenergic inhibitory and non-adrenergic non-cholinergic inhibitory nerves (Burnstock, Campbell and Rand, 1966; Campbell, 1970). These nerves can be correlated morphologically with three types of vesicles found in different axon profiles, for example, predominantly agranular vesicles in cholinergic nerves; predominantly small granular vesicles in noradrenergic nerves and predominantly large granular vesicles in non-adrenergic non-cholinergic nerves (Burnstock and Robinson, 1967). Recently, a fourth functional nerve type has been described (Furness and Costa, 1973) as non-cholinergic excitatory. There is as yet no histochemical data available on this nerve type. Intestinal smooth muscles are sparsely innervated by sympathetic nerve fibres (Read and Burnstock, 1969)

Vascular

The efferent nerves to the blood vessels, the vasomotor nerves, are principally from the sympathetic division of the autonomic nervous system. The nerves supplying most vascular smooth muscles are confined to the outside of the media, i.e. the adventitia (Falck, 1962; McLean and Burnstock, 1967). Known exceptions where many nerves are present within the media are sheep carotid artery (Keatinge, 1966), renal vein and artery (see Burnstock, 1970) cutaneous veins (Ehinger, Falck and Sporrong, 1966) and pulmonary artery (Verity and Bevan, 1968).

Blood vessels, in general, do not receive fibres from the parasympathetic nervous system; an exception is the nerve supply to the erectile tissues (see Goodman and Gilman, 1970).

The autonomic nerve-smooth muscle junctions

The electronmicroscopy of the innervation of smooth muscle was described for the first time by Caesa, Edwards and Ruska (1957) on the mouse urinary bladder. They established the existence of neuromuscular sypnapses with close apposition (70 to 200 Å) of nerve and muscle membranes. The idea that transmitters could be effective even when released from nerves separated by more than 200 Å from muscle was suggested by Gansler (1960) and 'en passage' release of transmitter was proposed by Richardson (1962) and Merrillees, Burnstock and Holman, (1963). Finally, the detailed relationship of nerves and muscles has been examined with serial electronmicroscope section sampling (Taxi, 1965; Thaemert, 1966; Bennett and Rogers, 1967; Merrillees, 1968).

There is a spectrum of type of innervation in different tissues (see Burnstock, 1970). The following can be generalized:

In the longitudinal muscle coat of the intestine, most vascular smooth muscles, uterus and ureter, close apposition (180 to 250 Å) of nerve and muscle is extremely rare and limited to discrete areas, where 'tight junctions' between muscle cells are prominent (Richardson, 1958; Rhodin, 1967; Thaemert, 1966; Yamauchi, 1964). Serial sections show that some muscle fibres are never sufficiently close

to nerve bundles to make direct action of diffused transmitter likely (Taxi, 1965). In the intestine of mouse, rat and guinea-pig there are no nerve bundles running inside the longitudinal muscle coat (Taxi, 1965). The sparse innervation of these muscles makes it likely that intermuscle fibre spread of excitation plays an important role (see Burnstock, 1970). This is supported by observations of an extensive system of intermuscle fibre junctions (tight junctions) in this muscle coat, viz, intestine: Bennett and Rogers (1967); vascular smooth muscle: Verity and Bevan (1968).

Few Neuro-muscular contacts of less than 800 Å have been observed in the longitudinal muscle coat of intestine and vascular smooth muscles (Bennett and Rogers, 1967; Bennett and Burnstock, 1968) and these authors have suggested that transmitter release from nerves up to 3,000 to 10,000 Å away from muscle cells is likely to be effective.

The density of close neuro-muscular junctions in the inner, circular muscle coat of intestine is higher than in the outer, longitudinal muscle coat (Thaemert, 1966; Rogers and Burnstock, 1966) and close apposition (200 Å) of nerve and muscle has been observed.

The Ionic Basis of Electrical Activity in Smooth Muscle

The membrane potential

Several theories have been proposed to explain the potential difference across the cell membrane. According to Troshin (1962) and Ling (1966) the membrane potential and ion distribution in

excitable cells should be attributed to the selective absorption of potassium ions to fixed negative charges within the cells. However, the most widely-accepted theory is the membrane hypothesis which originated from the work of Bernstein (1912). According to Bernstein the resting cell membrane was selectively permeable to potassium only and that this selectivity was lost during excitation, resulting in an indiscriminate penetration by other small ions such as sodium and chloride.

Bernstein's theory has since been elaborated through the work of Hodgkin and Huxley (1939, 1945) and this can be summarised as follows: Extracellular fluids contain relatively high concentrations of sodium and chloride and low concentrations of potassium, whereas intracellular fluids contain much potassium, little sodium and chloride, and the positive charges of the potassium ions are neutralised partly by large molecules with negative charges, including phosphate esters and peptides (see Katz, 1966). Potassium ions tend to diffuse out of the cell along their concentration gradient carrying positive charges to the outside of the membrane. The organic anions, to which the membrane is impermeable, are left behind and thus the charges are separated by the membrane which is therefore polarized. At equilibrium, there is no net diffusion of K^+ ions because of the operation of the Na^+/K^+ pump which carries K^+ ions into the cell, and chloride ions which enter the membrane along their concentration gradient tend to be driven out again by the negative charges on the inside and as a result there is little net diffusion of chloride ion through the membrane. For sodium ions, the concentration gradient

and the electrical forces are acting in the same direction and there is therefore a strong tendency for sodium ions to diffuse into the cell. However, in the resting state, the membrane is only slightly permeable to sodium ions. Furthermore, a metabolic process, known as the sodium pump (see later) continually drives sodium out of the cell against its concentration gradient so that in the resting cell, the net sodium gain is negligible.

When a membrane separating two solutions is permeable to only potassium ions, the potential difference E_K, set up across the membrane may be calculated by the Nernst equation (Nernst, 1889; cited by Bernstein, 1902):

$$E_K = \frac{RT}{Fn} \log_e \frac{[K]_o}{[K]_i} \qquad (1)$$

where R is the gas constant, T the absolute temperature, F Faraday's constant, $[K]_o$ and $[K]_i$ concentrations of potassium ions on the outside and inside respectively and n the valency of the ion.

In physiological situations, several ion species are involved, and the barrier between the inside and outside solutions has a limited and distinct permeability to each ion species (Boyle and Conway, 1941; Hodgkin and Huxley, 1939). The relationship between concentrations, permeabilities and potential most used in this complex situation is due to Goldman (1943) and is as follows:

$$E = \frac{RT}{F} \log_e \frac{P_{Na}[Na]_o + P_K[K]_o + P_{Cl}[Cl]_i}{P_{Na}[Na]_i + P_K[K]_i + P_{Cl}[Cl]_o}$$

where P_{Na}, P_K and P_{Cl} are permeability constants for the respective ions and the other terms have meaning as in equation 1. This expression is based upon the assumption that the voltage gradient through the membrane is constant and that the ions only move under the influence of diffusion and the electric field.

The 'sodium pump'

The maintenance of a steady state of ion distribution in cells is only possible if there is some active mechanism capable of extruding sodium continuously across the cell membrane (see Casteels, 1970). An active pumping system has been proposed first by Dean (1941) and has been fully investigated by Hodgkin and Keynes (1955) in the squid giant axon. This uphill movement of sodium from the cytoplasm to the outside depends on the presence of energy-yielding substrates, but the downhill movement in squid giant axon is not affected by inhibition of metabolism. Similar active sodium-extrusion, against an electrochemical gradient has been reported in other nerve fibres and skeletal muscle fibres (Caldwell, 1968), in invertebrate neurones (Kerkut and Thomas, 1965) and in smooth muscle cells (Casteels and Hendrickx, 1969; Taylor, Paton and Daniel, 1969). It is only by a continuous active exchange of ions that these cells are able to maintain their steady state, i.e. a low intracellular sodium content and a high potassium content. The amount of sodium extruded sometimes exceeds the amount of potassium taken up so that the pump acts as a direct source of current by transferring positive charges outward, and thus generating an 'electrogenic' component to the potential difference across the cell membrane. Such a pump can make the membrane

potential more negative than the potassium equilibrium potential in non-steady state conditions (Connelly, 1959; Kernan, 1962; Cross, Keynes and Rybova, 1965; Adrian and Slayman, 1966; Casteels, Droogmans and Hendrickx, 1971; Bolton, 1973).

For detailed discussion on whether the sodium pump is electrically-neutral (i.e. which links the outward transfer of each sodium ion to the inward transfer of a potassium ion so that there is no separation of electrical charge across the membrane) or electrogenic see reviews by Koketsu (1971) and Thomas (1972).

The Action Potential

The action potential may be defined as a response to depolarization during which the membrane undergoes further depolarization and may become polarized in the opposite direction to that of the resting membrane potential (see Holman, 1968). According to the ionic hypothesis of Hodgkin and Huxley (see Noble, 1966), this response is due to the dependence on membrane potential and time of the permeability or specific ionic conductances of sodium and potassium ions (g_{Na} and g_K).

The role of sodium ions in the generation of the action potential in smooth muscle is very much a subject of controversy. In the taenia coli, the amplitude of the action potential or spike is not dependent on the external sodium concentration

(Bülbring and Kuriyama, 1963). Spike activity persists for some time in the absence of sodium (Axelsson, 1961; Holman, 1958). The properties of the spike in taenia coli have recently been fully investigated by Brading, Bülbring and Tomita (1969) and these authors suggested that calcium rather than sodium is the main ion involved in the spike mechanism.

In the ureter, there is evidence to suggest that the spike component of the action potential is due to calcium entry and the plateau component is due to sodium entry (Kuriyama, 1970; Kuriyama and Tomita, 1970; Kobayashi, 1971).

The relationship between membrane potential, spike frequency and tension in smooth muscle

A close correlation between the development of tension and the electrical activity of smooth muscle was first demonstrated by Bülbring (1955) in the taenia coli. Using intracellular recordings she found that tension was directly proportional to the frequency of spike discharges which in turn was inversely related to the transmembrane potential. This inverse relation however, only holds when the transmembrane potential falls to a certain limit. In that case the isometric tension rises as a consequence of increased frequency of discharge. Numerous investigators have since confirmed and extended the observations of Bülbring, both for taenia coli and other types of smooth muscle (see Bülbring, Jones and Tomita, 1970).

Each spike or transient depolarization is normally followed by a brief mechanical response referred to as 'phasic response'. The mechanical response due to a sustained depolarization, consisting of a reversible shortening of the fibres or the development of tension is termed 'contracture' (see Axelsson, 1970). In both cases depolarization, whether transient or sustained, leads to or is associated with the activation of the contractile mechanism and a conversion of chemical energy into mechanical activity (Kuriyama, 1961; Marshall, 1962).

There are instances, usually under unphysiological conditions (e.g. impairment of metabolic activity and drastic changes in ionic environment) when the close connection between electrical and mechanical activity can be dissociated (Bülbring and Lullman, 1957; Durbin and Jenkinson, 1961; Edman and Schild, 1961,1962).

Changes in tension without changes in transmembrane potential (depolarized muscles).

In completely depolarized chick amnion, tension was increased by drugs which increase membrane permeability without any change of membrane potential (Evans, Schild and Thesleff, 1958).

Durbin and Jenkinson (1961) found that carbachol increased both inward and outward fluxes of various ions in a depolarized taenia coli and simultaneously increased the degree of contracture. Waugh (1962) also demonstrated that adrenaline and

noradrenaline could induce contractions in depolarized arterial muscles. In the depolarized portal vein noradrenaline increased, and isoprenaline in low concentrations decreased contracture without any measurable change in membrane potential (Axelsson, Johansson, Jonsson and Wahlstrom, 1966).

Various hypotheses have been advanced to explain the above phenomenon of tension changes which are dissociated from changes in membrane potential. (See Axelsson, 1970). Durbin and Jenkinson (1961) suggested that the carbachol contracture of depolarized smooth muscle was a consequence of a net movement of calcium ions into the cells, following the increase in permeability produced by the drug. Edman and Schild (1962) and Daniel (1963) postulated that 'bound' calcium plays a part in the contraction of uterine smooth muscle in polarized and depolarized smooth muscle.

Study of Drug Action

The mode of action of stimulating agents on smooth muscle can be studied with the use of known selective pharmacological blocking agents and of structural analogues. These compounds can help to determine direct and indirect actions of a drug. In addition, the mechanism of action of any chemical on excitable tissues, such as smooth muscle, can be studied by measuring changes in transmembrane electrical potential particularly if the agent is expected to act on the cell membrane.

A wide range of recording techniques have been utilized in the investigation of bioelectric potentials generated by smooth muscle and of the effects of drugs on membrane excitability. The techniques fall into either one of two categories, viz:

1) intracellular recording and 2) extracellular recording.

1) Intracellular

The most direct approach to the study of transmembrane potential changes involves the use of intracellular recording electrodes as described by Ling and Gerard (1949). This method has been successfully applied to several smooth muscle preparations, but most extensively to the uterus and the guinea-pig taenia coli (Bülbring, 1954a, 1954b; Burnstock, Holman and Prosser, 1963). Technical difficulties due to the small size (2 - 5 μ in diameter) and to the spontaneous mechanical activity of the muscle cells have prevented a more widespread use of this technique, although some of the difficulties have been circumvented by using small pieces of tissue and microelectrodes with long flexible tips (Holman, 1957, 1958) or suspended by very thin wires ("floating electrodes"; Woodbury and Brady, 1956). It is doubtful, however, whether there is sufficient seal of the puncture caused by the microelectrode even with a tip diameter of less than 0.5 μ to prevent leakage of intracellular contents which would affect membrane potential recordings in such small cells (Bülbring, 1954a).

2) Extracellular recording

The electrodes most commonly used for external recording are chlorided, coiled silver wires inserted into small glass tubes filled with Krebs-Ringer solution in agar and making contact with the tissue by thin cotton wicks at the tips of the tubes. (see Prosser and Bortoff, 1968). Extracellular recording therefore measures the overall electrical activity over a segment of a tissue rather than of individual cells. However, with external electrodes, both bipolar and monophasic recordings are possible.

The Sucrose-gap Technique

Stämpfli (1954) described a method for measuring the full value of the resting potential of peripheral nerve with external electrodes (Fig. 2a). His method is based on the theoretical calculation that the full value of the membrane potential can be measured with external electrodes on a core conductor, when the short-circuiting is negligible (Hodgkin and Rushton, 1946). Stämpfli obtained this condition by increasing the outside resistance of the preparation in the interpolar region by replacing most of the ions in the interstitial fluid with a nearly ion-free sucrose solution. If the membrane polarization of cells on both sides of this gap is unequal, current will flow between them.

In the real situation, the sucrose solution does not provide perfect insulation. Some current flow through the

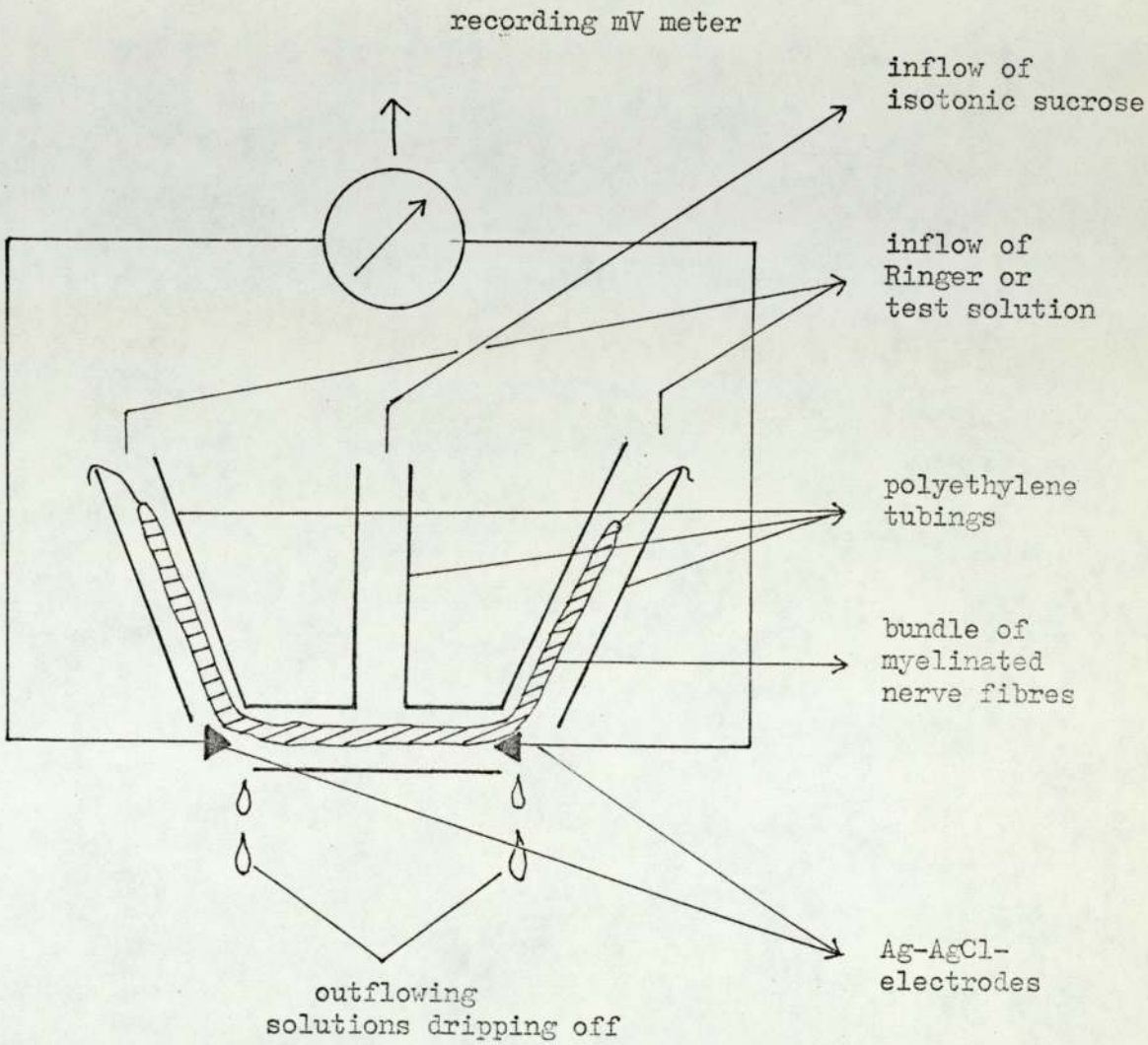

Fig. 2a. Arrangement of polyethylene tubes for recording membrane potentials. (simplified after Stämpfli, 1954)

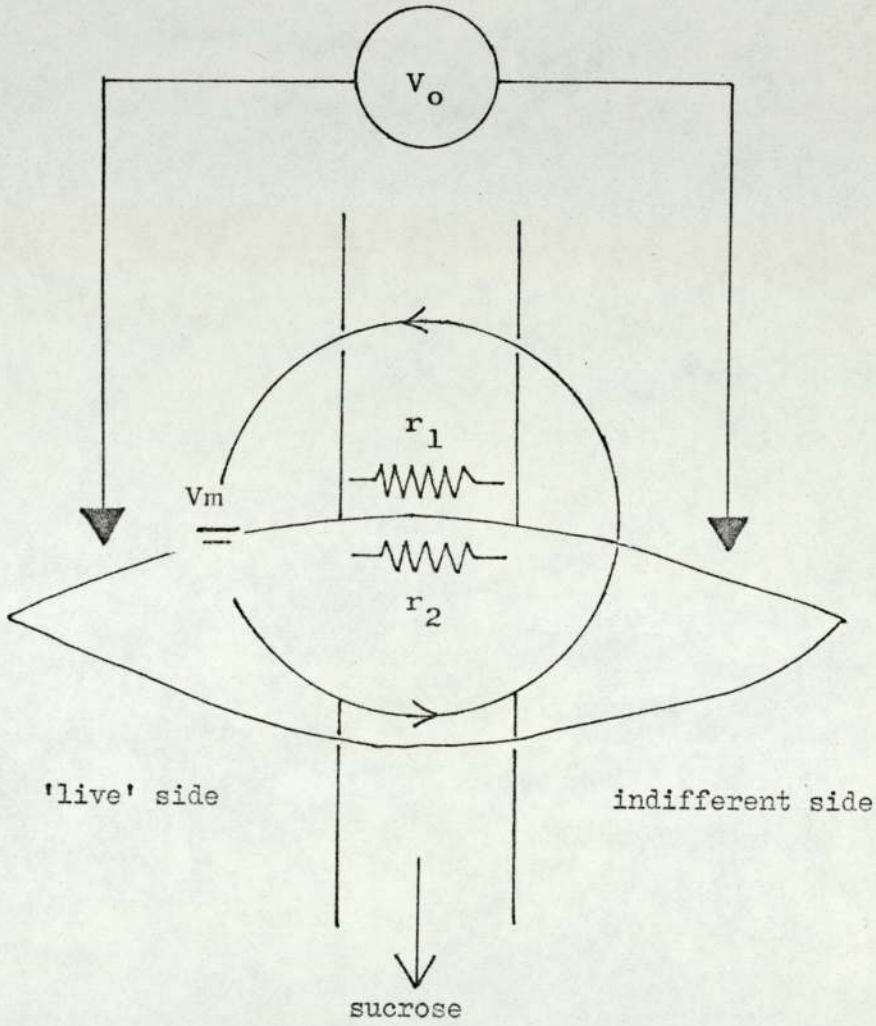

Fig. 2b. diagrammatic representation of a sucrose-gap arrangement showing short-circuiting pathway. The tissue is represented here as a single cell for simplicity. Further details refer to text.

sucrose will occur, and this will constitute a short-circuiting pathway (Fig. 2b). The true value of the membrane potential measured with external electrodes is reduced by the short-circuiting factor :

$$\frac{r_1}{r_1 + r_2}$$

where r_1 = external longitudinal resistance through sucrose

r_2 = internal longitudinal resistance of the cell.

An increase of r_1 to much higher values than r_2 will therefore tend to increase the short-circuiting factor to unity. The whole rationale of the sucrose-gap resides in this factor. The potential measured (V_o) with external electrode will therefore approximate the true membrane potential (V_m) of the tissue as expressed in the following equation:

$$V_o = V_m \left(\frac{r_1}{r_1 + r_2}\right)$$

In experiments with smooth muscle the situation is further complicated by the fact that no single smooth muscle cell is sufficiently long to span the width of the 'sucrose-gap' section (i.e. a minimum of 3 mm). The sucrose-gap method therefore provides a test of the hypothesis that the electronic spread of current from one cell to another ahead of the action potential is responsible for transmission between cells, through these inter-

cellular connections or 'nexuses' (Dewey and Barr, 1965). These authors suggested that the interiors of the cells must be connected by pathways of low enough resistance to allow electrical transmission between cells. These intercellular connections or 'nexuses' (Dewey and Barr, 1962) have been observed in various smooth muscles (see Barr and Dewey, 1968).

Application of the Sucrose-gap Technique

Berger and Barr (1969) summarized the following electrophysiological problems where sucrose-gap techniques can be utilized to advantage over other techniques.

1) Alterations of cellular membrane potentials which occur spontaneously or under the influence of certain drugs and ions may be measured with a single gap of constant width (Burnstock and Straub, 1958).

2) The existence of electrical coupling between cells can be proved or disproved by shunting the gap with a variable resistor. The presence of sufficiently low resistance connections between cells is shown by a spread of excitation across the blocked area when the shunt resistor bridges the gap (Barr and Berger, 1964). This method usually requires a sucrose gap of variable width.

3) The relationships between the current through cellular membranes and the voltage across them may be studied under voltage clamp conditions by using a double gap system in which a small area of the cell membrane (or of the preparation) is

electrically isolated by two adjacent sucrose gaps. This method was found to be suited to the study of current-voltage relations in the lobster axon (Julian, Moore and Goldman, 1962a,b), and recently in smooth muscle preparations (Kuriyama and Tomita, 1970; Ito, Kuriyama and Sakamoto, 1970).

Apart from the fact that with the sucrose-gap, recordings can only be obtained from groups of cells, there are other disadvantages which may need to be considered when analysing results obtained by this method, for example, the presence of sucrose solution in the extracellular space of the preparation and the contact of sucrose solution with saline are possible sources for artifacts which may influence electrical recordings. Liquid junction potentials could contribute to membrane values measured with such a technique. This is especially true in replacement studies where chloride ions in the saline are replaced by other ions of different mobility (Blaustein and Goldman, 1966; Burnstock and Straub, 1958).

In muscular tissues, cell movements during contractions seem to be responsible for another kind of recording problem when regions of muscle are pulled across the liquid junction (Berger and Barr, 1969). In cardiac and smooth muscle contraction where spontaneous electrical activity triggers rhythmic

contractions electrical recordings can be profoundly disturbed. Those artifacts cannot be eliminated by an 'isometric' recording technique, because although the total length of the preparation can be kept constant, segmental contraction may occur by stretching adjacent regions of the preparation.

Another disadvantage of the sucrose-gap method is the instability of the boundary where sucrose solution and saline contact each other. A slight change in the flow rate of one of the solutions will shift the boundary along the preparation. It is difficult to prevent artifacts when switching from saline to a test solution (Kerkut, personal communication). In particular, flow rates have to be carefully matched to keep the liquid boundary in the same position relative to the preparation. Some authors have minimized these artifacts by mechanically fixing the tissue as it passes each junction between solutions (Keatinge and Richardson, 1963) while others have used rubber membranes as partitions between solutions (König, 1962; Stämpfli, 1963). Nonetheless, these modifications cannot entirely eliminate the artifacts.

III PROSTAGLANDINS

In the results section of this thesis, evidence is presented which suggests that part of the action of angiotension on smooth muscles is mediated by a release of prostaglandins. It is pertinent therefore to review briefly the history of prostaglandins.

Early work by Kurzrok and Lieb (1930) established that human seminal fluid augments or inhibits spontaneously contracting isolated human uterine strips. Goldblatt (1933, 1935) and von Euler (1934, 1935) later independently demonstrated that seminal fluid also stimulated isolated intestinal smooth muscle. Von Euler (1936) also demonstrated that the active principle in an extract of human or sheep seminal fluid is a lipid-soluble acid. It was initially assumed that the active material was formed mainly in the prostate gland, hence the name "prostaglandin" (von Euler, 1935). This assumption proved to be wrong (Elliason 1959), but the original nomemclature has been retained (see Bergström, Ryhage, Samuelsson and Sjövall, 1963). The word "prostaglandin" is no longer used to refer to a single substance, but is a generic term for a family of closely related compounds, all derivatives of prostanoic acid, a basic 20-carbon atom skeleton (Bergström and Samuelsson, 1965; Pickles, 1967), (Fig. 3). Prostaglandins, although occuring in lesser amounts than those found in human or sheep seminal fluid, do have a widespread occurence in many tissues (see Horton, 1968).

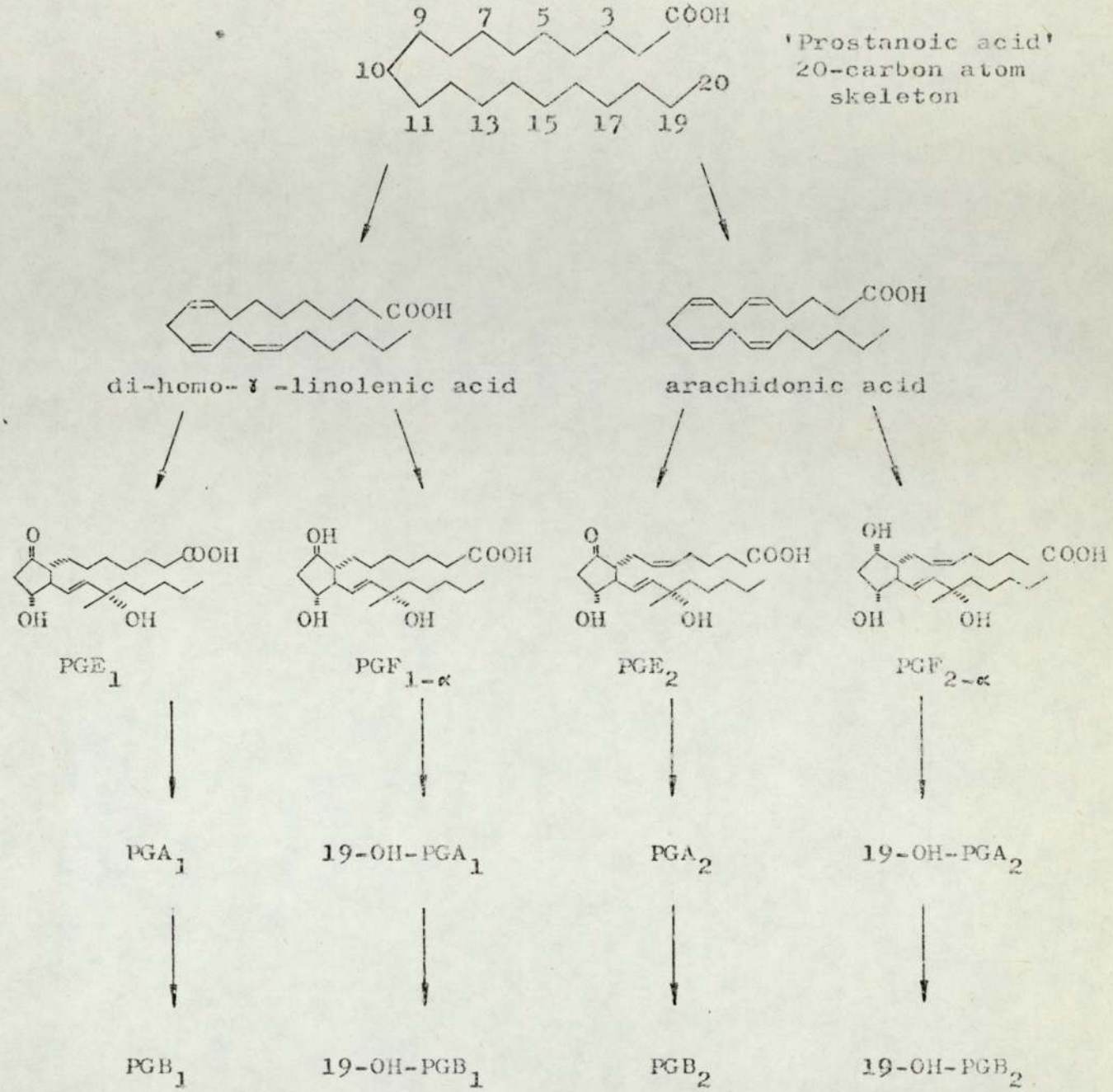

Fig. 3.

Structural formulae of the naturally occuring prostaglandins and their precursors. PGE_1 and $PGF_{1-\alpha}$ are from dihomolinolenic acid, PGE_2 and $PGF_{2-\alpha}$ are from arachidonic acid.
(Modified after Bergström, Carlsson and Weeks, 1968).

Chemistry, Occurence and metabolism

The prostaglandins are 20-carbon unsaturated fatty acids containing a five-membered ring. The particular biological properties of a prostaglandin depend primarily on the substituents of the ring. In the 9-position, a ketone group characterizes prostaglandins of the E series and a hydroxyl group characterizes those of the F series (Fig.3). Prostaglandins of the A series differ from those of the E series by the absence of a hydroxyl group in the 11-position and the presence of a double bond in the ring. The subscript numeral indicates the degree of unsaturation of the side chains, e.g. PGE_2 has two double bonds in its side chains and PGE_1 has one. Prostaglandins having similar degrees of unsaturation possess common precursors : arachidonic acid is the percursor for PGE_2 and $PGF_{2-\alpha}$ and di-homo-γ-linolenic acid is the precursor for PGE_1 and $PGF_{1-\alpha}$. PGE compounds readily undergo dehydration within the ring resulting in the formation of PGA compounds. (Fig.3). For further details on the chemistry of prostaglandins, see reviews by Samuelsson (1972) and Sih and Takeguchi (1973).

The prostaglandins are ubiquitous in their distribution. Verified isolations to date include sheep vesicular gland; lungs of sheep, cow, pig, guinea-pig, monkey, and man; sheep and bovine iris; bovine brain; calf thymus; bovine pancreas; and pig kidney, (see Bergström, Carlsson and Weeks, 1968; Horton, 1968; Samuelsson, 1972). Ambache, Brummer, Rose and Whiting (1966) have shown that PGE_2, $PGF_{2-\alpha}$, and possibly PGE_1 can be extracted from blood-free rabbit ileal longitudinal muscle sheets contain-

ing Auerbach's plexus. Furthermore, PGE_2 and $PGF_{2-\alpha}$ were found in a similar location in guinea-pig ileum (Ambache, Brummer, Whiting and Wood, 1966). Utilizing a paired comparison design these later workers also demonstrated that plexus-containing longitudinal muscle of the guinea-pig ileum has more prostaglandin as measured by bioassay than plexus-free longitudinal muscle from the same animals.

Three prostaglandins have been isolated from the rabbit renal medulla, namely, PGE_2, $PGF_{2-\alpha}$ and PGA_2 by Lee, Crowshaw, Takman, Attrep and Gougoutas (1967). However these authors thought that PGA_2 was a possible artifact produced from PGE_2 during extraction of the prostaglandins. Concurrently, Daniels, Hinman, Leach and Muirhead (1967) identified PGE_2 as the principal vasodepressor renal prostaglandin.

Studies of quantitative distribution may be misleading in that other studies have shown that the prostaglandins probably exist in precursor form and can be rapidly formed by chemical or nerve stimulation (Dorp, 1966; Ramwell, Shaw, Douglas and Poisner, 1966). The prostaglandin concentrations reported in tissues are more an indication of biosynthetic activity than of endogenous prostaglandin content (McGiff, Crowshaw, Terragno and Lonigro, 1970). The intestinal tissue of sheep and guinea-pig is capable of synthesizing prostaglandin (Dorp, 1966). Microsomal enzymes are capable of this biosynthesis, and a true dioxygenase is necessary, that is, both oxygen atoms on the cyclopentane ring come from the same molecule (Samuelsson, 1965).

All oxygen atoms in the molecule arise from molecular oxygen (Nugteren and van Dorp, 1965; Ryhage and Samuelsson, 1965). Prostaglandins E and F are not necessarily interconvertible but can arise from a common cyclic intermediate (Dorp, 1966).

The metabolic fate of prostaglandins has not been fully elucidated, however, it is believed that PGE compounds are metabolised into less polar compounds (Samuelsson, 1964; Samuelsson, 1965). In a recent study, McGiff, Terragno, Strand, Lee, Lonigro and Ng (1969) demonstrated that PGE and PGF but not PGA compounds are almost entirely removed from the blood on passage across the lung. PGE_2 and $PGF_{2-\alpha}$, although stable in blood are almost completely inactivated on passage across the lung (Ferreira and Vane, 1967). However, PGA compounds can escape destruction in the lung (McGiff et al, 1969).

Effects on Smooth Muscles

A) Intestinal

Isolated segments of gastro-intestinal smooth muscle of all species so far investigated (see Horton, 1968) contract in response to prostaglandins E_1, E_2, E_3, F_1 and $F_{2-\alpha}$. Qualitative differences in the response to the E's and the F's have been reported (Bergström, Eliasson, Euler, and Sjövall, 1959; Horton and Main, 1965). On the isolated rabbit jejunum, the response to $F_{2-\alpha}$ is slower in onset and reaches its peak more slowly than the response to E_1 (Horton and Main, 1965). On this preparation the responses to E_1 are reduced in size if sodium ions in the bathing fluid are partially replaced by lithium ions, but E_1

contractions are not blocked by botulinum toxin in a concentration which abolished the response to nicotine (Miyazaki, Ishizawa, Sunano, Syuto and Sakagami, 1967). In contrast E_1 contractions of the guinea-pig ileum are partially blocked by atropine, although the degree of block cannot be increased by increasing the concentration of atropine in the bath (Horton, 1965). From this result it is suggested that there is a small nervous component in the contractile response of the guinea-pig ileum to prostaglandins (Horton, 1968). The stimulating action of prostaglandin on guinea-pig ileum is not inhibited by antihistamines, hexamethonium, d-tubocurarine, nicotine, cocaine, tryptamine, lysergic acid diethylamide, or dihydroergotamine (Eliasson, 1959).

The isolated rat fundus is particularly sensitive to both prostaglandin E_1 and $F_{2-\alpha}$ (Coceani and Wolfe, 1966). Contractions are not affected by the presence of atropine, papaverine or 5-hydroxytryptamine-blocking drugs. Responses are potentiated by procaine, bretylium, dichlorisoprenaline, ascorbic acid, doubling the calcium ion concentration or increasing the potassium concentration. Responses to prostaglandins are reduced by isoxsuprine, noradrenaline, reduction in bath temperature, reduced oxygenation, carbon monoxide, sodium azide, cyanide ions or a reduction in calcium ion concentration. From these results, Coceani and Wolfe (1966) concluded that the prostaglandin action is a direct one on smooth muscle, closely connected to an oxidation reaction which requires enzymes or coenzymes in the reduced state

and that calcium ions are intimately involved in the contraction. A similar conclusion was arrived at by Paton and Daniel (1967) in their study on the rat uterus and fundus strip. These authors also demonstrated that polarization of the membrane is not a requirement for contractile activity induced by the prostaglandins, provided that calcium is present in the bathing fluid.

B) Vascular

The effects of the prostaglandins on the cardiovascular system appear to be directed to specific areas of vascular smooth muscle and vary with the particular prostaglandin and with species. For example, prostaglandin E_1 injected intravenously lowers the systemic arterial blood pressure in all species so far investigated whereas prostaglandin $F_{2-\alpha}$ raises the blood pressure in the dog, rat and spinal chick (Horton and Main, 1967; Du Charne and Weeks, 1967) but lowers it in the cat and rabbit (Änggard and Bergström, 1963).

The fall in arterial blood pressure may be due partly to vasodilation, since prostaglandins dilate blood vessels in skeletal muscle and skin (Änggard and Bergström, 1963; Horton, 1963; Horton and Main, 1963, 1965), and prostaglandin E_1 also antagonizes the vasoconstrictor action of the catecholamines, vasopressin and angiotensin (Holmes, Horton and Main, 1963). In the blood-perfused rabbit and cat lung preparation prosta-

glandin E_1 has a vasodilator potency about equal to that of adrenaline, but unlike adrenaline the effect is neither abolished nor reversed by propranolol and is unaffected by phentolamine (Hauge, Lunde and Waaler, 1967).

The pressor action of $PGF_{2-\alpha}$ in the dog and the rat is thought not to be due to a venoconstrictor action, and is dependent upon an intact sympathetic nerve supply to the veins (Du Charne and Weeks, 1967). The increased venous return increases cardiac output thus accounting for the rise in arterial blood pressure. The mechanism of the pressor action of $PGF_{2-\alpha}$ in the spinal chick is unknown but the response is not blocked by hexamethonium, phenoxy-benzamine or propranolol (Horton and Main, 1967).

Prostaglandins E_2, F_1 and A_1 have potent actions in reducing blood pressure in renal hypertensive dogs (Muirhead, Daniels, Pike and Hinman, 1967) and in human hypertension (Lee, 1967). Since prostaglandins E_2 and A_2 occur in the kidney (Lee, Covino, Takman and Smith, 1965), the possibility of a normal hormonal role in controlling blood pressure has been suggested.

Other actions

Prostaglandins have extremely potent actions on systems as different as adipose tissue, nerve cells, platelets, respiratory tract, reproductive tract, central and peripheral nervous system. For detailed reviews covering these aspects

of the biological actions of the prostaglandins, see reviews by Bergström *et al* (1968); Ramwell and Shaw (1970) and Weeks (1972).

THE AIM

It is apparent that angiotensin has multiple sites of action both in whole animals and in isolated organs. The complexity of angiotensin action can be related to the structure and innervation of smooth muscle. It is also apparent that many investigations have been undertaken in an attempt to explain the mechanism of action of the peptide on smooth muscle. The phenomenon of angiotensin tachyphylaxis however remains unresolved.

There is evidence to show that acetylcholine and noradrenaline are involved in the contractile effect of angiotensin in intestinal and vascular smooth muscles. Similarly, however, there is evidence to suggest that these transmitters are not always involved in angiotensin-induced contractions. These results led to the postulation that angiotensin has both a direct and an indirect action on smooth muscle.

The present study was undertaken to clarify the situation at present prevailing in this field. By pharmacological blockade, individual steps in the excitatory pathway could be tested as sites of drug action (direct or indirect) provided the blocking agents could be shown to exert selective action in the experimental conditions used. The first part of this thesis deals with such studies on angiotensin action in a number of smooth muscle preparations.

In most excitable tissues, development of tension is invariably accompanied by membrane depolarization. There has been conflicting reports (see Axelsson, 1970) regarding the correlation between angiotensin-induced increase in tension and electrical activity. This aspect of angiotensin action was studied on the taenia coli of the guinea-pig using the sucrose-gap apparatus.

Recently, evidence has indicated that angiotensin can cause a release of prostaglandins from several isolated organs. An attempt was made to establish a possible involvement of prostaglandins in angiotensin-induced contractile responses. The results are presented in the last section of this thesis.

SECTION TWO

EXPERIMENTAL METHODS

EXPERIMENTAL METHODS

I ISOLATED INTESTINAL AND VASCULAR SMOOTH MUSCLE PREPARATIONS USED IN INITIAL PHARMACOLOGICAL STUDIES

i) The guinea-pig ileum preparation

Guinea-pigs from both sexes (Hartley strain) weighing between 300 and 450 grams were killed by a blow on the head and bled from a large incision in the neck. The ileum was excised and 3 - 4 cm long segments were removed from the preterminal region (about 10 cm above the ileo-caecal junction) and suspended in a 15 ml organ bath containing Krebs solution (for composition see end of Methods) maintained at 34° C. In some experiments Tyrode solution (for composition see end of Methods) was used instead. The muscle was initially loaded with 1 gram tension. Increases in tension were recorded with an isometric transducer (Devices type 2ST02). The signals were amplified by DC preamplifier (Devices DC 2D) and permanent records were made with a pen recorder (Devices M2).

Transmural stimulation was effected with bipolar platinum electrodes of the type described by Paton (1955). Supramaximal stimuli were delivered from a square wave stimulator with frequencies of from 0.2 to 10.0 Hz and pulse width of 0.5 - 1.0 ms.

ii) The guinea-pig taenia coli preparation

Guinea-pigs were killed as above. The caecum was exposed and carefully fixed in position by pins, so that the taenia coli

lay on top of the caecum. A small incision was made between the taenia coli and the underlying circular muscle to separate the two. A piece of cotton was tied round the taenia coli to facilitate manipulation of the preparation while it was very carefully separated from the underlying muscle using fine scissors. The taenia coli was transfered to Krebs solution and stretched to its in situ length. Lengths of 3 - 4 cm were cut and suspended in a 10 ml organ bath containing Krebs solution at 32 °C. The initial load on the muscle was 1 gram. Increases in tension were recorded with an isometric transducer as in the guinea-pig ileum.

iii) The rat stomach fundus strip preparation

The rat stomach fundus strip was prepared according to the method described by Vane (1957). Rats of both sexes (Wistar strain) weighing 200 - 350 grams were stunned by a blow on the head and then bled. The stomach was dissected free from the abdomen and placed in Tyrode solution. The fundus is a translucent ballon-like tissue. It is separated from the pyloric antrum by a definite ridge on the mucosa. The fundus was dissected out from the stomach with a band of pyloric tissue attached which serves as a marker. A cut was made along the lesser curvature of the fundus and the contents washed away. Two or three incomplete transverse cuts (depending on the tissue size) were made from alternate sides so as to preserve the longitudinal muscles. The resultant strip was about 4 - 5 cm long. Cotton was tied to each end and the strip was gently stretched and any protrusions and fringes of pyloric tissues trimmed away to give a clear thin strip which was then mounted in a 20 ml bath containing Tyrode solution at 36° C. The muscle was initially loaded with 2 gram tension. Contractile responses were

recorded with an isotonic transducer (Devices type R2502 - 2) on a pen recorder (Devices M2).

iv) The rat ileum preparation

Rats were killed as above. The ileum was excised and segments 2 - 4 cm long were removed from the preterminal region and suspended in a 15 ml bath containing Tyrode solution at 36° C. The initial tension on the muscle was 1 gram. Changes in tension were recorded with an isometric transducer as in the guinea-pig ileum.

v) The rat ascending colon preparation

The rat colon was prepared as described by Regoli and Vane (1964). The ascending colon can be identified by its diagonal striations and it is immediately adjacent to the caecum. The whole tissue was excised, washed and suspended in a 15 ml bath with rat colon Ringer solution (Gaddum, Peart and Vogt, 1949). The bath temperature was maintained at 36°C. The muscle was loaded with a 2 gram tension and changes in tension were recorded isometrically.

vi) The chick rectum preparation

The chick rectum preparation was prepared as described by Mann and West (1950). Chickens of 7 - 10 days old were killed by cervical fracture. The rectum was exposed, removed, washed and suspended either in a 10 ml organ bath or for superfusion experiments in a polythene jacket with the solution flowing over the tissue at a rate of between 8 - 10 ml per minute The muscle was loaded with 1 gram tension. Changes in tension were recorded with an isometric transducer.

vii) The rabbit ileum preparation

Rabbits weighing 1 - 3 Kg were killed by a blow on the back of the neck and bled out. Sections of ileum about 3 - 4 cm long were removed and carefully cleared of any contents from the lumen. The tissue was suspended in a 20 ml bath containing Tyrode solution at 36°C. The muscle was initially loaded with 2 - 4 grams tension and changes in tension were recorded isometrically.

viii) The rabbit aortic strip preparation

Rabbits were killed as above. The descending thoracic aorta was removed and cut spirally according to the method described by Furchgott and Bhadrakom (1953). In some experiments, the abdominal aorta was used. The strip, about 3 - 4 cm long and about 5 mm in width was suspended in a 20 ml bath containing Krebs solution at 36°C. Isometric contractions were recorded on a pen recorder (Devices M2). Initial tension was 2.0 grams. The strip was allowed to equilibrate for at least an hour before the administration of drugs.

ix) The rat portal vein preparation

Rats were killed as before (see fundus strip preparation). The portal vein was exposed and cleared of adherent tissue. Silk sutures were inserted through the wall of the vessel and a 1 - 2 cm length was removed and suspended vertically in a 10 ml bath containing Krebs solution at 34°C. The passive tension applied to the portal vein was 400 - 600 mg. Contractions were measured isotonically and recorded on a pen recorder (Devices M2). At least an hour was allowed for the preparation to equilibrate before commencement of experiment.

II THE SUPERFUSED ORGAN SYSTEM USED FOR THE DETECTION OF PROSTAGLANDINS

The method was essentially similar to that described by Ferreira and Vane (1967). The effluent perfusing the isolated guinea-pig ileum was allowed to superfuse a series of isolated assay tissues (Fig.4). The three assay organs used were as follows:

i) rat stomach strip (Vane, 1957)

ii) rat colon (Regoli and Vane, 1964)

iii) chick rectum (Mann and West, 1950).

These tissues were chosen because of their sensitivity to prostaglandins and specificity was further increased by infusing a mixture of antagonists which rendered the assay tissues insensitive to catecholamines, acetylcholine, 5-hydroxytryptamine and histamine. The antagonist reagent consisted of Krebs solution with the following drugs per 100 ml. :

methysergide 2 mg

phenoxybenzamine 1 mg

propranolol 20 mg

hyoscine hydrobromide 1 mg

mepyramine 1 mg

The antagonist solution was perfused across the assay organs at 0.1 ml per minute.

Standard agonists were tested for their direct effect on the assay organs by adding the agent to the Krebs solution after it had passed through the ileum. The response of the ileum to angiotensin was determined by injecting the drug into the direction of flow of the Krebs solution perfusing the tissue by means of an infusion apparatus. The ileum was perfused with Krebs solution at a rate of

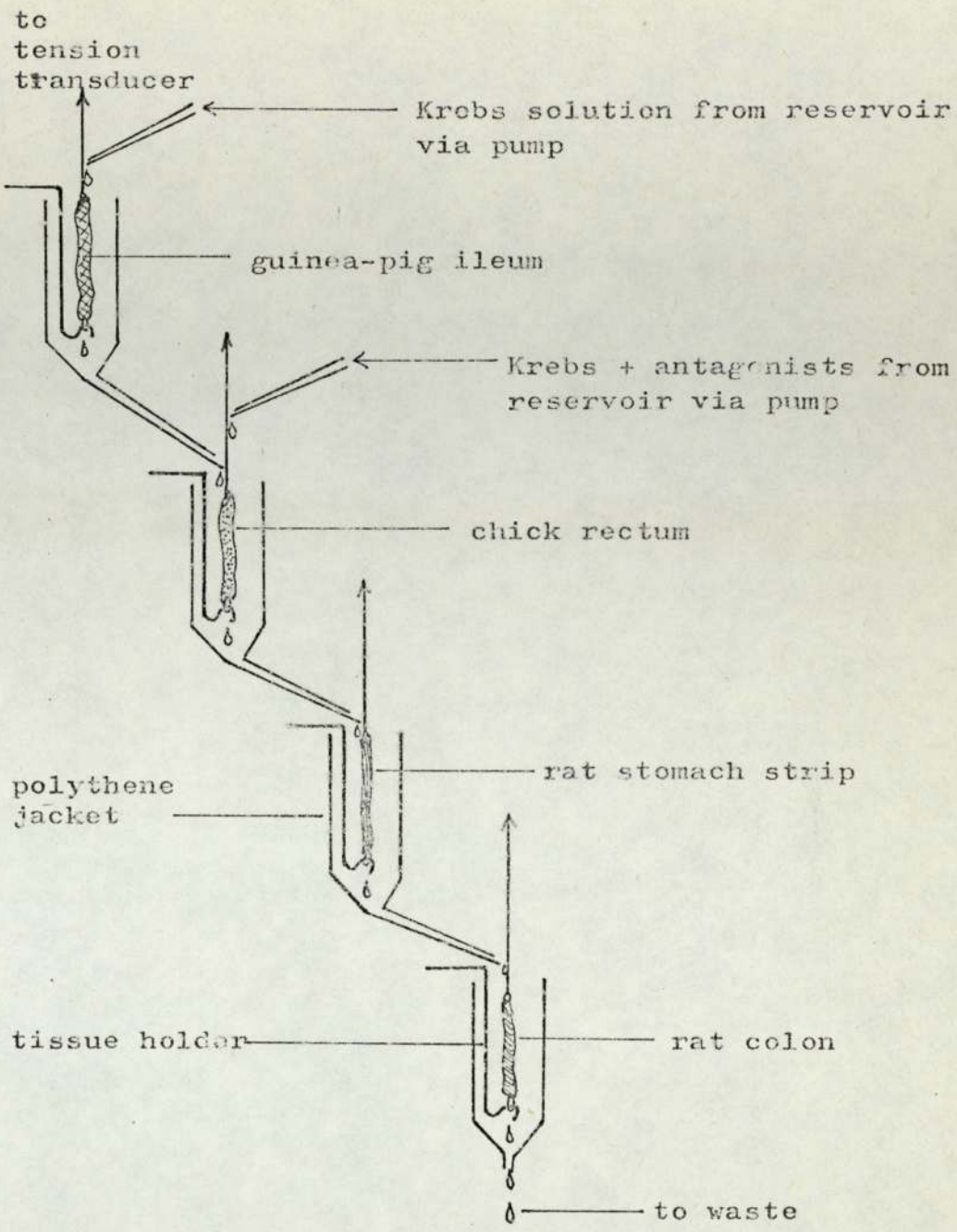

Fig. 4. The superfused organ system for the detection of prostaglandins.
A diagramatic representation of the arrangement of tissues and perfusion fluids used.

8 - 10 ml per minute. The Krebs solution was maintained at 36° C. Changes in tension and contractions were recorded with isometric and isotonic transducers respectively. The signals were amplified by DC preamplifier and permanent records were made with a 4-channel pen recorder (Devices M4).

III THE PITHED RAT PREPARATION

Male or female rats (Wistar strain) weighing 200 - 300 grams were anaesthetised with pentobarbitone (Nembutal) 60 mg/kg injected intraperitoneally; atropine sulphate 1 mg/kg was administered by the same route immediately after the pentobarbitone. The trachea was cannulated, artificial respiration started and the animal pithed by the method of Shipley and Tilden (1947) using a steel pithing rod 1.5 mm in diameter, prepared as described by Gillespie and Muir (1967). Positive pressure artificial respiration was achieved using a Palmer small animal respirator adjusted to deliver 20 ml/kg body weight in each experiment. The right jugular vein was then cannulated with polythene tubing (Portland Plastics, PP 30) previously filled with 0.9% saline containing 10 units/ml heparin. The right common carotid artery was cannulated with polythene (PP 30) filled with heparinized saline, and the arterial blood pressure measured by means of a blood pressure transducer (Devices/C.E.C. type 4-327-L221) connected to a Devices M2 pen recorder.

In experiments where stimulation of the sympathetic outflow was carried out, the preparation was injected intravenously with (+) - tubocurarine hydrochloride (3 mg/kg).

Sympathetic stimulation.

Electrical stimulation of the spinal sympathetic outflow was performed as described by Gillespie and Muir (1967). A length of steel wire was attached firmly under the skin. The indifferent electrode, a steel hypodermic needle inserted subcutaneously into the left leg, was connected to one pole of a square wave stimulator (Scientific and Research Instruments Ltd). The other pole was connected to the pithing rod. The sympathetic outflow was stimulated with supramaximal strength pulses (usually 80 volts) of 1 ms duration at frequencies ranging from 0.1 - 1.0 Hz applied for periods of 40 seconds and repeated at intervals of not less than 20 minutes.

Heart rate measurement.

In a few experiments, heart rate was measured by means of a Devices Instantaneous Ratemeter (Type 2751) triggered by the blood pressure signal.

Route of administration of drugs.

Drugs were injected intravenously in volumes not exceeding 0.2 ml and flushed in with a further 0.1 ml of saline. Infusions were made using a Palmer automatic injection apparatus at rates up to 0.05 ml/minute.

IV ELECTRICAL RECORDING OF TRANSMEMBRANE POTENTIAL USING THE SUCROSE-GAP TECHNIQUE

i) The sucrose-gap apparatus

The sucrose-gap apparatus was constructed according to the principles described by Stämpfli (1954). The bath was drilled out of a perspex block as illustrated in Fig. 5. The vertical channels through which Krebs solution flowed were of 1 mm in diameter and the central channel through which isotonic sucrose solution flowed was 2 mm in diameter. The sucrose gap was 5 mm in width with a diameter of 0.5 mm.

ii) Mounting of the taenia coli

The taenia coli of the guinea-pig was dissected as previously described for organ bath study. A length of the taenia, 15 - 20 mm in length was teased to size so as to fit the sucrose-gap bath.

Cotton was tied to both ends of the strip. One end of the cotton was threaded through a needle and this was first passed through the horizontal sucrose-gap, carrying with it the taenia coli, so that about equal length of the strip was exposed on either side. The cotton was then passed upwards through the vertical channel. Great care was taken during this procedure, so that the tissue was not stretched or grossly injured. The same was done for the other end of the strip, so that each end of the taenia coli was suspended in the vertical channel through which Krebs solution flow. The cotton at each end of the strip was secured by means of close-fitting

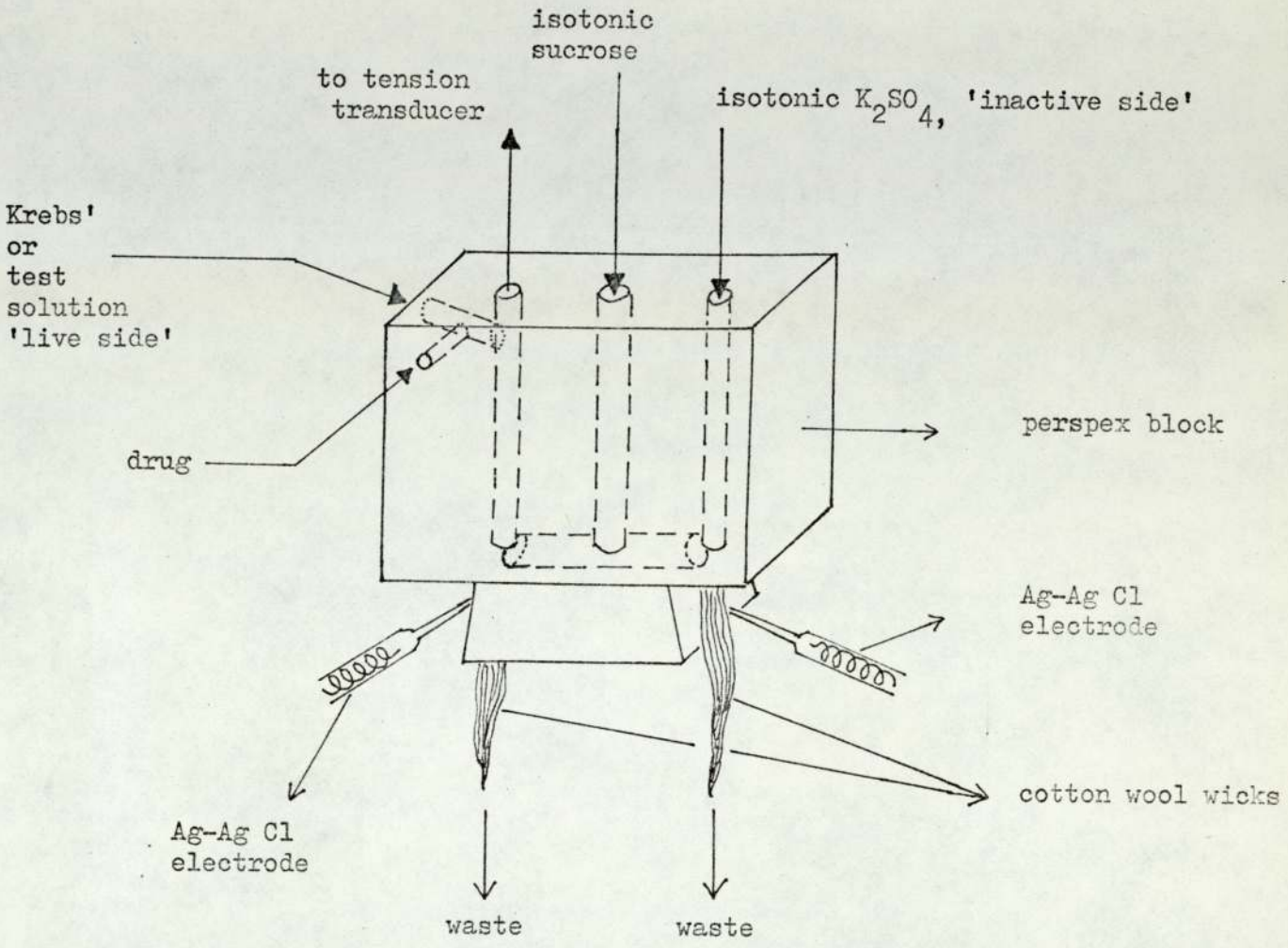

Fig. 5. The Sucrose-gap bath.

The channels were drilled out of a perspex block (for dimensions see text). Drug or test solution was introduced into the 'active' side via the small side-tube.

polythene tubing. Both ends of the strip were perfused with Krebs solution at the start of the experiment. Thirty minutes later, the perfusion fluid of one side was changed over to isotonic K_2SO_4 or KCl solution, thus making the side "inactive". By this procedure, the membrane potential could be measured. The gap-section was perfused with isotonic sucrose solution which had been passed through an ion-exchange column (Elgastat) before entering the gap. The flow rate of each perfusion fluid ranged between 3 - 5 ml per minute and was kept constant throughout any one experiment. The Krebs solution was maintained at 30 $\pm$ 1 °C, while the sucrose and K_2SO_4 solutions were kept at room temperature.

iii) Electrical recordings

A pair of silver-silver chloride electrodes in contact with the tissue through cotton wicks recorded the potential difference between the sucrose-Krebs junction and sucrose-K_2SO_4 junction.

The side perfused with K_2SO_4 , termed "inactive" was at earth potential and the side perfused with Krebs solution termed "active" , was connected via a unity gain preamplifier (Electronics for Life Sciences, wide-band electrometer) and displayed on a cathode-ray oscilloscope (CRO) (Tektronix type RM 502 A) or recorded on a Devices DC 2 pen recorder . In the majority of experiments, the end of the strip of the active side was attached by cotton thread to a Devices isometric transducer (Type 2ST02) for simultaneous recording of mechanical activity (Fig. 6).

Fig. 6. The sucrose-gap apparatus.
For details refer to text.

In a few experiments, both the CRO and the pen recorder were used for simultaneous visual display and recording of events. In such instances, the signal for the pen recorder was fed from the output of the CRO.

iv) Addition of drugs

All drugs were diluted in Krebs solution and injected in volumes not exceeding 0.02 ml (20 µl) by means of a micro-syringe (Hamilton) into the perfusion fluid of the active side. In a few experiments, drug solutions were introduced into the active side by means of a 3-way tap, after passing through a heating coil. However, during the change over of solutions, marked artefacts were encountered. The use of a 3-way tap was abandoned in preference to the micro-syringe.

V DRUGS AND SOLUTIONS USED

Unless indicated otherwise drugs were generally dissolved in 0.9% saline and added in volumes not exceeding 0.5 ml directly into the organ bath. Immediately after the response was maximal they were washed out by rinsing the bath with the appropriate physiological salt solution. Antagonist drugs were applied to the preparations for a minimum of 15 minutes (unless otherwise indicated) before adding agonists and maintained in contact with the preparations throughout the test period.

i) Preparation of indomethacin solution

Indomethacin was prepared by dissolving in slight excess sodium carbonate solution (0.2%) made up to the desired volume with either Krebs or Tyrode solution. If the solution was cloudy, a trace of solid sodium carbonate was added and the pH immediately adjusted to 7.3 - 7.6 with HCl. The solution of indomethacin was freshly prepared prior to each experiment.

ii) Preparation of prostaglandin-E_2 and $F_{2-\alpha}$ solutions

Prostaglandin-E_2 (PGE_2) was prepared as the sodium salt as follow:

A solution of sodium carbonate 0.2 mg/ml (20 mg of anhydrous sodium carbonate made up to 100 ml) in water or isotonic sodium chloride was prepared. PGE_2 was dissolved in 95% ethanol (0.1 ml/mg of PGE_2). 0.9 ml of the sodium carbonate solution for each milligram of prostaglandin was then rapidly added to the alcoholic prostaglandin solution. If the solution remained

cloudy, a trace of solid sodium carbonate was cautiously added. The final pH should be between 6 and 7.5 and this was checked with pHdrion paper. The stock solution was then stored frozen and dilution made as required.

Prostaglandin-$F_{2-\alpha}$ ($PGF_{2-\alpha}$) was available as $PGF_{2-\alpha}$-tromethamine salt, and this was dissolved in distilled water. A stock solution of 1mg/ml was prepared and stored frozen.

iii) Preparation of physiological salt solutions

The composition of physiological salt solutions used are given in Table III. All solutions were made up with distilled water. In addition the following modifications were made to Krebs solution for sucrose-gap studies.

a) High sodium solution was prepared by adding weighed quantities of sodium glutamate or sodium benzenesulphonate to the normal Krebs solution.

b) Low sodium solution was prepared by replacing the deficit of sodium chloride by an equiosmolar quantity of Tris (Hydroxymethyl) amino-methane chloride and titrated with HCl to give pH 7.3 - 7.4.

c). Sodium-free solution was prepared by replacing the NaCl with equiosmolar quantity of Tris-chloride and the $NaHCO_3$ with $KHCO_3$. The solution prepared in this way would contain 25 mM K.

d) High potassium solution was prepared by adding weighed quantities of KCl to the normal Krebs solution.

e) Potassium-free solution was prepared by replacing the potassium salts with sodium salts.

f) Calcium-free and calcium excess salt solutions were prepared by omitting $CaCl_2$ and adding required quantities of $CaCl_2$ to the normal Krebs solution respectively. The osmolarity of the solution was not corrected in these instances.

g) Low chloride solutions were prepared by substituting NaCl with sodium glutamate.

Isotonic KCl solution was prepared by dissolving 8.94g of KCl in 1L of distilled water (120 mM) and isotonic K_2SO_4 solution was prepared by dissolving 22g of K_2SO_4 in 1L of water (127 mM).

Isotonic sucrose solution was prepared by dissolving 10% sucrose (Aristar, BDH) (weight/volume) in glass-distilled deionized water.

Table III

Composition of physiological salt solutions used

	Krebs		Tyrode		Rat Colon Ringer	
	g/L	mM/L	g/L	mM/L	g/L	mM/L
NaCl	6.90	118.0	8.0	137.0	9.00	156.0
KCl	0.35	4.7	0.20	2.70	0.40	5.4
$MgCl_2$ $(6H_2O)$	-	-	0.26	1.10	-	-
$MgSO_4$ $(7H_2O)$	0.29	1.2	-	-	-	-
NaH_2PO_4	-	-	0.05	0.40	-	-
KH_2PO_4	0.16	1.18	-	-	-	-
$NaHCO_3$	2.10	25.0	1.00	11.90	0.14	1.68
$CaCl_2$ $(2H_2O)$	0.37	2.5	0.26	1.76	0.05	0.32
Glucose	1.00	5.5	2.00	11.00	1.00	5.5
Aeration	O_2:95% CO_2: 5%		air		air	

DRUGS USED

DRUG	SUPPLIER
Acetylcholine bromide	B.D.H. LTD.
(-)-adrenaline hydrochloride	B.D.H. LtD.
Angiotensin amide (CIBA) (Hypertensin)	CIBA Ltd.
Bradykinin	Sigma Ltd.
Guanethidine monosulphate	CIBA Ltd.
Hexamethonium bromide	Koch-Light Ltd.
Histamine acid phosphate	B.D.H. Ltd.
5-hydroxytryptamine creatine sulphate	Sigma Ltd.
Hyoscine hydrochloride	B.D.H. Ltd.
Indomethacin	M.S.D. Ltd.
Mepyramine maleate	May & Baker Ltd.
Methysergide	Sandoz Ltd.
Nicotine hydrogen(+)-tartrate	B.D.H. Ltd.
L-noradrenaline bitartrate	Sigma Ltd.
Pempidine tartrate	May & Baker Ltd.
Phentolamine	CIBA Ltd.
Procaine hydrochloride	B.D.H. Ltd.
Propranolol	I.C.I.
Prostaglandin-E_2	A gift from Dr. J.E.Pike Upjohn Ltd., Kalamazoo, U.S.A.
Prostaglandin-$F_{2-\alpha}$	ditto
Tetrodotoxin	Sigma Ltd.
Vasopressin	Sigma Ltd.

All concentrations and doses given in the text refer to the forms and salts listed above.

SECTION THREE

EXPERIMENTAL RESULTS

PART ONE

THE ACTION OF ANGIOTENSIN ON INTESTINAL AND VASCULAR SMOOTH MUSCLES

CHAPTER I THE ACTION OF ANGIOTENSIN ON ISOLATED SMOOTH MUSCLE PREPARATIONS

A) INTESTINAL SMOOTH MUSCLE

Various smooth muscle preparations have been used for a quantitative comparison of angiotensin with other drugs and some like the rat uterus (Paiva and Paiva, 1960; Khairallah and Page, 1961), rabbit aortic strip (Helmer, 1957, 1964) and rat colon (Regoli and Vane, 1964a)have been used to study the mechanism of action of angiotensin on smooth muscle. Isolated intestinal preparations from various species invariably contract to angiotensin.

Tachyphylaxis to angiotensin has been reported in the rabbit ileum, guinea-pig ileum (Bisset and Lewis, 1962; Khairallah and Page, 1962), human colon (Fishlock and Gunn, 1970) and ilea of the mouse, rat and gerbil (Goldenberg, 1967).

Godfraind et al (1966a) first described a fast and a slow component in the contractile response of the isolated guinea-pig ileum to angiotensin. A similar biphasic effect of angiotensin has been described by Goldenberg (1967) in the isolated ilea of the mouse, rat and gerbil.

In this chapter, experiments are described which were performed in order to determine :

1) Whether a similar biphasic action of angiotensin could be elicited in other intestinal smooth muscle preparations viz:

guinea-pig ileum and taenia coli, rat colon, ileum and stomach strip and rabbit ileum. 2) Whether these preparations show signs of tachyphylaxis to repeated or continued administration of large amounts of angiotensin. The sensitivity of these intestinal preparations to angiotensin was compared with that of another polypeptide, bradykinin and of other known agonists.

RESULTS

Isolated preparations of the guinea-pig, rat and rabbit ilea contracted to angiotensin with a biphasic response, which was first described by Godfraind et al (1966a) for the guinea-pig ileum. In the rat colon, rat stomach strip and the guinea-pig taenia-coli preparations, biphasic responses to angiotensin could not be demonstrated. The sensitivity of these intestinal preparations to angiotensin and other agonists is given in Table IV. All the preparations contracted with a latency of between 5 to 30 seconds after addition of angiotensin to the bath, the response was maximum within 60 to 120 seconds (Table V). Repeated or continued administration of high concentrations ($>10^{-}$ M) of angiotensin to these preparations caused prominent tachyphylaxis, however, this could be minimised or even abolished by increasing the time interval between successive exposure to the drug. Table V summarizes the minimum time interval to prevent the occurence of tachyphylaxis required for each of the tissues examined.

The following is a more detailed account of the observations made on the action of angiotensin on these isolated intes-

tinal preparations. Because of the similarity in the responses to angiotensin between the ilea of the rat, rabbit and guinea-pig, detailed description will be confined to the guinea-pig ileum. The only qualitative difference between the responses of these three tissues to angiotensin was that of sensitivity which was of the following order: guinea-pig ileum : rabbit ileum : rat ileum (TableIV).

1.1. Guinea-pig ileum

Three qualitatively identifiable forms of responses to angiotensin were observed depending upon the concentration used. At low concentrations ($<5 \times 10^{-9}$M) the ileum responded after a latent period of some 20 to 30 seconds, with a progressive increase in tension co-incident with rhythmic contractions, the maximum response was reached in about 90 seconds (Fig.7a). Higher concentrations ($5 \times 10^{-9} - 5 \times 10^{-8}$M) caused a reduction in the latent period and a biphasic response. There was an initial fast "spike" contraction followed by a small transient fall then a further slow progressive contraction which reached maximum in about 60 - 90 seconds, superimposed by rhythmic contractions which continued until wash out (Fig.7b). These results are similar to those described by Godfraind, et al,(1966a). These authors referred to the inital fast contraction as the fast component and the second plateau phase as the slow component (see Fig. 8).

With maximum or supramaximum concentrations of angiotensin ($>10^{-7}$M), the response rose sharply to a peak, then fell to a

Preparations	Angiotensin	Acetylcholine (Threshold concentrations)	Bradykinin	Source
Guinea-pig ileum	2 - 20	2 - 20	200	Regoli and Vane, 1964a.
	0.4 - 0.8	-	0.4 - 1	Bisset and Lewis, 1962.
	1 - 10	5 - 10	200	*
Guinea-pig taenia coli	2 - 20	-	200 R	Regoli and Vane, 1964a.
	10 - 20	5 - 10	500 R/C	*
Rat colon	0.2 - 2	2 - 20	20 - 200	Regoli and Vane, 1964a.
	500	-	1000	Bisset and Lewis, 1962.
	0.1 - 5	5 - 20	500	*
Rat ileum	20 - 200	200	200	Regoli and Vane, 1964a.
	50 - 100	100	500	*
Rat stomach strip	0.2 - 2	2 - 20	2 - 20 R	Regoli and Vane, 1964a.
	2 - 10	10	20 R	*
Rabbit ileum	5 - 20	5 - 10	-	*

Table IV. Sensitivity of various isolated intestinal preparations to angiotensin and other agonists. Threshold concentration expressed as dose-range in nanograms per millilitre.
* results obtained in this study.
C and R denotes contraction and relaxation of the tissue respectively.

Parameters	Preparations					
	Guinea-pig ileum	Taenia coli	Rat colon	Rat ileum	Rat stomach strip	Rabbit ileum
Biphasic response	yes	no	no	yes	no	yes
Latent period (seconds)	5 - 20	5 - 20	5 - 10	5 - 20	5 - 15	10 - 30
Time to maximum contraction (seconds)	60 - 90	60 - 120	60 - 90	60 - 90	60 - 90	60 - 120
Concentration to effect biphasic response	$5x10^{-9}M$	-	-	$10^{8}M$	-	$10^{-8}M$
Minimum dose interval to avoid tachyphylaxis (minutes)	5*	15	5	10	5	10

Table V . Action of angiotensin on various parameters for isolated intestinal preparations. Concentrations expressed in Moles.
* with two washes between doses (see text).

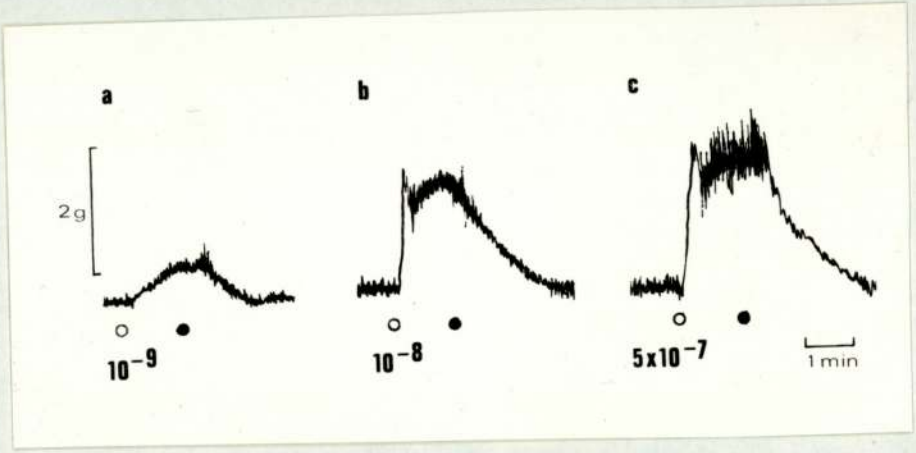

Fig.7. Isolated guinea-pig ileum preparation
Contractile responses to a) 10^{-9}M
b) 10^{-8}M and c) 5×10^{-7}M angiotensin.
Open circles indicate addition of angiotensin,
closed circles indicates removal of angiotensin.

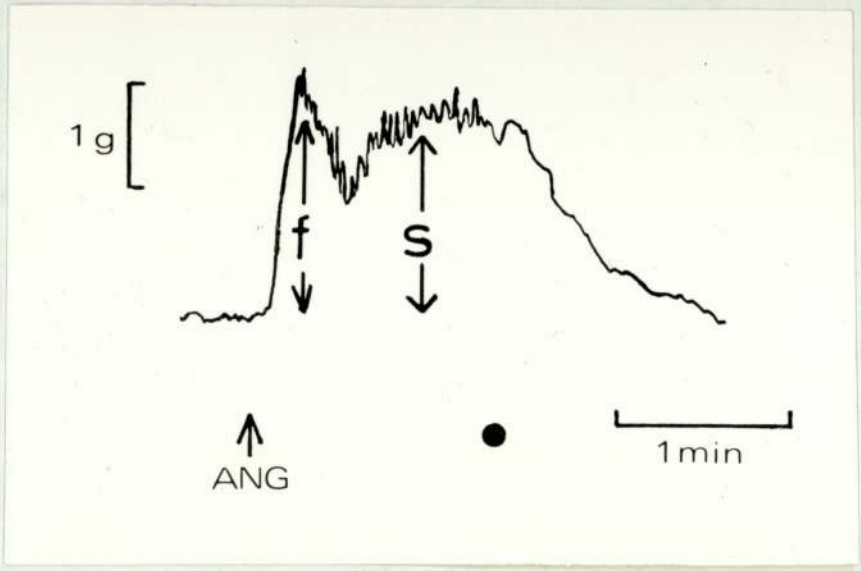

Fig. 8. Guinea-pig ileum preparation. Contractile response to 2 x 10^{-8}M angiotensin, on fast chart speed showing the fast (f) and slow (s) components. Angiotensin added at arrow and removed at closed circle.

plateau, upon which a series of high amplitude rhythmic contractions was superimposed (Fig. 7c).

The magnitude of the slow component of the angiotensin response was found not to be dependent upon the amplitude of the fast component. This is in agreement with the findings of Goldenberg (1967) on the ilea of the mouse, gerbil and rat. However, the relative magnitude of the slow and fast components is a function of the concentration of angiotensin under the experimental conditions used (Fig. 9). The optimum of the fast component attains 80 to 90% of the maximum response to acetylcholine, on the other hand, the slow component reaches only 55 to 75% of this same maximum response.

Tachyphylaxis was not observed when an interval of 8 minutes as suggested by Robertson and Rubin (1962) was allowed between doses. However, it was found that this interval could be reduced to 5 minutes if two or more washes were interspersed between each contraction.

The guinea-pig ileum was equally sensitive to acetylcholine or angiotensin, but of the order of 10 times less sensitive to bradykinin (Table IV). Bradykinin, like angiotensin contracted the ileum but the response was not biphasic.

1.2. Guinea-pig taenia coli

Biphasic responses were not apparent even with concentrations of up to 10^{-7} M (Fig. 10). The tissue relaxed slowly after each exposure to angiotensin, which necessitated an interval

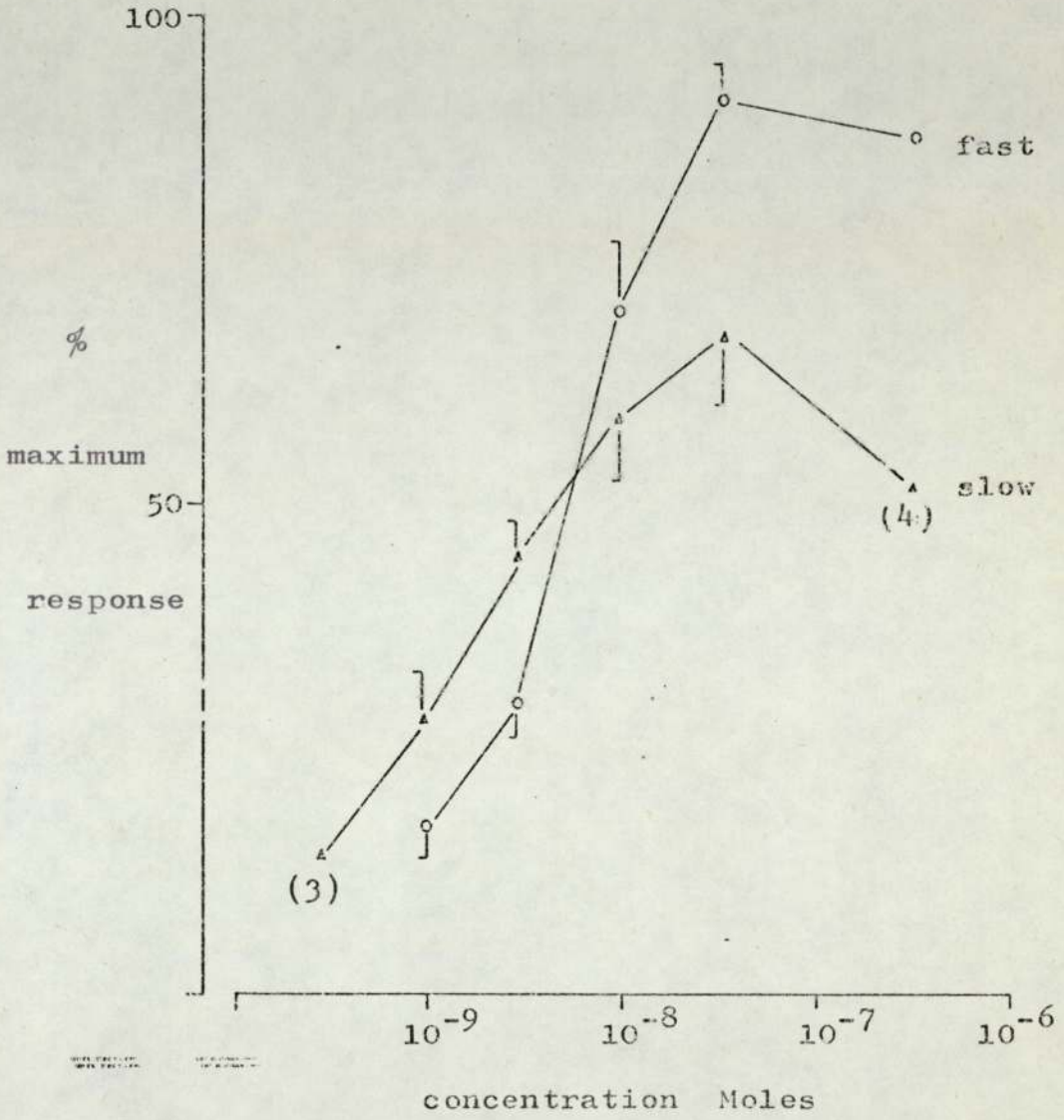

Fig. 9 . Concentration effect curves of angiotensin on isolated guinea-pig ileum. Fast (∘) and slow (▵) component determined as in Fig. 8 . Responses expressed as a percentage of the maximum contraction evoked by acetylcholine. Each point is the mean of 6 experiments unless otherwise indicated in brackets.

of at least 10 minutes between successive applications of the drug. Contractile responses were reproducible and the relationship between dose and response was linear within the range 5×10^{-9} M and 5×10^{-8} M. However, with higher concentrations, responses became variable and no dose-response relationship could be established. Tachyphylaxis was apparent with high concentrations ($>5 \times 10^{-8}$ M), but could be minimized with a 15 minute dose interval (Table V).

Bradykinin (5×10^{-8} M) relaxed the preparation, while high concentrations ($>10^{-7}$ M) often caused an initial relaxation which was followed by a small contraction. As with the higher concentrations of angiotensin, no strict dose-response relationship could be established. Similar results have been reported by Regoli and Vane (1964 a).

1.3. Rat Colon

The rat colon was found to be very sensitive to angiotensin in agreement with the findings of Regoli and Vane (1964 a) who suggested it as a preparation for the assay of angiotensin. The threshold concentration was found to be 10^{-10}M. The form of the response to various concentrations of angiotensin was rather similar to that described earlier for the guinea-pig ileum, except that the initial fast component was not apparent even with a concentration (10^{-7} M) which caused maximum contraction (Fig. 10b). Tachyphylaxis was not observed with a 5 minute dose-interval. However, after a very high concentration (10^{-6} M), the tissue

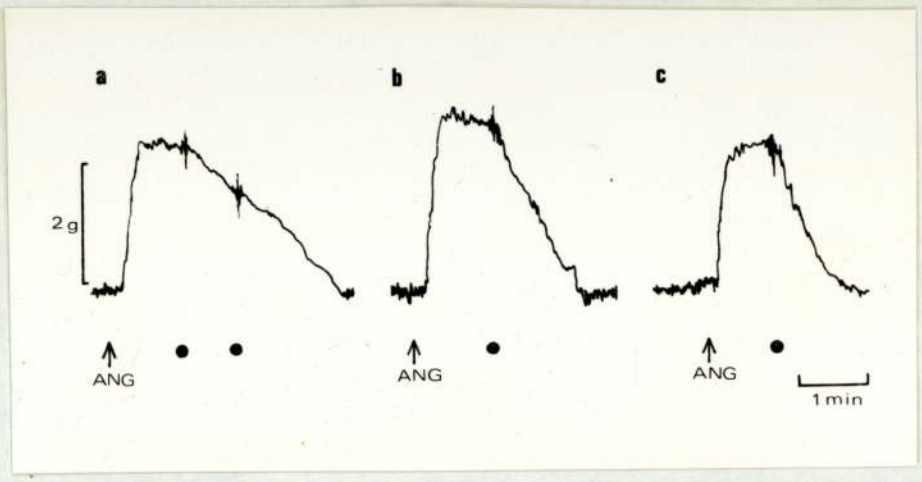

Fig. 10. Isometric contractions of a) guinea-pig taenia coli; b)rat colon and c) rat stomach strip to angiotensin (10^{-7}M)(ANG) added at arrows and removed at closed circles.

became desensitized to angiotensin for a period of 30 to 40 minutes.

Bradykinin was found to cause either a cessation of spontaneous activity or relaxation or both. The relative unresponsiveness of the rat colon to bradykinin has been previously reported by Bisset and Lewis (1962).

1.4. Rat stomach strip

Regoli and Vane (1964a) reported that the sensitivity of the rat stomach strip to angiotensin was the same as that for the guinea-pig ileum. The rat stomach strip contracted to a threshold concentration of 5×10^{-9} M, and maximum contractions were obtained with a concentration of 10^{-7} M. These results are in agreement with those of Regoli and Vane (1964a).

Biphasic contractile response to angiotensin could not be demonstrated within the range of concentrations used in this study (Fig.10c). Tachyphylaxis was not seen with the dose interval used (5 minutes). The latent period was shorter than that for the guinea-pig ileum.

In contrast to angiotensin, bradykinin (10^{-8} to 10^{-7} M) relaxed the preparation.

B) VASCULAR SMOOTH MUSCLE

INTRODUCTION

Vascular smooth muscles of different regions in the body respond to angiotensin with consistent individualities, for example, most arterial vessels show contraction and prominent tachyphylaxis, while renal resistance vessels are non-responsive to angiotensin (Bohr and Uchida, 1967). The effect of angiotensin on veins has scarcely been investigated (vide infra).

Information concerning the action of angiotensin on isolated veins is scanty and often contradictory. Page and Bumpus (1961) and Folkow, Johansson and Mellander (1961) reported that angiotensin was unable to effectively stimulate venous smooth muscle, and suggested that its contribution to a pressor action *in vivo* would be rather insignificant. However, other workers found that angiotensin was active in stimulating preparations of pulmonary, hepatic portal, mesenteric and saphenous veins of the dog (Somlyo and Somlyo, 1966), anterior mesenteric, external jugular and posterior caval veins of the rabbit (Sutter, 1965) and the portal vein of the rat (Bohr and Uchida, 1967; Blair-West, McKenzie and McKinley, 1971; Carruba, Mandelli and Mantegazza, 1973).

Tachyphylaxis to angiotensin is a prominent feature in arterial and venous smooth muscles (Somlyo and Somlyo, 1966; Bohr and Uchida, 1967; Khairallah *et al*, 1966). The mechanism underlying angiotensin tachyphylaxis remains controversial (see Introduction).

In this section, the action of angiotensin and other agonists on the rat portal vein preparation was examined and compared to those on the rabbit aortic strip, a preparation which has long qualified as a test system for angiotensin (Helmer, 1957).

RESULTS

The sensitivity of the rabbit aortic strip, rat portal vein and guinea-pig portal vein to angiotensin, noradrenaline and adrenaline is summarized in Table VI, which also shows the sensitivity of the rat and guinea-pig portal veins to acetylcholine. The threshold concentration of angiotensin required for development of tension by the aortic strip and rat portal vein was 5×10^{-9} M and 5×10^{-11} M respectively. Maximum responses were obtained with a concentration of 10^{-6} M for the aortic strip and of the order of 5×10^{-7} M for the rat portal vein. The guinea-pig portal vein did not respond to angiotensin even with a concentration of up to 10^{-5} M (5 preparations).

1.5. Rabbit Aortic Strip

Following the addition of angiotensin into the bath and a latent period of 15 to 60 seconds, the tension developed progressively, reaching a maximum in 2 to 3 minutes. The preparation did not show diminishing responses to repeated

administration of angiotensin over a period of 6 to 8 hours, provided an interval of 15 to 20 minutes was allowed between successive applications of the drug. This contrasts with the results of Bohr and Uchida (1967) who reported that responses to low concentrations of angiotensin were abolished in strips that had been left in the bath for periods of more than 6 hours.

In the rabbit aortic strip noradrenaline and angiotensin were equiactive (Table VI). However, the maximum contraction induced by angiotensin was always less than that induced by noradrenaline or adrenaline. (Fig. 11). These findings are in agreement with those of other workers (Helmer, 1957, 1964; Khairallah, et al, 1966)

1.6. Rat Portal Vein

The rat portal vein showed regular rhythmic contractions with frequencies ranging from 3 to 12 per minute. Following the addition of angiotensin in concentrations as low as 5×10^{-11}M there was an increase in the frequency of contractions. Increasing concentrations of angiotensin caused further increases in frequency of contractions superimposed on a slow contracture.

Similar observations were made with noradrenaline and acetylcholine. The rates of onset of action and of recovery were much slower for angiotensin than for the other two agonists. (Fig. 12).

Tachyphylaxis was not apparent with adrenaline, noradrenaline or acetylcholine with a 5 minute dose cycle. For angiotensin, an interval of 8 to 10 minutes was necessary to reproduce responses over a 6 hour period. This longer dose interval for angiotensin is probably related to the longer time-course of action of angiotensin.

Dose-effect curves to angiotensin, noradrenaline, adrenaline and acetylcholine are illustrated in Fig. 13 . The maximum contraction obtainable with acetylcholine was less than that for angiotensin or the catecholamines.

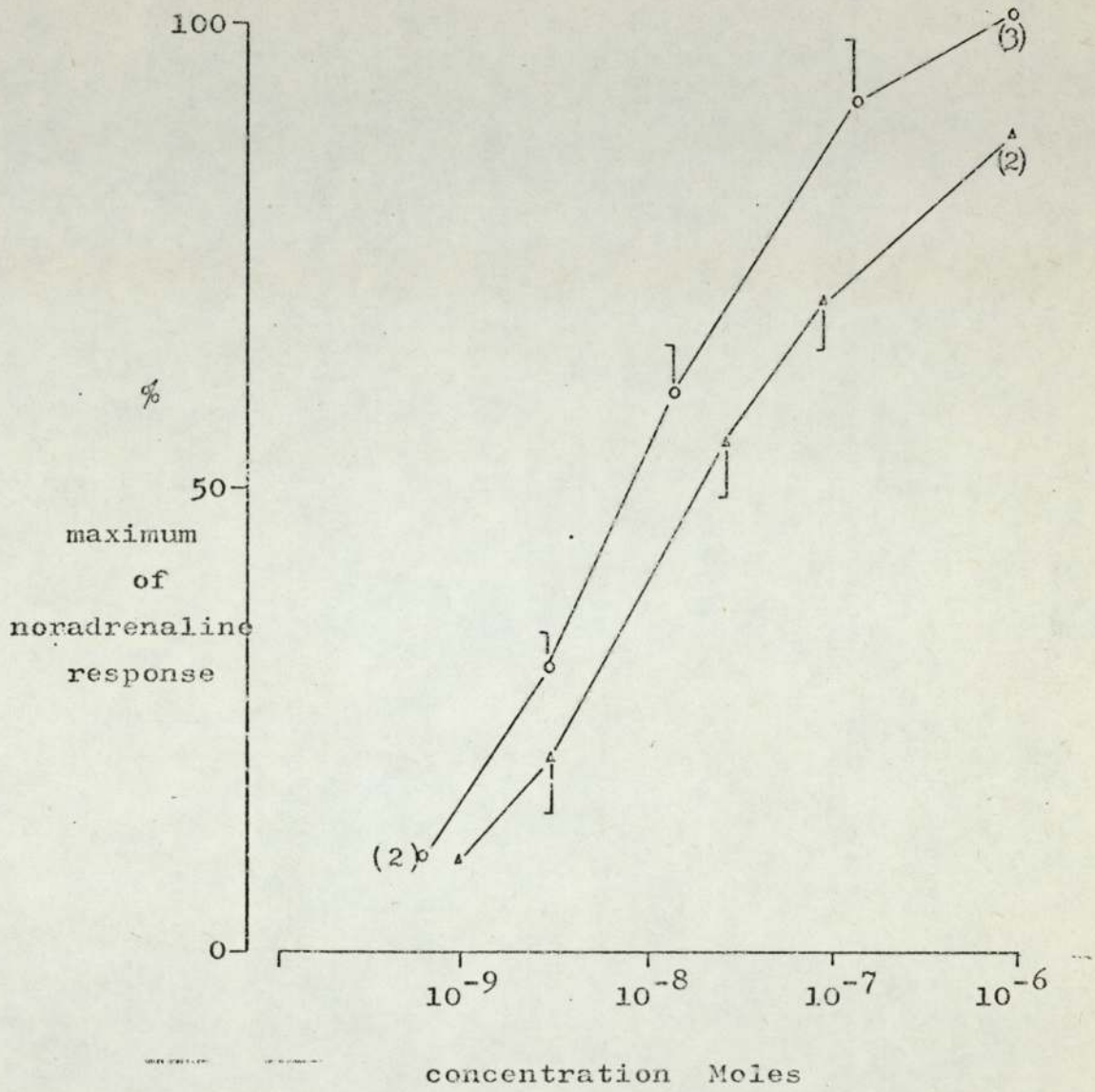

Fig. 11 . Concentration effect curves to noradrenaline (○ — ○) and angiotensin (△ —△) on the rabbit aortic strip. Contractions expressed as % maximum of noradrenaline response. Each point is the mean of 5 experiments unless otherwise indicated in brackets.

Agonists	Preparations			Source
	Rabbit aortic strip	Rat portal vein	Guinea-pig portal vein	
Angiotensin	$5x10^{-9}$	$5x10^{-11}$	10^{-4}	*
	10^{-9}	10^{-11}	-	Bohr & Uchida, 67.
	-	10^{-10}	10^{-5}	Blair-West et al, 71
Noradrenaline	$2.6x10^{-9}$	$5x10^{-9}$	$2.6x10^{-8}$	*
	10^{-9}	-	-	Bohr & Uchida, 67.
	-	10^{-9}	10^{-8}	Blair-West et al, 71
Adrenaline	$5x10^{-9}$	$5x10^{-9}$	10^{-8}	*
Acetylcholine	-	$4x10^{-8}$	$3.6x10^{-6}$	*
	-	10^{-9}	10^{-7}	Blair-West et al, 71.
	-	10^{-8}	10^{-6}	Carruba et al, 73.

Table VI. Sensitivity of various isolated vascular smooth muscles to angiotensin and other agonists. Threshold concentrations expressed in Moles.
*values obtained in this study.

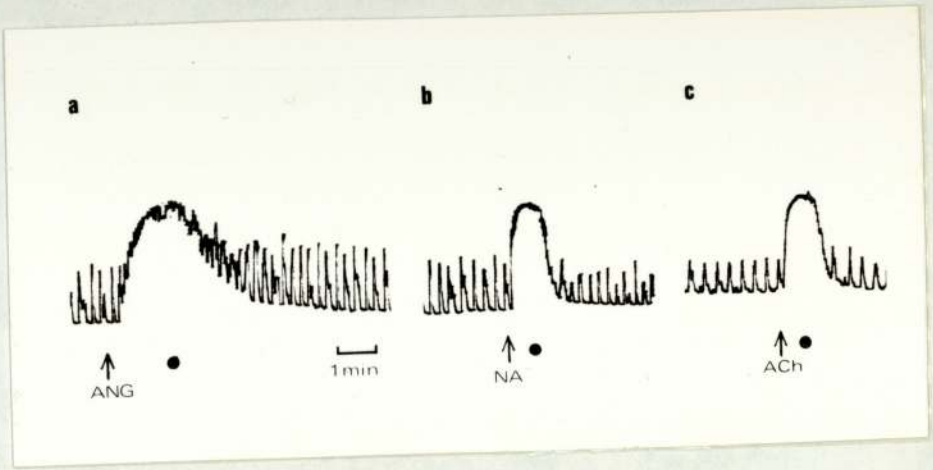

Fig. 12. Isotonic contractions of rat portal vein to a) angiotensin (5×10^{-9}M)(ANG); b) noradrenaline (10^{-8}M)(NA) and c) acetylcholine (5×10^{-6}M)(ACh). Drugs were added at arrows and removed at closed circles.

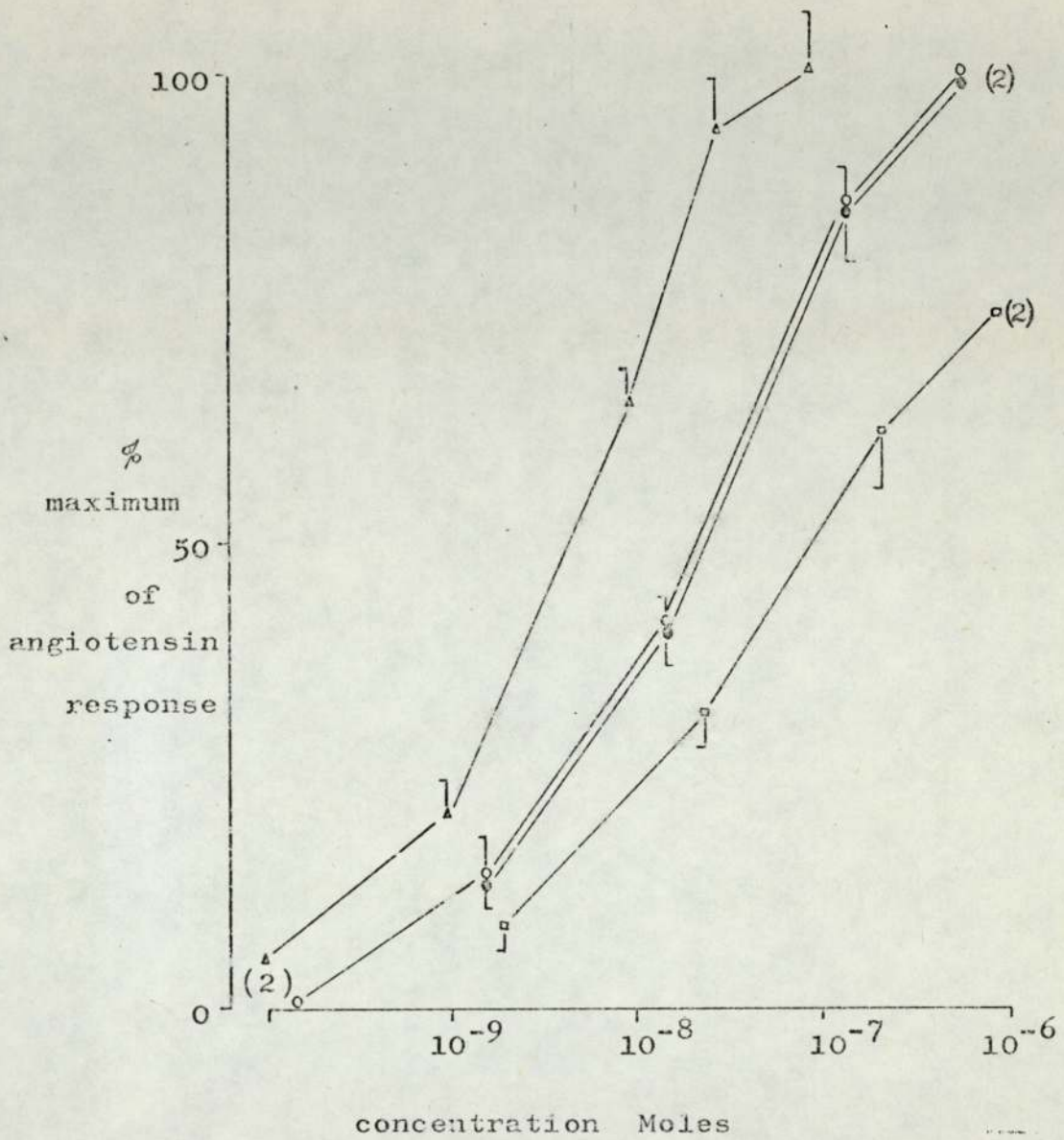

Fig. 13. Concentration effect curves to angiotensin (▵ — ▵), noradrenaline (○ — ○), adrenaline (• — •) and acetylcholine (□ — □) on the isolated rat portal vein. Contractions expressed as % maximum of angiotensin response. Each point is the mean of 5 experiments unless otherwise indicated in brackets.

DISCUSSION

The results presented in the first part of this chapter confirm the results of other workers by demonstrating the presence of biphasic response of the guinea-pig ileum and rat ileum to angiotensin, and further demonstrated similar biphasic responses in the rabbit ileum. However, in the ilea of the rat and rabbit biphasic responses could be demonstrated only with high concentrations of angiotensin. The sensitivity of the various intestinal smooth muscle preparations to angiotensin is in general agreement with the findings of Regoli and Vane (1964 a).

The nanopeptide, bradykinin has been shown to produce slow contractions of many intestinal smooth muscle preparations, including the guinea-pig and rabbit ileum (Rocha E Silva, 1970). The action of bradykinin on intestinal smooth muscle was therefore compared with that of the octapeptide angiotensin. Bradykinin was found to be relatively inactive on the rat colon and rat ileum, about 10 to 20 times less active on the guinea-pig ileum and caused relaxation of the rat stomach strip and guinea-pig taenia coli. Therefore, apart from its slow time course of contraction in the guinea-pig ileum, the action of bradykinin is quite different from that of angiotensin. Angiotensin invariably contracts isolated intestinal smooth muscle preparations of most species and of different regions in the same species. These results indicate that the mode of action of the two peptides on intestinal smooth muscle are probably different.

The analysis of the concentration-effect curve for angiotensin on the guinea-pig ileum shows that the maximum response produced by the peptide is always lower than that produced by acetylcholine. The slow component is a curve with an optimum that is characteristic of a partial agonist. The concentration of angiotensin that evokes the fast component is higher than 5×10^{-9} M; lower concentrations cause only a slow response. These results indicate that responses to higher concentrations (above 5×10^{-9}M) of angiotensin are more complex and probably involve separate mechanisms.

Most isolated or denervated vascular smooth muscle with the exception of the aorta are very insensitive to angiotensin (Zimmerman, 1962; Laverty, 1963; dela Lande & Rand, 1965; McGregor, 1965 & Somlyo et al, 1965). The rabbit aortic strip contracts to low concentrations of angiotensin and has been used for the assay of angiotensin (Helmer, 1957, 1964). Recently, it has been shown (Bohr & Uchida, 1967; Blair-West et al, 1971) that the rat portal vein preparation is very sensitive to angiotensin. The results presented in this chapter are in agreement regarding the sensitivity and overall time-course of contraction to angiotensin in the rabbit aortic strip and rat portal vein preparation as reported by various workers.

Dose-response curves of angiotensin and noradrenaline on the rat portal vein show that maximal responses to both agonists were approximately equal. However, in the rabbit aortic strip,

maximal responses to angiotensin were lower than to noradrenaline. The mode of action of angiotensin on these two preparations would therefore appear to be different. It is noteworthy that the action of angiotensin on the rat portal vein is greater than that of other drugs considered to be active on venous smooth muscle, i.e. noradrenaline, adrenaline and acetylcholine.

Tachyphylaxis to angiotensin has been reported in various isolated smooth muscle preparations (see Introduction). The observation that tachyphylaxis to angiotensin in the smooth muscle preparations described in this chapter could be minimized by lengthening the dose-interval suggests that tachyphylaxis could be due to a depletion of secondary transmitter. A longer interval between stimulation would presumably allow the replenishment of transmitter store and restore contractile responses to angiotensin. On the other hand, the phenomenon could be due to the slow dissociation of angiotensin or substance released by angiotensin from receptor sites in view of the observation that tachyphylaxis could be minimised by repeated washing. The long latent period required for onset of contraction, the relatively long time required for the contraction to reach maximum and the biphasic responses observed in some intestinal smooth muscle preparations are indications that the action of angiotensin may be partly indirect and perhaps due to the release of a secondary transmitter or transmitters. Possible exceptions to this hypothesis are the action of the peptide on the rat colon and rat portal vein, where tachyphylaxis was apparently absent and the latent period similar to that of other agonists, i.e. acetycholine and noradrenaline.

CHAPTER II THE ACTION OF ANGIOTENSIN ON ISOLATED INTESTINAL SMOOTH MUSCLE PREPARATIONS IN THE PRESENCE OF SPECIFIC ANTAGONISTS

The possibility of an indirect component in the contractile response of some intestinal smooth muscle preparations to angiotensin was indicated in the preceding chapter. Studies on isolated intestinal smooth muscle preparations by various authors indicate that angiotensin has both direct and nerve-mediated indirect actions (see Introduction). However, in the rat colon (Regoli and Vane, 1964a) and human colon (Fishlock and Gunn, 1970) no indirect action can be demonstrated. Studies on the isolated guinea-pig ileum suggest a cholinergic mechanism prevails in the indirect nerve-mediated action (Khairallah and Page, 1961; Robertson and Rubin, 1962).

While it is generally agreed that in certain species angiotensin can stimulate intestinal nervous elements to release acetycholine, the precise site of angiotensin action on the nerves remains unknown. Actions on the preganglionic nerve endings and on the postganglionic nerve endings have separately been proposed (vide infra). Ross et al (1960) found that the action of angiotensin on the guinea-pig ileum could be blocked by atropine and postulated that angiotensin acted on the post-ganglionic cholinergic fibres. Khairallah and Page (1961, 1963) studied a series of ganglion blocking agents on the guinea-pig ileum and found that angiotensin contraction could be inhibited by nicotine and tetra-methyl-ammonium (TMA) but not by hexame-thonium. These authors concluded that angiotensin was acting

mainly by stimulating the ganglion cells in the myenteric plexus of Auerbach.

An action of angiotensin on postganglionic nerve endings in guinea-pig ileum has been substantiated by Panisset (1967) who showed that a subthreshold concentration of angiotensin enhanced the release of acetylcholine from a co-axially stimulated preparation. He also showed that the same concentration of angiotensin increased the output of acetylcholine from the preganglionic nerve endings of the perfused cat superior cervical ganglion.

A unifying view of the action of angiotensin on the nervous elements has been proposed by Blair-West and McKenzie (1966). These authors showed that atropine, hemicholinium and tetrodotoxin, each depressed the response of the guinea-pig ileum to angiotensin by approximately the same extent. They suggested that angiotensin has a stimulant action on intramural excitatory ganglion cells together with an action on post-ganglionic nerve terminals. Similar conclusions were reached by Beleslin (1968) who found that blockade of the peristaltic reflex in the guinea-pig ileum whether by ganglion blocking agents, morphine, adrenaline or atropine could be restored by angiotensin. From these resultsm Beleslin suggested that angiotensin might stimulate 3 different sites on the intramural nervous network, viz: preganglionic nerve endings, ganglion cells and post-ganglionic nerve endings.

For preparations in which biphasic responses to angiotensin can be demonstrated there is evidence to suggest that cholinergic blocking agents (e.g. atropine) and agents which impair

neuronal function (e.g. procaine) can selectively inhibit the fast component of angiotensin induced contractions of the ileum of the guinea-pig (Godfraind et al 1966a) mouse and gerbil (Goldenberg, 1967).

The long latency of action of angiotensin, which is fully described in previous chapter suggests that it may be having an indirect action on the smooth muscle preparations and may not be acting at the smooth muscle cell membrane by simple depolarization. The following series of experiments were undertaken in order to determine whether an indirect, nerve-mediated action of angiotensin is demonstrable in tissues which do not respond by a biphasic contraction.

The muscarinic receptor blocking agent used in these experiments was hyoscine and not atropine as has been used by previous workers (Khairallah and Page, 1961; Blair-West and McKenzie, 1966), since Paton and Rosales (see Day and Vane, 1962) have shown that hyoscine is a more selective blocker of muscarinic receptors than atropine.

RESULTS

The effect of selective antagonists on the contractile responses to angiotensin and acetylcholine on the guinea-pig, rat and rabbit ilea is shown in Table VII. None of the antagonists used had any inhibitory effect on contractions induced by angiotensin on the rat colon, rat stomach strip and taenia coli. A detailed description of the selective antagonists used on the responses to angiotensin is given below.

2.1. Muscarinic receptor blocking agent

Hyoscine in concentrations (10^{-8} - 10^{-6}M) which abolished submaximal contractions to acetylcholine had no effect on the angiotensin induced contractions of the colon, ileum on stomach strip of the rat or the taenia coli of the guinea-pig. This same concentration of hyoscine also abolished the fast component of the angiotensin response on the rabbit and guinea-pig ileum (Fig. 14) but did not cause any significant reduction of the slow component (see Table VII). These results are in agreement with those of Godfraind et al (1966a) and Goldenberg (1967) in their studies using atropine on the guinea-pig ileum and rat ileum respectively.

2.2. Ganglion blocking agents

Hexamethonium at 10^{-5}M and pempidine at 10^{-4}M were without effect on the angiotensin responses in all the intestinal preparations examined (Table VII). The same concentration of hexamethonium however effectively abolished the responses to nicotine and transmural stimulation in the guinea-pig ileum.

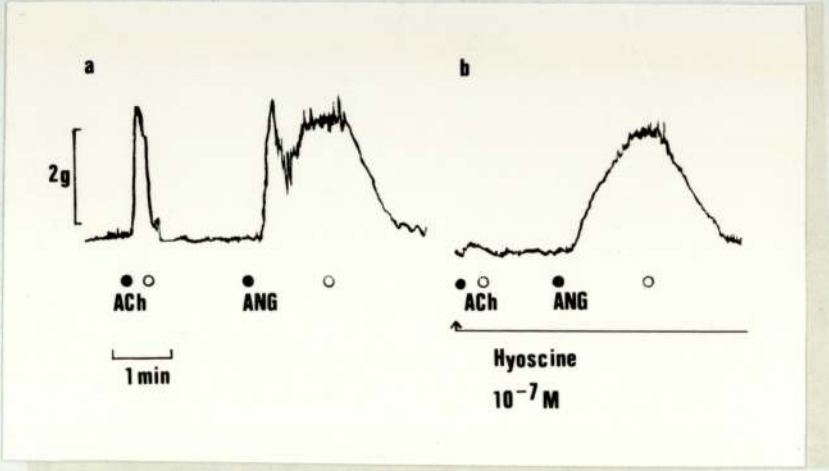

Fig. 14. Isometric contractions of guinea-pig ileum to acetylcholine (10^{-7}M)(ACh) and angiotensin (10^{-8}M)(ANG). (a) = control responses (b) = responses in the presence of hyoscine (10^{-7}M). Drugs added at closed circles and removed at open circles.

Blocking agents	% mean depression of control								
	Guinea-pig ileum			Rat ileum			Rabbit ileum		
	ANG		ACh	ANG		ACh	ANG		ACh
	fast	slow		fast	slow		fast	slow	
Hyoscine 10^{-7}M	100*	12±1	100* (n=10)	6±2	4±1 (n=5)	100*	97±2*	10±3 (n=6)	100*
Hexamethonium 10^{-5}M	3±1	4±1	11±3 (n=6)	7±1	2±0 (n=4)	8±1	6±2	5±1 (n=4)	12±1
Tetrodotoxin 10^{-7}M	100*	46±2	5±1 (n=10)	100*	37±2* (n=5)	6±2	100*	41±3* (n=5)	10±1
Procaine 10^{-5}M	100*	38±1*	8±2 (n=4)	100*	28±1* (n=4)	10±1	100*	39±3* (n=4)	11±2

Table VII. Effect of pharmacological blocking agents on submaximal contractions of the guinea-pig, rat and rabbit ilea to angiotensin (ANG) and acetylcholine (ACh)

* denotes significant reduction

($P < 0.05$).

2.3. Agents which impair nerve function

Tetrodotoxin at 10^{-7}M , a concentration known to cause a functional denervation of smooth muscle (Gershon, 1967) abolished the responses of the guinea-pig ileum to nicotine and transmural stimulation. The same concentration of tetrodotoxin abolished the fast component and reduced the slow component (by 35 - 51% (mean 46.1 ± 2.7%, n = 5)) of the angiotensin response in the ileum of the guinea-pig (Fig.15), and caused a similar reduction (see Table VII) of the angiotensin responses of the ilea from the rat and rabbit. Procaine (10^{-5}M) also abolished the fast component and reduced the slow component of the angiotensin response in these preparations although the reduction of the slow component was not as great as with tetrodotoxin.

The respones of the other intestinal preparations; rat stomach strip, rat colon and guinea-pig taenia coli were not affected by tetrodotoxin or procaine.

2.4. Histamine and 5-hydroxytryptamine receptor blocking agents

Mepyramine (10^{-6}M) and methysergide (10^{-5}M) which are selective antagonists to histamine and 5-hydroxytryptamine respectively had no effect on the contractile responses to angiotensin in all of the intestinal smooth muscle preparations examined. These results are in agreement with the findings of Blair-West et al (1967) on the guinea-pig ileum.

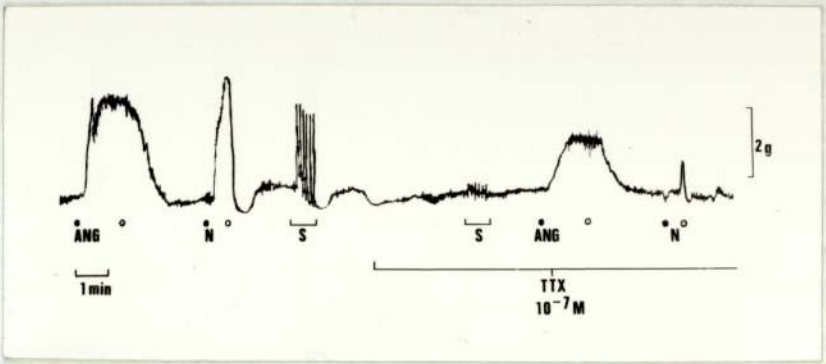

Fig. 15. Isometric contractions of guinea-pig ileum to angiotensin (10^{-8}M)(ANG); nicotine (2 x 10^{-7}M)(N) & transmural stimulation (0.5 Hz)(S).
(a) = control responses (b) = responses in the presence of tetrodotoxin (10^{-7}M). Drugs added at closed circles and removed at open circles.

DISCUSSION

The action of angiotensin on isolated intestinal smooth muscle preparations of the guinea-pig, rat and rabbit was analyzed using known pharmacological blocking agents. Both direct and nerve-mediated indirect actions of angiotensin were demonstrated in the ileal preparations of the guinea-pig, rat and rabbit, while only direct action could be demonstrated in the guinea-pig taenia coli, rat and colon and rat stomach strip. The results presented in this chapter emphasize the diversity of angiotensin action in intestinal smooth muscle.

Evidence to substantiate that the fast component of the angiotensin response in the ileum of the guinea-pig, rat and rabbit was mediated via the stimulation of intramural nervous elements was achieved by nerve blocking agents such as procaine and tetrodotoxin. Tetrodotoxin (10^{-7}M) which is known to cause functional denervation by blockade of propagating action potentials in nerve (Kao, 1966 and Gershon, 1967) and procaine (10^{-5}M) which is known to block nervous conduction (see Weatherall, 1968), abolished the fast component of the angiotensin response in these smooth muscle preparations. The concentration of tetrodotoxin used has been shown to have no inhibitory effect on smooth muscle of the guinea-pig ileum (Gura, Mori and Watanabe, 1966).

The results obtained with the muscarinic receptor blocking agent, hyoscine, suggest that the fast component of the angiotensin response on the guinea-pig and rabbit ileum but not

the rat ileum is mediated via a cholinergic pathway. These results are in agreement with those of other workers cited.

In an attempt to determine whether angiotensin has an action on the ganglion cells or presynaptic nerve endings, the ganglion blocking agents hexamethonium and pempidine were used. These agents had no effect on the fast or the slow component of the angiotensin contraction in the preparations examined, but the same concentration of these agents effectively abolished the responses to transmural electrical stimulation. Khairallah and Page (1961) using guinea-pig ileum reported that angiotensin responses were reduced after ganglion blockade with mecamylamine or with nicotine. Goldenberg (1967) reported similar effect with mecamylamine in the ileum of the rat, mouse and gerbil. Inhibition of angiotensin contractions by mecamylamine, however, does not indicate angiotensin has a ganglion stimulant action in intestinal smooth muscle, since there is evidence that part of mecamylamine's blocking action on autonomic ganglia is due to inhibition of the mechanism for transmitter release (see Bowman, Rand and West, 1968). Furthermore, Blair-West and McKenzie (1966) have shown that mecamylamine did not reduce angiotensin responses in the guinea-pig ileum and similar findings have been extended to the ileum of the rat and rabbit in this study, by using another ganglion blocking agent, pempidine. These results suggest that angiotensin does not stimulate preganglionic nerve endings to release acetylcholine, and that its depolarizing action on autonomic ganglia (Haefely, 1972) may involve receptor sites other than those stimulated by nicotine.

This conclusion is in agreement with the general concensus that receptors for angiotensin on the ganglion are different from those activated by acetylcholine (Feldberg and Lewis, 1964; Lewis and Reit, 1965). It has also been postulated that the ionic mechanisms underlying the ganglionic actions of angiotensin are different from those involved in nicotinic stimulation (Haefely, 1972).

In the present study, the exact site of action of angiotensin on the intrinsic motor nerve supply of the intestine could not be determined. However, the results presented here together with those of other workers, strongly indicate that angiotensin has two sites of action on the intramural nervous network, at the ganglion and at the postganglionic nerve endings.

The action of angiotensin on the rat colon, rat stomach strip and guinea-pig taenia coli, does not seem to involve a cholinergic nervous component, as tetrodotoxin and hyoscine had no effect on the contractile response. The possible involvement of other "local hormones" such as histamine and 5-hydroxytryptamine seems unlikely, since antagonists to these agents were without effect on the contractile response. A direct action only for angiotensin on the rat colon has been suggested by Regoli and Vane (1964 a).

The slow component of the angiotensin contractions of the ileum of the guinea-pig, rat and rabbit were not affected by selective antagonists of histamine or 5-hydroxytryptamine,

thus confirming the report of Walaszek, Huggins and Smith (1963). However, the slow component was significantly reduced in the presence of tetrodotoxin but not hyoscine. This finding is at variance with the report of Blair-West and McKenzie (1966) that hyoscine and tetrodotoxin each reduced the angiotensin response on the guinea-pig ileum by about the same extent. These authors, however, did not differentiate between the fast and the slow component. The observation that tetrodotoxin but not hyoscine reduced the slow component by about 46% suggests that part of the slow component is nerve mediated, releasing transmitter or neurohumors unaffected by blocking agents or that the ionic currents mediating the slow component are susceptible to tetrodotoxin action.

The main points which arise from this study may be summarized as follows :

1) The mode of angiotensin action varies between smooth muscles from different species and even from different muscles of the same species.

2) An indirect nerve-mediated component of the angiotensin contractile response is not a uniform finding in intestinal smooth muscle, and some seemingly indirect actions of angiotensin do not appear to rely on the release of acetylcholine.

3) The direct action of angiotensin appears to involve specific receptor sites which are different from those of acetylcholine, histamine, 5-hydroxytryptamine and bradykinin.

4) These results argue against the use of generalization in describing the mode of action of angiotensin on smooth muscle even for muscles of the same type (i.e. intestinal muscle) from different species.

CHAPTER III THE ACTION OF ANGIOTENSIN ON ISOLATED VASCULAR SMOOTH MUSCLE PREPARATIONS IN THE PRESENCE OF SPECIFIC ANTAGONISTS

The mode of action of angiotensin on vascular smooth muscle remains poorly understood despite extensive investigation on isolated vascular preparations and on whole animal studies including man. Angiotensin is one of the most potent pressor substances known and its effect on systemic blood pressure is believed to be due both to a direct action on vascular smooth muscle and to indirect mechanisms (Zimmerman, 1962; Feldberg and Lewis, 1964, 1965; Lewis and Reit, 1966) including actions on the central nervous system (Halliday and Buckley, 1962; Scroop and Whelan, 1966; Scroop and Lowe, 1969 and Deuben and Buckley, 1970).

In the anaesthetised cat, the pressor action of angiotensin injected intra-arterially is largely due to the release of endogenous adrenomedullary amines (Lewis and Reit, 1966; Feldberg and Lewis, 1964, 1965). The strong stimulant action of angiotensin on the adrenal medulla led Lewis to propose the possibility that the vasoconstriction caused by intravenous injection of angiotensin might be mediated via liberation of noradrenaline from the sympathetic nerve endings at the vascular wall. Since then, both direct and indirect actions of angiotensin have been reported in several vascular preparations. Liebau *et al* (1965) showed that the catecholamine content of aortic strips from rats was significantly reduced after incubation with

angiotensin, furthermore, angiotensin tachyphylaxis in these strips could be restored by incubating with noradrenaline. These authors interpreted these findings as indicating that the action of angiotensin on vascular smooth muscle was mainly indirect, mediated by a liberation of noradrenaline. A noradrenaline mediated, indirect action has since been demonstrated in several vascular smooth muscle preparations and in the whole animal (see Introduction). Furthermore, angiotensin has been shown to accelerate catecholamine biosynthesis in sympathetically innervated tissues such as rat and guinea-pig atria and rat vas deferens (Boadle, Hughes and Roth, 1969).

However, a direct action of angiotensin on vascular smooth muscle is favoured by some workers. Khairallah et al (1966) demonstrated that the tachyphylaxis of arterial strips of cat, dog, sheep and rat could be abolished following incubation with a plasma fraction rich in angiotensinase A. Bohr and Uchida (1967) reported a similar reversal of tachyphylaxis when vascular strips were incubated with kidney extract. These authors suggested that tachyphylaxis is caused by saturation of the receptor sites with angiotensin which inhibits further stimulation. A direct action of angiotensin on helical arterial strips of canine coronary, renal and carotid arteries and on strips of the thoracic aorta from guinea-pigs and rats has also been proposed by Walter and Bassenge (1969) who demonstrated that following noradrenaline incubation, tachyphylaxis could not be abolished or reduced, and the action of angiotensin could not be modified after blockade of adrenergic alpha-receptors. Similar conclusions

were arrived at by Blair-West et al (1968) in their study of angiotensin action on the rabbit ear artery.

The rabbit aortic strip, because of its sensitivity and lack of tachyphylaxis to angiotensin, has been extensively used for the study of angiotensin action (Helmer, 1957, 1964; Khairallah et al, 1966). Both direct and indirect actions have been reported by Suzuki and Matsumoto (1966) while a direct action of angiotensin on this preparation is favoured by other workers (Khairallah et al, 1966; Rioux et al, 1973). In view of the complexity of angiotensin action on arterial smooth muscle, the mode of action of angiotensin was further examined on the rat portal vein preparation and rabbit aortic strip, in order to determine whether the constrictor action of angiotensin is mediated by liberation of noradrenaline from sympathetic nerve endings or if the constrictor action is caused by a direct action on the vascular smooth muscle itself.

RESULTS

The effects of various specific antagonists on the constrictor response of angiotensin on the rabbit aortic strip and rat portal vein are as follows:

3.1. α - and β - adrenoceptor blocking agents

Phentolamine, an α - receptor blocking agent at a concentration of 10^{-7} - 10^{-6}M did not modify the contraction of angiotensin on the rabbit aortic strip (4 preparations) or the rat portal vein (6 preparations). The same concentrations of phentolamine had no effect on acetylcholine responses on the rat portal vein, but abolished the responses to noradrenaline and adrenaline on both preparations. These results indicate that the stimulant action of angiotensin and acetylcholine is not effected via interaction with α - receptors.

The β - adrenoceptor blocking agent, propranolol at 10^{-6}M had no antagonizing effect on the contractile response to angiotensin and noradrenaline on the preparations examined. The response to acetylcholine on the rat portal vein was similarly unaffected by the same concentration of propranolol. β - adrenoceptor activation by these agonists therefore seems unlikely.

3.2. Adrenergic neurone blocking agents

Guanethidine (10^{-6} - 10^{-4}M) had no effect on the responses of the rabbit aortic strip to angiotensin or noradrenaline (4 pre-

parations). The same concentration of guanethidine did not affect the responses to angiotensin, noradrenaline or acetylcholine on the rat portal vein (3 preparations). Similar results have been described by Blair-West et al (1971) on the rat portal vein. Guanethidine is known to interfere with the release of noradrenaline from adrenergic nerve junctions with vascular smooth muscle (Goodman and Gilman, 1970). The lack of effect of guanethidine here suggests that an indirect noradrenaline mediated action of these agonists is unlikely.

3.3. Agents which impair nerve conduction

Tetrodotoxin (10^{-7}g/ml) did not affect the resting tension of the rabbit aortic strip or the rat portal vein. Responses to angiotensin or the catecholamines on the rabbit aortic strip were not affected by tetrodotoxin, nor were the responses to angiotensin, noradrenaline and acetylcholine on the rat portal vein. However, tetrodotoxin (10^{-6}g/ml) did reduce the amplitude of the rhythmic spontaneous contractions of the rat portal vein by about 20% (3 preparations). (Fig. 16).

3.4. Specific antagonists to histamine and 5-hydroxytryptamine

Concentrations of mepyramine (10^{-7}M) and methysergide (10^{-5}M) which blocked the responses to histamine and 5-hydroxytryptamine respectively did not modify the responses to angiotensin on the preparations examined. These results indicate that the myotropic action of angiotensin on the rat portal vein and rabbit aortic strip is not effected by the stimulation of hista-

minergic or 5-hydroxytryptaminergic receptors which are present in these preparations (Blair-West et al, 1971; Rioux et al, 1973).

3.5. Muscarinic receptor blocking agent on the rat portal vein

Acetylcholine has been shown to be a potent stimulant on the rat portal vein (see Chapter II). Hyoscine (10^{-6}M) abolished submaximal responses to acetylcholine but was without effect on the responses to angiotensin.

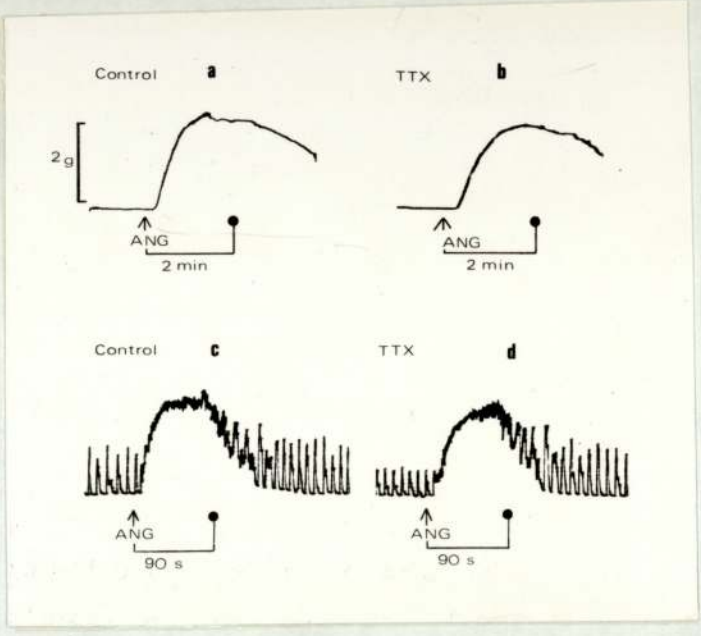

Fig. 16. Isometric contractions of rabbit aortic strip (a and b) and isotonic contractions of rat portal vein (c and d) induced by angiotensin (5×10^{-8} M)(ANG) before (a and c) and after 10 minutes exposure (b and d) to tetrodotoxin (10^{-6} M)(TTX). Angiotensin added at arrows and washed out at .

DISCUSSION

The mechanism of action of angiotensin on the rabbit aortic strip and the rat portal vein has been examined by using selective pharmacological antagonists. According to Lewis& Reit (1966) and Liebau et al (1965) the vasoconstrictor effect of angiotensin is mediated via a release of noradrenaline from sympathetic nerve endings. Such an action of angiotensin has since been demonstrated in several sympathetically innervated vascular preparations by various workers. Thus, Suzuki and Matsumoto (1966) found that α- adrenoceptor blocking agents such as yohimbine, dibenamine and dihydroergotamine were capable of reducing the contractile responses of the rabbit aortic strip to angiotensin by 20 - 30% and that in vitro reserpinization of the strip which abolished the contraction due to tyramine also depressed the contraction due to angiotensin by 20 - 30%.

The results presented in this chapter however do not support the hypothesis of Liebau et al (1965). If the action of angiotensin is by way of liberating noradrenaline, it should be possible to block or reduce the effect of angiotensin by adrenoceptor blocking agents. The contractile response of angiotensin on the rabbit aortic strip and the rat portal vein was not affected by the blockade of α or β - adrenoceptor with phentolamine or propranolol respectively. These results are in agreement with the observations of Walter and Bassenge (1969) on the aortic strips of rats, guinea-pigs and dogs and of Blair-West et al (1971) on the rat portal vein preparation.

Recently, Rioux et al (1973) have similarly reported the lack of effect of phentolamine on angiotensin induced contractions of rabbit aortic strips.

Contrary to the report of Suzuki and Matsumoto (1966) that bretylium, an adrenergic neurone blocking agent, caused a reduction of angiotensin contraction in the rabbit aortic strip, agents which impair nerve function like tetrodotoxin, and other adrenergic neurone blockers, such as guanethidine, were without effect on the angiotensin contraction on the rabbit aortic strip and rat portal vein preparation. These results indicate angiotensin does not have an action along the postganglionic sympathetic nerve fibre and further support the proposition that angiotensin action is not mediated via a release of noradrenaline. Blair-West et al (1971) arrived at a similar conclusion after examining the effect of guanethidine on the rat portal vein and Rioux et al (1973) also demonstrated that high concentration of cocaine or tetrodotoxin had no effect on the stimulant action of angiotensin on the rabbit aortic strip. Turker and Karahneyinoglu (1968) were also unable to detect any difference in the contractile response to angiotensin in the presence of cocaine in rabbit aortic strips taken from normal and reserpinised animals.

The specificity of angiotensin receptor in the rabbit aorta was further demonstrated by the lack of effect of methysergide and mepyramine at concentrations that completely abolished the vasoconstrictor effect of 5-hydroxytryptamine and histamine

respectively. This adds support to the suggestion that angiotensin action on the rabbit aortic strip is a direct one.

In the previous chapter, acetylcholine was shown to contract the rat portal vein preparation. However, possible cholinergic mediation of the responses to angiotensin was excluded by the failure of hyoscine and tetrodotoxin to depress them. Similar results have been reported by Blair-West et al (1971) and Carubba et al (1973) using atropine. The possibility that angiotensin might cause a local release of 5-hydroxytryptamine and histamine seems unlikely, since antagonists to these agents were without effects on the responses to angiotensin. The presence of intrinsic autonomic ganglia accessible to exogenous pharmacological agents also seems unlikely, since Blair-West et al (1971) have demonstrated that the ganglion blocking agent pempidine did not reduce the response to angiotensin and the ganglion stimulant [illegible], DMPP had no effect on the preparation.

The results presented in this chapter together with those of other workers cited, strongly suggest that the action of angiotensin on the rabbit aortic strip and rat portal vein is not mediated via the release of noradrenaline from sympathetic nerve endings in the vascular wall. Furthermore, the myotropic action of angiotensin appears to involve receptor sites which are different from those of acetylcholine, histamine and 5-hydroxytryptamine. However, that angiotensin can release some yet unknown local vasoconstrictors in the rabbit aortic strip remains feasible, in view of its relatively long latent period compared to noradrenaline.

PART TWO

AN ELECTROPHYSIOLOGICAL STUDY ON THE MODE OF ACTION OF ANGIOTENSIN ON SMOOTH MUSCLE

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INTRODUCTION

It is known that activation of the contractile mechanism by electrical events of the cell membrane is not the only physiological way of activation in smooth muscle (see Introduction). In recent years, experimental evidence has accumulated which indicates that vasoactive substances may evoke contractions of vascular smooth muscle which are not strictly connected with changes of the membrane potential (Somlyo and Somlyo, 1968; Johansson, 1971; Peiper, Griebel and Wende, 1971).

The action of angiotensin on the electrical activity of smooth muscle, such as depolarization and spike generation has not been extensively studied. Recently, several actions of angiotensin on membrane activity have been reported (Keatinge, 1966; Orlov and Plekhanov, 1968; Somlyo and Somlyo, 1966, 1968, 1970). However, very few of the observations of different workers are in agreement with one another and the types of smooth muscle studied have been mainly confined to vascular tissue.

Angiotensin contracts the rabbit aortic strip, but the contractile response is not accompanied by membrane depolarization (Shibata and Briggs, 1966). Orlov and Plekhanov (1968) reported similar results on the rabbit aortic strips and carotid artery.

These authors concluded that angiotensin is capable of freeing calcium ions from an intracellular site without changing the permeability of the cell membrane.

Conflicting results were obtained by Keatinge (1966) on sheep and dog carotid arteries. Angiotensin, acetylcholine, noradrenaline and bradykinin caused depolarization and simultaneous contraction of this tissue. Contractions could be maintained with each of these agents, despite the fact that repolarization of the membrane had started. During exposure to high potassium medium, the tissue could still be contracted by the four agents, without any detectable electrical changes. Keatinge therefore concluded that the myotropic effect of angiotensin and other agonists is, at least in part, independent of changes of membrane potential.

Cuthbert and Sutter (1965) reported that the action potential discharge, induced by angiotensin, of the rabbit mesenteric veins, occurs in parallel with the contractile response. The effect of angiotensin under a condition where action potentials were blocked by high potassium ions in the medium, was greatly reduced, compared to noradrenaline. From these results, these authors concluded that the action of angiotensin is more dependent on membrane excitation than that of noradrenaline. Angiotensin has also been shown to cause a graded depolarization, proportional to the amplitude of the contraction, in the pulmonary artery of the rabbit and dog (Somlyo and Somlyo, 1968).

The contractile effect of angiotensin on the guinea-pig taenia coli is associated with membrane depolarization and an increase in spike frequency (Ohashi, Nonomura and Ohga, 1967). This observation is consistent with the view that contractile or constrictor substances produce their effects through primary bioelectric changes in the smooth muscle membrane.

In this section, experiments are described to elucidate further the electrical changes of smooth muscle associated with tension development produced by angiotensin.

CHAPTER I THE CHANGES IN MEMBRANE ACTIVITY AND TENSION INDUCED BY ANGIOTENSIN ON THE TAENIA COLI AS STUDIED BY THE SUCROSE-GAP METHOD

The results obtained in this thesis and those of other workers suggest that angiotensin can influence the contractions of smooth muscle by acting a) indirectly through the release of a neurotransmitter or b) by the release of prostaglandins or c) by a direct action on the smooth muscle cell membrane or d) on the coupling between excitation and contraction or by a combination of some or all of these sites. It was concluded that the action of angiotensin on the taenia coli of the guinea-pig was a direct one on the smooth muscle membrane and that neuronal intervention or release of prostaglandins, if any, did not contribute significantly to the contractile response. This latter conclusion is consistent with the concept of functional innervation of the taenia coli (vide infra). Electron microscopy has revealed that only small nerve bundles (containing 3 to 5 axons) actually enter the muscle bundles of the taenia coli (Bennett and Rogers, 1967) and that close apposition of nerve and muscle is extremely rare (see Burnstock, 1970). In view of these observations, the taenia coli appears to be a convenient preparation for investigating the electro-physiological basis of the direct action of angiotensin.

The sucrose-gap method originally described by Stämpfli (1954) for measuring resting membrane potentials with external

electrodes has been variously modified and adapted (Burnstock, 1958; Burnstock and Straub, 1958) for studying the effects of ions, drugs and of electrical stimulation on the resting and action potentials of smooth muscles. In addition, Bülbring, Burnstock and Holman (1958) and Bülbring and Burnstock (1960) have altered the apparatus so that tension changes could be recorded simultaneously with those of the membrane potential. The sucrose-gap method allows a quick change of the bathing solution, since the solutions are continuously flowing, so that the whole sequence of changes in membrane activity and in tension caused by test solutions could be continuously recorded over long periods. Furthermore, this method records the sum of the effects of each cell rather than the activity of a single cell as in the case with intracellular microelectrode studies, and therefore is more suitable for pharmacological study. The underlying theory of sucrose-gap recording and its advantages over cellular microelectrode study has been discussed (see Introduction).

A modified sucrose-gap apparatus (see Methods) has been used to correlate changes in membrane activity and tension caused by angiotensin on the taenia coli in the hope of clarifying the mechanism of its direct action on the cell membrane of smooth muscles.

RESULTS

Preliminary experiments were made to determine the approximate membrane potential and the characteristics of action potentials during normal activity.

1.1. Membrane potential

The membrane potential recorded when one end of the tissue was perfused with isotonic potassium chloride was within the range of 18 - 20 mV. The mean value was 19.6 $\pm$ 0.67 mV (n = 5). An average value of 21.3 mV was reported by Burnstock and Straub (1958) using the sucrose-gap method.

When instead of potassium chloride, potassium sulphate (K_2SO_4) was used, a much greater depolarization was observed (Table IX). The value ranged from 38 to 55 mV, with a mean of 48.3 $\pm$ 0.27 mV (n = 10). This value is close to the resting potentials of 56.1 mV and 51.5 mV reported with isotonic K_2SO_4 Ringer by Burnstock and Straub (1958) using sucrose-gap and Holman (1958) using cellular micro-electrodes respectively. However, this membrane potential is lower than the 60 mV as reported by Bülbring in 1954 with microelectrode studies. (Table IX).

1.2. Normal activity

Ten to fifteen minutes after the preparation had been depolarized with isotonic K_2SO_4 solution at one end, spontaneous rhythmic contractions began to occur. These contractions were associated with bursts of uneven spike activity. The amplitude of

Ringer	Membrane potential Depolarization KCl	K_2SO_4	Spike Amplitude	Spike Frequency	Temperature	Source
Krebs	19.6±0.67	48.3±0.27	8.4 ± 1.2	0.3 - 0.7	29°C	this study
Locke	21.3	56.1	14	0.6-0.8	25°C	Burnstock and Straub, 1958. (sucrose-gap)
Krebs	-	51.5±0.36	59.3±0.27	0.7-0.8	35°C	Holman, 1958 (microelectrode)
Krebs	-	60.0±9.18	10	1.0	35°C	Bülbring, 1954. (microelectrode)

Table IX . Membrane potential amplitude, action potential (spike) amplitude and spike frequency of the guinea-pig taenia coli, measured with sucrose-gap technique. Depolarization and spike amplitude in millivolts, spike frequency expressed as sec^{-1}.

contraction was less than that observed with conventional techniques, where only tension was recorded. The size and configuration of the action potentials showed considerable variation (see control in Fig. 17a). This is predictable since the electrode is in contact with a number of cells and some of which may be firing asynchronously. In addition, the membrane potential often showed slow fluctuations of about 5 to 8 mV, each rise and fall lasting about 3 minutes.

In some preparations, however, the spikes became larger and more regular after about 30 minutes. In general , the shape of the spikes showed an initial slow phase of depolarization of 2 - 3 mV, which led to a fast phase 'the spike', and was followed by a rapid repolarization with hyperpolarization. These observations are similar to those described by Burnstock (1958) and Burnstock and Straub (1958). The largest spike recorded during normal activity was about 12 mV, the average spike amplitude was 8.4 ± 1.2 mV ($n = 20$). These values are close to those of Burnstock and Straub (1958), but are much smaller than those recorded from a single fibre with intracellular electrodes (Table IX).

The spike frequency varied between 0.3 and 0.7 per second. This range is close to that reported by Burnstock and Straub (1958). However, a frequency of 1/second at 35 °C had been reported by Bülbring (1954).

These observations seem to justify the application of the sucrose-gap bath described (see Method) for studying the changes

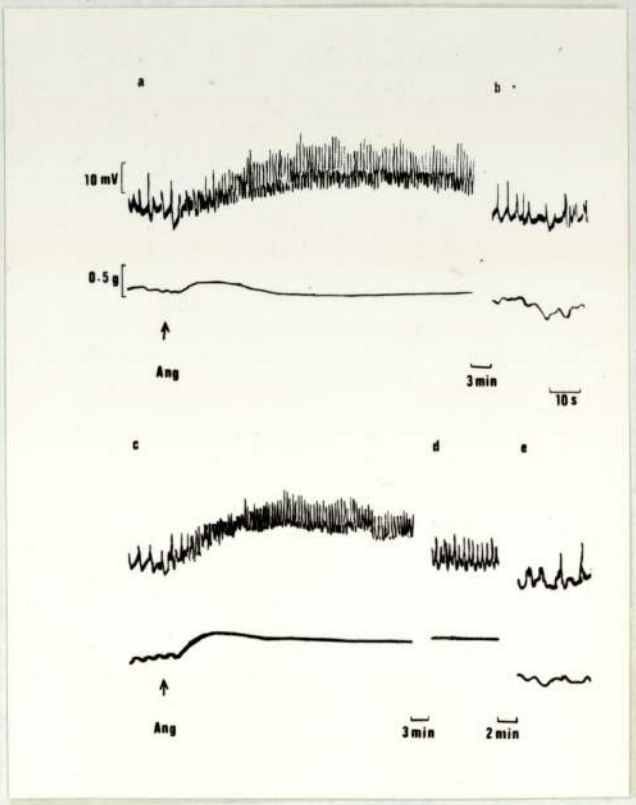

Fig. 17a. Changes in the electrical (upper record) and mechanical (lower record) activity of guinea-pig taenia coli to 10^{-7}M (a) and 10^{-6}M (c) of angiotensin (Ang). (b) 3 minutes after (a) and (d) & (e) responses 3 and 2 minutes after (c).

in membrane potential, spike activity and tension produced by brief application of drugs on to the tissue.

1.3. Effects of angiotensin on membrane activity and tension

Concentrations of angiotensin between 5×10^{-8}M and 10^{-6}M accelerated the spike discharge and depolarized the membrane by 3 to 8 mV. These changes in electrical activity were associated with an increase in muscle tension. In some preparations, an increase in spike frequency occured without any noticeable change in membrane potential. The course of events following the administration of angiotensin and the recovery after its removal is shown in Fig. 17a. When angiotensin reached the preparation, there was a latent period ranging from between 5 to 15 seconds, during which the membrane activity and tension did not change appreciably. This latent period was followed by an increase in spike frequency with large regular spikes, the maximum recorded amplitude was 18 mV. This spike activity lasted for about 30 to 40 seconds, after which the spike gradually decreased in amplitude. During this time, the membrane showed a gradual depolarization and reached a new level, where it was maintained. The maximum depolarization often coincided with the maximum increase in tension, but sometimes occured a few seconds before or after. After the maximum depolarization and maximum increase in tension, which collectively shall be refered to as 'maximum response', was reached, the tension gradually declined to the original level or slightly below. The membrane potential however, recovered more slowly and also returned

to a higher value than that recorded initially. A similar increase in membrane potential after recovery from acetylcholine depolarization has reported by Burnstock and Straub (1958), Bülbring and Burnstock (1960) and Bülbring and Kuriyama (1963) on the taenia coli and by Bolton (1971) on the guinea-pig ileum.

The average rate of depolarization was greater than that of repolarization. The average rates of depolarization and repolarization by 10^{-7}M angiotensin were 0.19 $\pm$ 0.04 mV and 0.13 $\pm$ 0.03 mV per second respectively. This slow rates of onset of depolarization and repolarization appeared to be responsible for the long duration of angiotensin action. With increasing concentrations of angiotensin, the latent period became progressively shorter, while the overall time-course of the response became longer. Table X summarizes the effect of different concentrations of angiotensin on the latent period, spike activity, depolarization, average rates of depolarization and repolarization and tension development on the taenia coli.

1.4. Comparison of the responses of acetylcholine with angiotensin

The changes in electrical and mechanical activities induced by acetylcholine on the taenia coli have been extensively studied elsewhere (e.g. Burnstock and Straub, 1958; Bülbring and Kuriyama, 1963; Bülbring and Burnstock, 1960). Responses induced by angiotensin were therefore compared with those of acetylcholine to facilitate interpretation of the results.

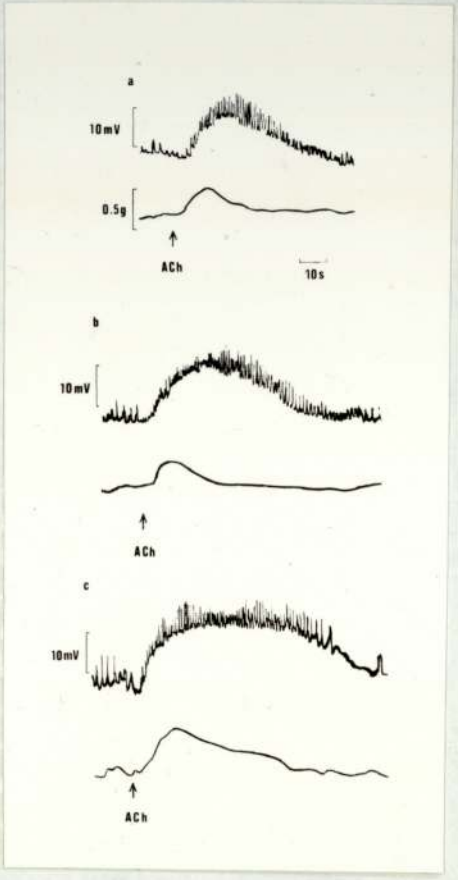

Fig. 17b. Changes in the electrical (upper record) and mechanical (lower record) activity of guinea-pig taenia coli to 4×10^{-8}M (a); 4×10^{-7}M (b) and 1.8×10^{-6}M (c) of acetylcholine (ACh).

The changes in membrane potential, spike discharge and tension produced by brief application of acetylcholine or angiotensin were qualitatively similar, except for the longer duration of action of angiotensin (Fig.17a,b). The average duration of responses to submaximal concentrations of acetylcholine and angiotensin were 48.3 $\pm$ 0.37 and 285 $\pm$ 0.86 seconds respectively (n = 10).

The depolarization caused by acetylcholine or angiotensin was a function of the concentration applied (Fig. 18). Low concentrations of the drugs ($< 10^{-8}$M for acetylcholine and $< 5 \times 10^{-8}$M for angiotensin) did not produce any detectable membrane depolarization. With higher concentrations, the membrane was depolarized, spike frequency increased initially and then gradually declined. These results are expressed graphically in Fig. 18. Table X also compares the various parameters of responses induced by acetylcholine and angiotensin.

1.5. Effect of repeated applicationsof angiotensin

Tachyphylaxis to angiotensin has been demonstrated in several smooth muscle preparations if the interval between doses was insufficient for the tension to return to its resting state (see Part I). This phenomenon was therefore further examined on the membrane activity of the taenia coli.

With all effective concentrations of angiotensin (10^{-8}M - 10^{-6}M) when applied at intervals of less than 15 minutes,

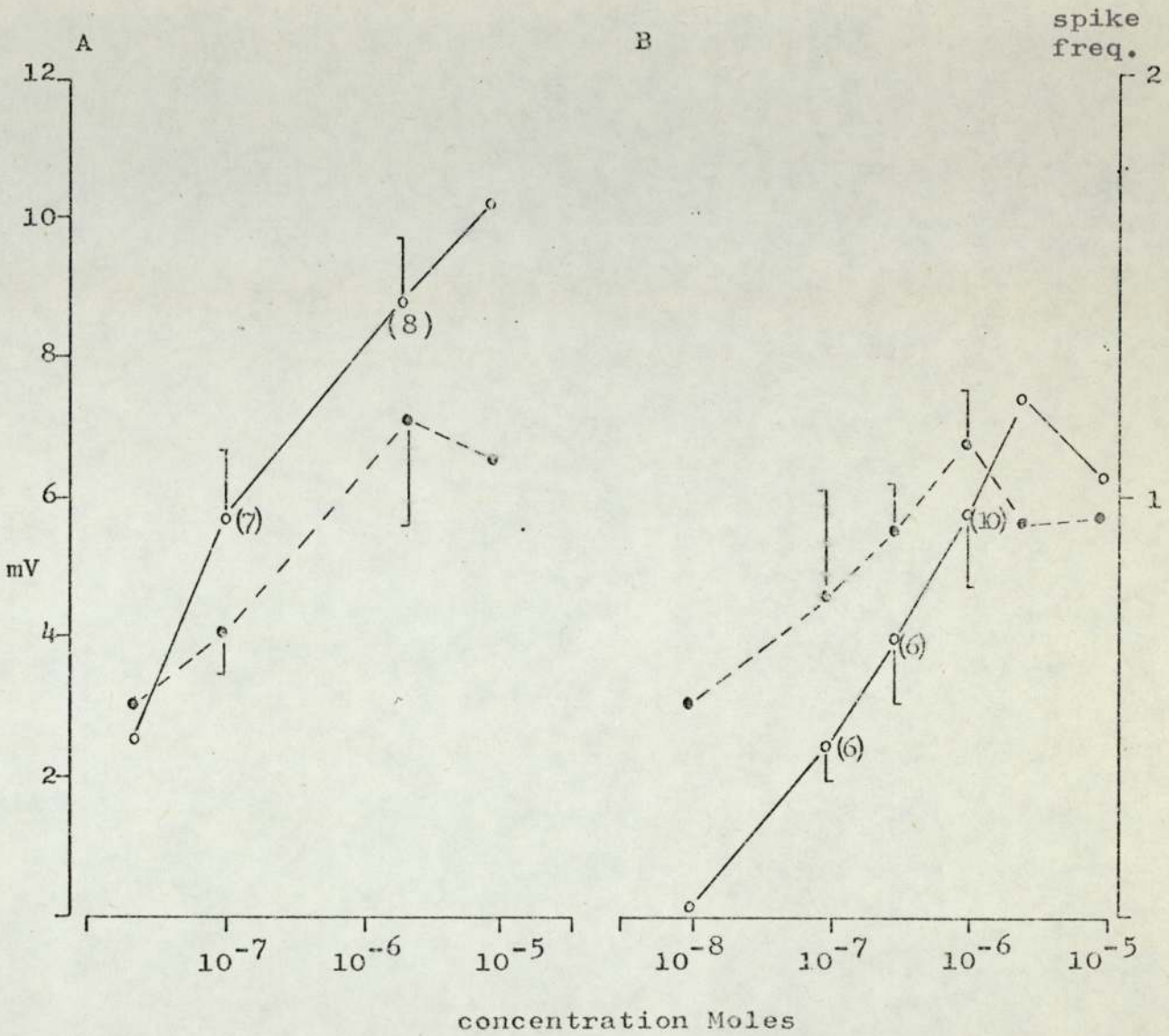

Fig. 18. Concentration effect curves of membrane depolarization (o—o) and spike frequency (•—•) to acetylcholine (A) and angiotensin (B). Each point is the mean of 4 observations unless otherwise indicated in brackets.

tachyphylaxis was evident from the increased latent period, reduced spike frequency and tension and in the rate of depolarization. By increasing the dose interval, tachyphylaxis observed with submaximal concentrations of angiotensin (5×10^{-7}M) could be minimized but not abolished. With a dose interval of between 20 to 30 minutes, concentrations of angiotensin (10^{-8}M - 10^{-7}M) gave fairly reproducible resonses over a 3 hour period. However, higher concentrations greater than 5×10^{-7}M, angiotensin gave eratic responses and the tissue often failed to recover. Tachyphylaxis of depolarization was more pronounced than tachyphylaxis to tension. Table XI summarizes the responses to repeated applications of angiotensin at various time intervals.

Tachyphylaxis to acetylcholine was also observed if the dose interval was less than 5 minutes.

Drug Concentration m	Latent Period sec.	Mean rates of depolarization mV	repolarization mV	Depolarization mV	Spike Frequency sec^{-1}	Duration sec.	Tension	n
Angiotensin								
10^{-9}	15.3	-	-	-	0.49(n=2)	210	4	2
10^{-8}	10.8	0.07±0.09	0.03±0.01	2.85±0.18	0.58±0.05	260	6	8
10^{-7}	8.1	0.19±0.04	0.13±0.03	5.71±0.96	0.98±0.04	285	12	7
$5x10^{-7}$	3.2	0.25±0.04	0.15±0.01	7.30±0.79	0.70±0.07	320	15	6
10^{-6}	3.6	0.21(n=3)	0.15(n=3)	5.63(n=3)	0.82(n=3)	310	13	3
Acetylcholine								
10^{-8}	10	0.18(n=2)	0.11(n=2)	3.62(n=2)	0.86(n=2)	40	11	2
10^{-7}	7	0.34±0.09	0.13±0.04	5.91(0.72)	0.92±0.04	45	18	6
10^{-6}	4	0.53±0.04	0.29±0.03	8.54±0.65	1.28±0.09	65	20	8
10^{-5}	3	0.65±0.07	0.29±0.08	11.35±1.21	1.02±0.21	80	19	4

Table X. Changes in membrane parameters, duration of action, latent period and tension to different concentrations of angiotensin and acetylcholine. Each value is the mean of at least 5 experiments ± SEM; unless otherwise indicated. Increase in tension expressed as arbitrary units. Spike frequency is calculated from the ratio :-

No. of spikes/10 seconds. Details refer to text.

Dose Interval	Latent Period seconds	Average Depolarization mV	Spike frequency % of control	Duration seconds	Tension
Control	8.1	5.8	150%	290	12
5 mins.	12.3	0	116%	120	3
15 mins.	10.7	2.4	130%	210	7
20 mins.	7.9	4.9	150%	300	10

Table XI

The effect of repeated applications of angiotensin (10^{-7}M) at different dose intervals on membrane activity and tension development on the guinea-pig taenia coli. Tension expressed as arbitrary units.

DISCUSSION

a) The sucrose-gap apparatus and the study of drug action

The sucrose-gap apparatus described in this thesis, is particularly suited to the observation of drug action. It allows the long term simultaneous monitoring of membrane potential and tension. The construction of the bath as a single unit instead of 3 separate components (Stampfli, 1954) minimizes cost and eliminates bath assembly. However, some of the inherent difficulties related to sucrose-gap study (see Introduction) cannot be entirely eliminated. Maintaining a steady flow rate throughout an experiment is difficult since smooth muscle is spontaneously active and the recording of tension aggravates the problem.

The recordings of tension are not always comparable with those obtained in a conventional organ bath, possibly due to the fact that the tissue is bent into a U-shape, such that only a small segment of it is in actual contact with the test solution and possibly due to damping by the inert part of the tissue in parallel with the 'live' part. One must assume that this influence remains constant throughout an experiment; even then, however, one cannot be sure that the damping element would bear a direct simple relationship to the displacement.

Since the 'live' side of the apparatus was of very small volume, drug or test solutions could be changed rapidly. In preliminary experiments, drugs were introduced from a reservoir by means of a 3-way tap. Although this method allowed a quick change over of test solutions, it caused prominent artifacts and

necessitated a rather long exposure time of the tissue to drug solutions (1 - 3 minutes). It was found that under such condition, the taenia coli loses its reactivity to angiotensin upon subsequent exposure.

In the majority of experiments performed, drugs were introduced in small volume (see Method) via a small side tube so that the drug flow was in line with the perfusion fluid. This method of application minimized 'change over' artifacts and had the advantage of immediately presenting the drug in the concentration to be tested. One must realize that the exact concentration of the drug cannot be calculated due to a slight dilution with the main perfusing solution. Nevertheless, with such brief exposure to drugs and especially to drugs which cause tachyphylaxis, the responsiveness of the tissue can be maintained over several hours.

Movement which could dislodge an intracellular micro-electrode has little effect with the sucrose-gap technique, at worst, a clearly distinguishable movement may be encountered. However, in these experiments these complications appeared rarely and were easily recognizable when they did occur. As previously discussed (see Introduction), the size and shape of the sucrose-gap recorded potentials differs from those recorded with intra-cellular microelectrodes. However, this fact does not detract from the value of the method, since when drug effects are evaluated, control experiments are always necessary. One must, however, consider the possibility that a drug effect may be confused with an artifact characteristic of the method. For example, a drug may produce an

apparent effect on membrane phenomena by affecting the individual cells. The sucrose-gap recording is obtained from the population of cells at the first interface in the 'live' side, and this also may introduce factors not seen with cellular microelectrode recording. In general, this 'averaging' effect would be expected to damp out variability occuring between the individual electrical units.

b) The effect of angiotensin on membrane activity

The membrane potential of 52 mV recorded in this study for the taenia coli is very close to the value of 56 mV reported by Burnstock and Straub (1958) using a similar technique. The relationship between electrical and mechanical activity of the taenia coli at rest and during stimulation by acetylcholine is in accordance with the results of Bülbring and Burnstock (1960). These results form the basis upon which the effects of angiotensin can be compared.

The results presented in this chapter show that the action of angiotensin on the taenia coli was to cause depolarization and an increase in spike frequency, which was coincident with a development in muscle tension. These results are in agreement with those of Ohashi *et al* (1967).

An increase in spike discharge and development of isometric tension occured simultaneously during angiotensin action. However, less correlation was found between the mechanical response and the change in membrane potential. In some cases, no change in

membrane potential was found during the contraction of the taenia coli. In other cases, the maximum increase in tension occured before maximum depolarization was reached. These results suggest that angiotensin induced changes in tension are a direct consequence of changes in the spontaneous spike activity and that the ionic basis involved in action potential generation may be different from that involved in membrane depolarization. In the taenia, it is believed that spikes are due to an inward flowing calcium current rather than sodium current (Brading and Tomita, 1968; Bülbring and Tomita, 1969).

The membrane of the taenia coli is unstable and has the tendency to fire spontaneously (Bülbring, 1954, 1955). The inherent oscillation of excitability and of membrane polarization or 'slow waves' (Holman, 1968) are believed to be due to a periodically changing rate of active processes at the membrane affecting its stability and causing periodical shifts in the balance of ion fluxes (Bülbring and Burnstock, 1960). The main ionic current involved is that of sodium (Bülbring and Kuriyama, 1963; Bolton, 1971). Therefore, if angiotensin was applied during the depolarization phase, a further change in membrane potential may not be very obvious, in contrast, if angiotensin was applied when the membrane potential was high, any reduction in potential may be more prominent. The same reasoning may also explain the reduction in responses to angiotensin observed when the interval between two successive applications of angiotensin was short. Responses to acetylcholine were reproducible even when a shorter interval than that for angiotensin was used. This could be due to the

fact that the average rates of depolarization and of repolarization are faster than those of angiotensin.

When the maximum response to acetylcholine was reached, the membrane repolarized and then hyperpolarized. This latter phase of repolarization has been referred to as 'after-hyperpolarization' by Bolton (1973). A similar 'after-hyperpolarization' was observed when angiotensin was applied to the taenia coli. The significance of this effect will be discussed in the next chapter.

In summary, the results presented in this chapter, suggest that the modified sucrose-gap bath is applicable to the study of drug action on the electrical and mechanical activities of smooth muscle, even though the difficulties which are inherent in the use of the sucrose-gap technique could not be eliminated. The results obtained with angiotensin on the taenia coli, suggests that the mechanical responses induced by the peptide are due to excitation of the membrane, i.e. depolarization and increase in spike discharge. The pattern of changes in membrane activity is similar to that caused by acetylcholine, except for the longer time course of response of angiotensin. This result suggests that similar underlying ionic mechanisms may be involved in both actions, but the actions of the two drugs on their specific receptors may be different.

CHAPTER II EFFECTS OF CHANGES IN IONIC ENVIRONMENT ON THE ACTION OF ANGIOTENSIN ON THE TAENIA COLI

In the preceding chapter, it was shown that the increase in tension induced by angiotensin on the taenia coli was accompanied by a simultaneous increase in membrane activity, in the form of increased spike discharge, or membrane depolarization or both. Similar events were observed for acetylcholine.

The mechanism of action of acetylcholine on smooth muscle membrane is thought to resemble its action on the motor end-plate (Burnstock, 1958). At the motor end-plate, it has been shown that acetylcholine increases the permeability to sodium, potassium and possibly other free ions which are present on either side of the membrane. (Fatt and Katz, 1951; del Castillo and Katz, 1954, 1955). These results have been confirmed by Takeuchi and Takeuchi (1959, 1960) who demonstrated that at the frog motor end-plate, acetylcholine caused an increase in the conductance of the membrane to sodium, potassium and calcium, but had little effect on the chloride conductance.

The effects of acetylcholine on membrane potential, spike activity and tension have been studied on the taenia coli in different ionic environments by Bülbring and Kuriyama (1963). These authors came to the conclusion that acetylcholine exerted its effect by a non-selective increase of membrane permeability. It is possible that the action of angiotensin on smooth muscle membranes may also be explained in terms of ionic permeability. The

effects of changes in ionic environment of the action of angiotensin on the guinea-pig taenia coli was therefore examined. Acetylcholine was used for comparison.

RESULTS

In all experiments, the concentrations of angiotensin and acetylcholine used were 5×10^{-7}M and 1.8×10^{-6}M respectively. These concentrations of the drugs were found to give fairly reproducible responses over a three hour period (see previous Chapter).

2.1. The effect of angiotensin in sodium deficient and sodium excess solutions

When 50% of the sodium chloride was replaced with Tris-chloride, the membrane activity and tension scarcely changed. The effects of angiotensin and acetylcholine were the same as in normal physiological solution.

When the muscle was exposed to a solution containing 7mM sodium (+ 130mM Tris), spontaneous discharge gradually decreased and after an hour the spike frequency was reduced from the normal 0.4 (in normal Krebs) to 0.01 per second and continued indefinitely at this low frequency, while the membrane became slightly depolarized. Under these conditions, angiotensin depolarized the membrane slightly and increased spike discharge. The tension development followed these changes closely. (Fig. 19). The magnitude of the response was less than in normal solution. A similar pattern of changes in membrane activity and tension were observed with acetylcholine (Fig. 20).

In sodium-free Krebs, the membrane was slightly depolarized by about 5 to 10 mV over one hour. Spike amplitude and frequency

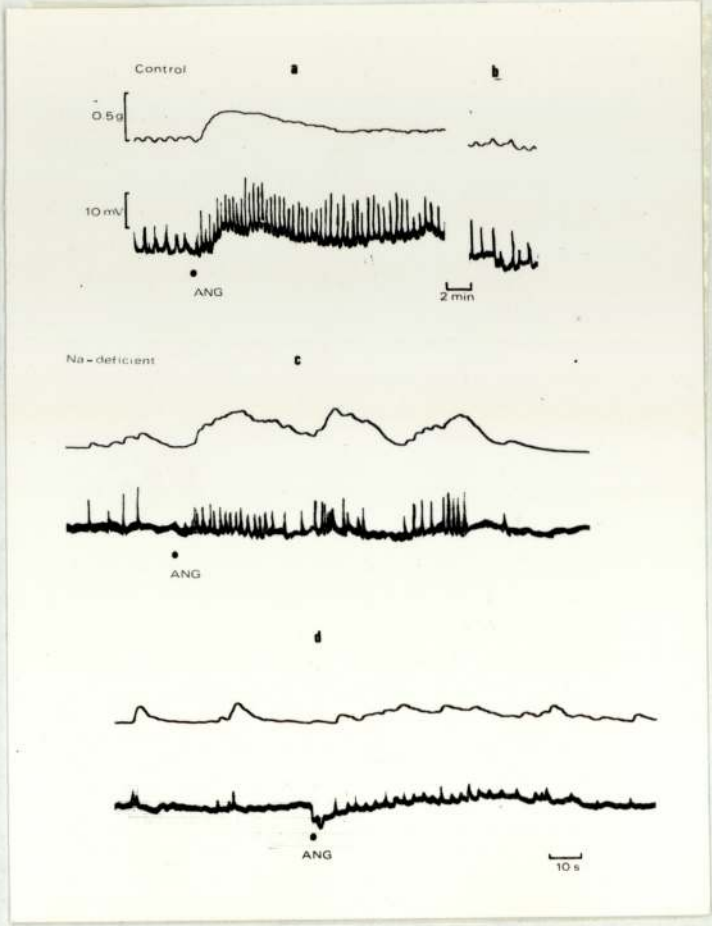

Fig. 19. Effect of sodium deficient Krebs (7 mM Na^+) on the mechanical (upper record) and electrical (lower record) activity of the guinea-pig taenia coli produced by angiotensin (5 x 10^{-7}M) (ANG) : (a) control responses to angiotensin; (b) recovery; (c) 15 minutes after exposure to 7mM Na^+-Krebs and effect of angiotensin; (d) effect of angiotensin after 40 minutes in sodium-deficient Krebs.

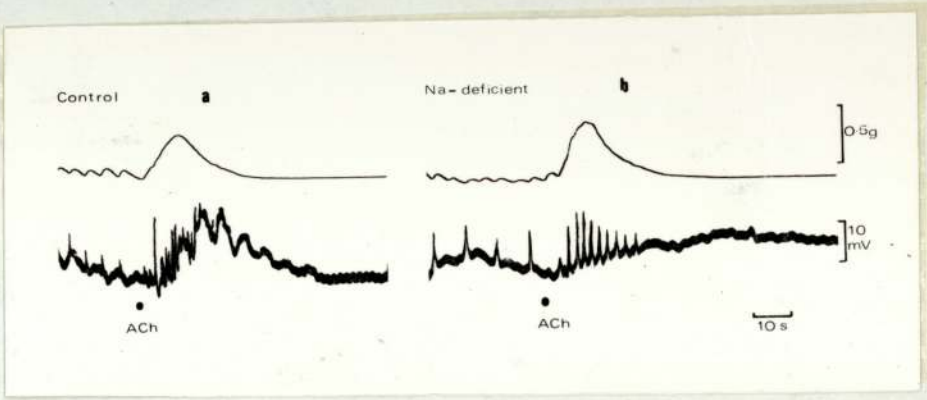

Fig. 20. Effect of acetylcholine (1.8×10^{-6} M)(ACh) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli; (a) control Normal Krebs; (b) 20 minutes after exposure to 7 mM Na^+-Krebs.

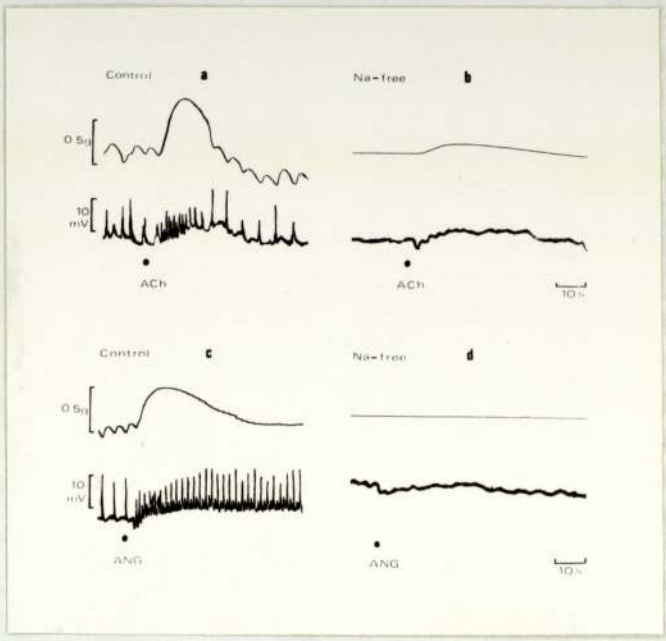

Fig. 21. Comparison of the effects of acetylcholine (ACh) (a and b) and angiotensin (ANG) (c and d) on the mechanical (upper record) and electrical (lower record) activity of the guinea-pig taenia coli in normal Krebs (a and c) and sodium-free Krebs (b and d).

gradually declined and eventually ceased after about one hour. After this time, angiotensin was without effect, however, acetylcholine still caused a small depolarization and contraction (Fig. 21).

In solutions containing excess sodium, the membrane was slightly depolarized and developed large local potentials triggering multiple spike discharge. The effects of angiotensin on membrane activities were greatly enhanced and prolonged by about 2 to 3 times (Fig. 22). The effects of acetylcholine were also enhanced, but were less prolonged than those of angiotensin (Fig. 23).

2.2. The effect of angiotensin in potassium-free and potassium excess solutions

Potassium free solution caused an initial small (about 4mV) depolarization and increased the frequency and amplitude of the spike discharge. After about ten minutes, the membrane began to repolarize and then hyperpolarized to a potential about 6 mV above the initial value. The spike frequency gradually decreased and ceased after about 60 minutes. When angiotensin was applied while spontaneous activity was still present, the effect was essentially the same as in normal solution.

When the spike activity had been greatly reduced, angiotensin had no appreciable effect on membrane activity nor tension (Fig. 24).

The effect of acetylcholine in potassium free solution followed the pattern of changes induced by angiotensin closely,

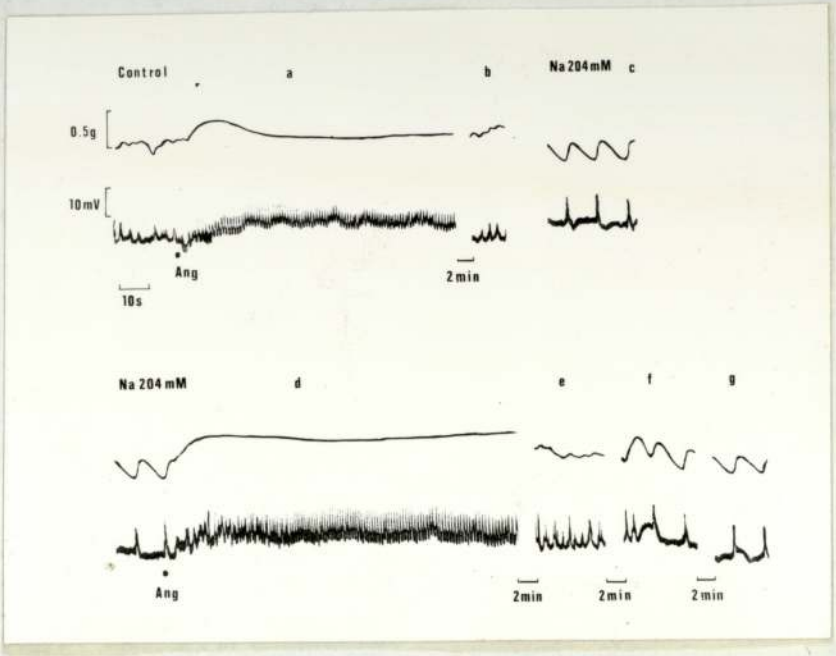

Fig. 22. Effect of angiotensin (5×10^{-7}M)(Ang) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli:(a) control responses to Ang in normal Krebs;(b) recovery; (c) spontaneous activity following 10 mins exposure to high Na^+(204 mM) Krebs; (d) responses to Ang after 20 mins in high Na^+Krebs;(e),(f) & (g) recovery at 2 mins interval after (d).

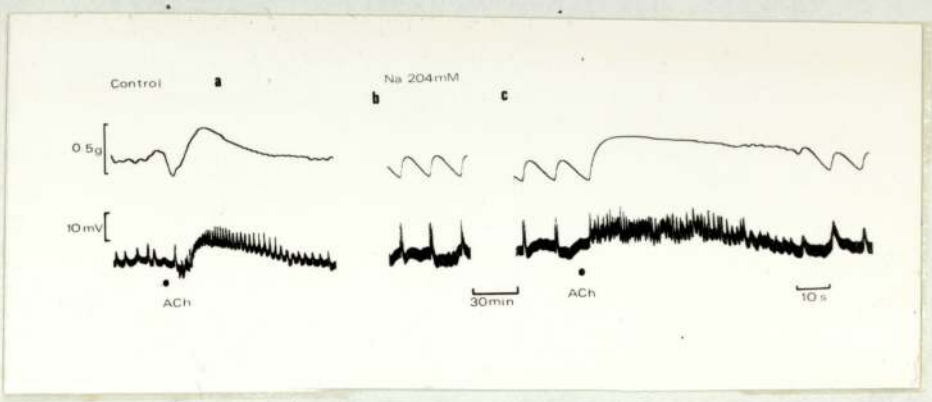

Fig. 23. Effect of acetylcholine (ACh)(1.8×10^{-6}M) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli: (a) control responses to ACh in normal Krebs; (b) spontaneous activity following 10 mins exposure to high Na^+-Krebs (204 mM); (c) responses to ACh after 40 mins in high Na^+-Krebs.

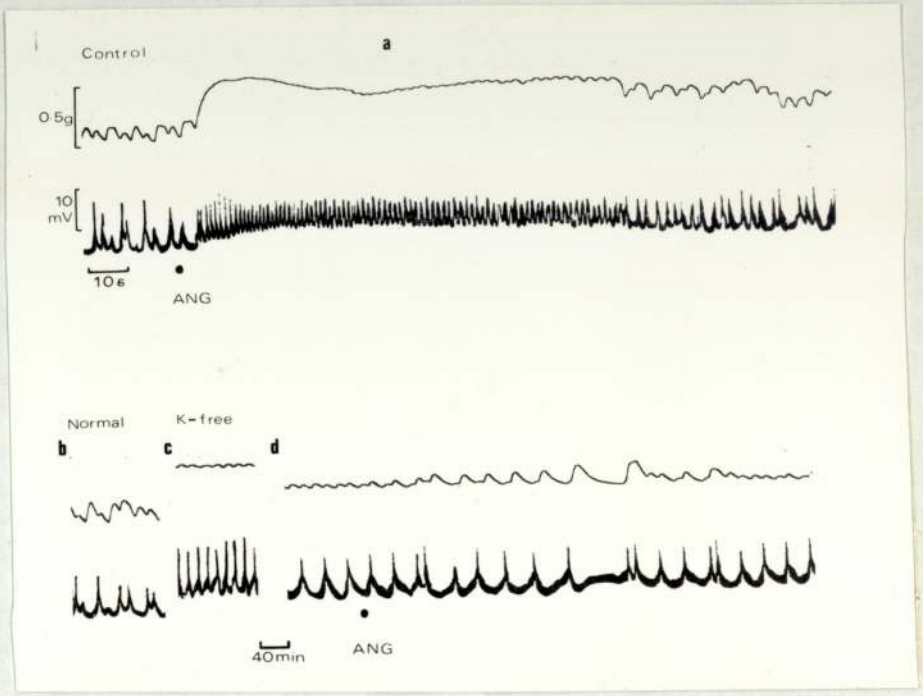

Fig. 24. Changes in the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli to (a) angiotensin (5×10^{-7}M)(ANG); (b) 15 mins after adding ANG; (c) 10 mins after changing to K^+-free Krebs; (d) ANG (5×10^{-7}M)40 mins after changing to K^+-free Krebs.

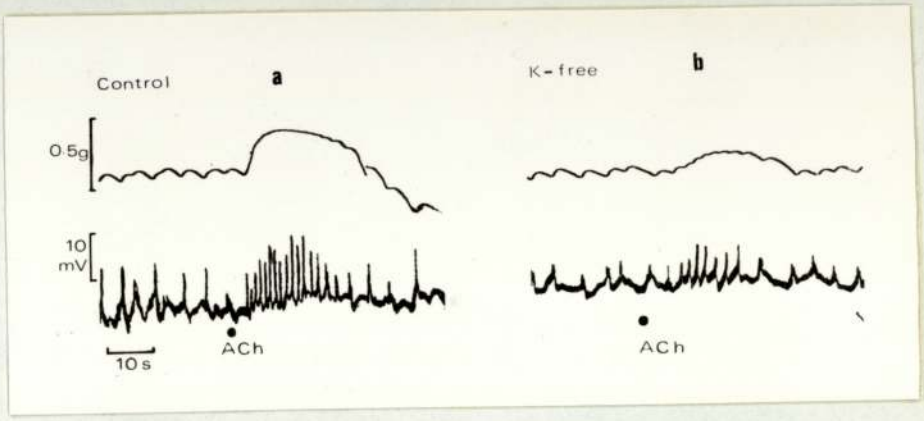

Fig. 25. Effect of acetylcholine (1.8×10^{-6}M) (ACh) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli; (a) normal Krebs and (b) following 30 minutes exposure to K^+-free Krebs.

with the exception that, at the time when angiotensin was without effect on the tissue, acetylcholine still initiated or increased the spike discharge and produced an increase in tension. However, these activities were of lesser magnitude than those in normal Krebs. (Fig. 25).

Excess potassium (18mM) depolarized the membrane and increased spike frequency and amplitude, together with an increase in tension. The effect of angiotensin on the electrical activity became biphasic. There was an initial increase in spike frequency associated with a small increase in tension which lasted for about 10 seconds. The tension then fell rapidly to control values (i.e. before addition of high K^+) with a concomittant decrease in spike frequency. During this time, the membrane was depolarized (Fig. 26). A similar course of events was observed for acetylcholine (Fig. 27).

2.3. The effect of angiotensin in chloride-deficient solution

The effects of reducing chloride concentration and replacing it with proportionate amount of benzenesulphonate ($C_6H_5SO_3$) were, in general depolarization of the membrane and an increase in spike discharge and spike amplitude. However, after 30 minutes continuous spike discharge ceased, and bursts of spikes appeared alternating with quiescient periods. The effect of angiotensin during the initial phase of chloride deficiency was to enhance spike discharge and membrane depolarization. When continuous spike activity had ceased, angiotensin evoked spike discharge in groups of rapid bursts and the membrane was slightly depolarized. These changes were accompanied by an increase in tension. (Fig. 28).

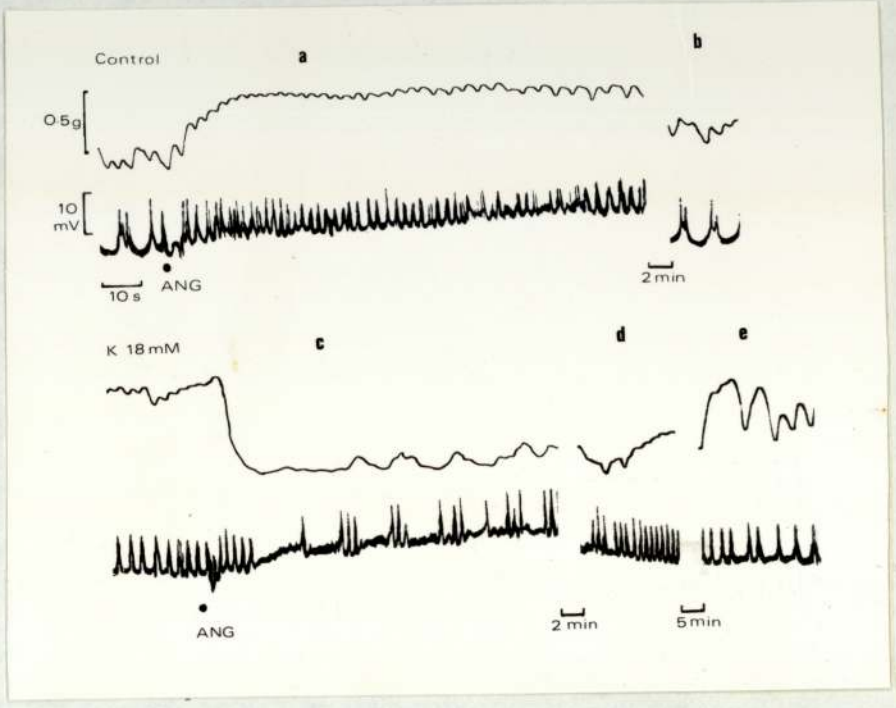

Fig. 26. Effect of angiotensin (5×10^{-7} M)(ANG) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli. (a) response to ANG in normal Krebs; (b) recovery after ANG; (c) ANG following 20 minutes exposure to high K^+ (18 mM Krebs). (d) & (e) responses 2 and 5 mins after (c).

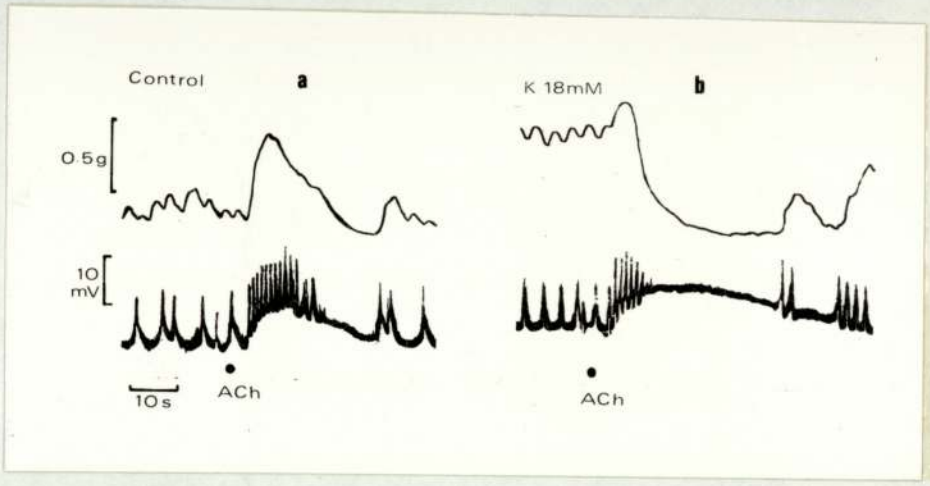

Fig. 27. Effect of acetylcholine (1.8×10^{-6}M)(ACh) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli; (a) ACh in normal Krebs; (b) ACh following 15 mins exposure to high K^+(18 mM) Krebs.

Under these conditions, the effect of acetylcholine was essentially similar to that of angiotensin.

2.4. The effect of angiotensin in calcium-free solution and in the presence of excess calcium

In the absence of calcium, membrane activity ceased after about 40 minutes. Angiotensin did not initiate spike discharge, depolarize the membrane or produce an increase in tension.

Excess calcium (7.5mM) did not change the membrane activity or tension significantly. The effect of angiotensin, however, was enhanced (Fig. 29). The depolarization caused by the peptide increased from 6 mV (in normal Krebs) to about 8 mV, and the spike frequency was increased from 0.4 to 1.6 per second compared to 0.7 to 1.1 per second in normal Krebs. The potentiation in excess calcium was rather similar to that observed in excess sodium. A similar enhancement of acetylcholine response was observed in excess calcium. (Fig. 30).

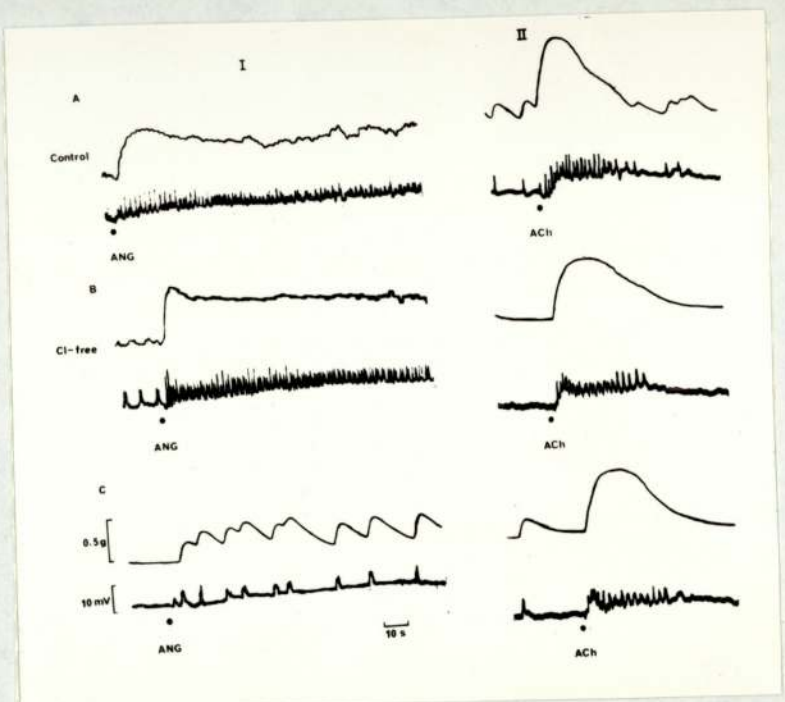

Fig. 28. Comparison of the effect of angiotensin (5×10^{-7}M)(ANG)(panel I) with acetylcholine (1.8×10^{-6}M)(ACh)(panel II) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli: A) control responses in normal Krebs; B) responses after 15 mins (ANG) and 25 mins (ACh) in Cl-deficient Krebs; C) responses after 30 mins (ANG) and 40 mins (ACh) in Cl-deficient Krebs.

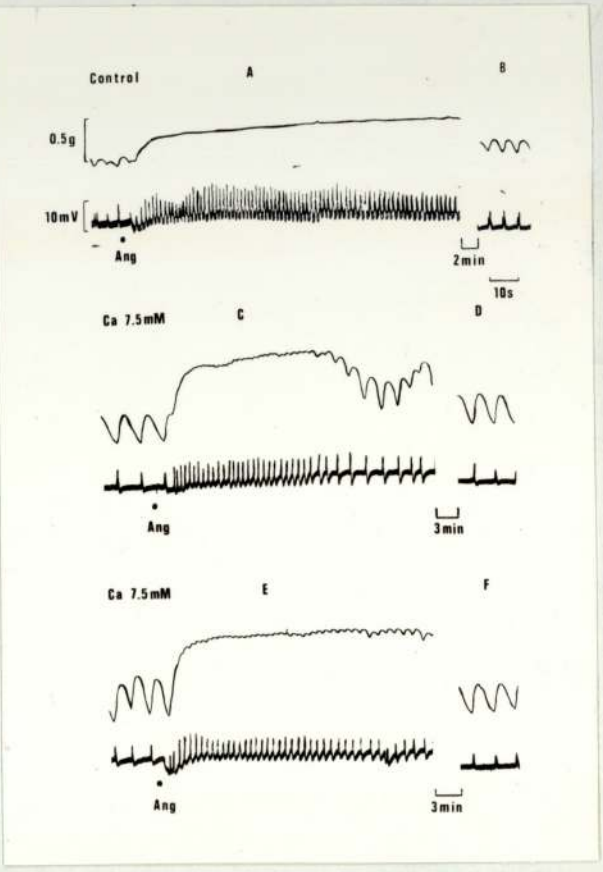

Fig. 29. Effect of angiotensin (5×10^{-7}M)(ANG) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli: (A) control responses in normal Krebs; (B) responses 2 mins after (A); (C) responses following 20 mins exposure to high Ca^{2+}-(7.5mM) Krebs; (E) responses following 40 mins exposure to high Ca^{2+}-Krebs; (D) & (F) responses 3 mins after (C) & (E).

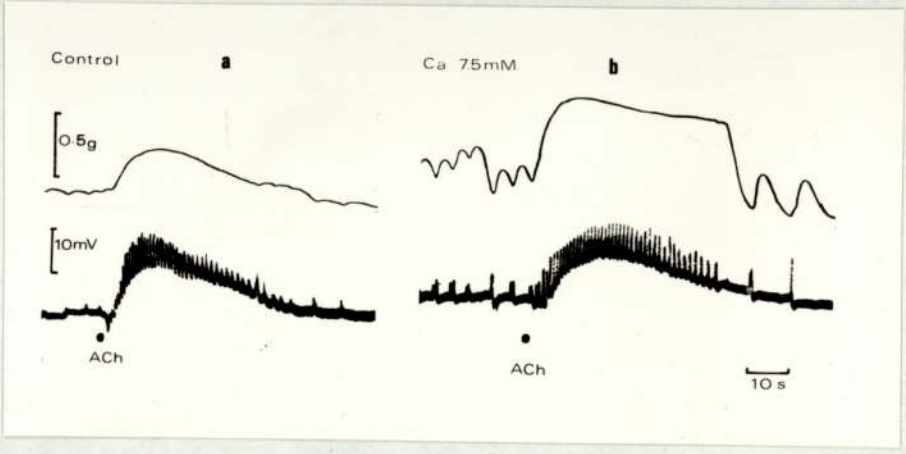

Fig. 30. Effect of acetylcholine (1.8×10^{-6}M)(ACh) on the mechanical (upper record) and electrical (lower record) activity of guinea-pig taenia coli: (a) control responses in normal Krebs; (b) responses following 15 mins exposure to high Ca^{2+}-(7.5mM) Krebs.

DISCUSSION

On the taenia coli of the guinea-pig, acetylcholine depolarizes the membrane, increases spike frequency (Burnstock, 1958; Bülbring and Kuriyama, 1963) and membrane conductance (Kuriyama, 1970). Ion flux studies on smooth muscle indicate that acetylcholine increases the permeability to potassium, sodium, chloride, bromide and calcium ions (Durbin and Jenkinson, 1961; Jenkinson and Norton, 1967; Burgen and Spero, 1968).

In the present study, it was shown that the action of angiotensin was reduced when the concentration of sodium or calcium and to a lesser extent potassium or chloride ions was reduced and enhanced when the concentration of either sodium or calcium was increased. These results suggest that angiotensin causes a change in the permeability of the membrane to most of these ions.

In the absence of sodium, angiotensin was without effect, whereas acetylcholine still caused a small response. These results suggest that the action of angiotensin on the taenia is mainly due to an increase in sodium permeability of the membrane. This is supported by the observation that in the presence of excess sodium, the depolarization and spike frequency induced by angiotensin were enhanced and the effect was prolonged. The potentiation of the effect of angiotensin on membrane

activity by excess sodium, may be related to the increase in mechanical response of the guinea-pig ileum in excess sodium caused by angiotensin (Blair-West and McKenzie, 1966) and vice versa.

When excess potassium had increased the frequency of spike discharge and tension, angiotensin caused a small further acceleration of spike discharge associated with a transient increase in tension. This observation may be related to the effects of acetylcholine and carbachol described by Evans, Schild and Theseleff (1953) and Edman and Schild (1961, 1962) on the completely depolarized membrane, suggesting a change in calcium permeability. When smooth muscle is completely depolarized by isotonic potassium chloride or sulphate solution, there is an initial contracture, which is followed by partial or complete relaxation (Durbin and Jenkinson, 1961a). Under these conditions the muscle still produces a fully reversible contractile response to the administration of acetylcholine (Schild, 1964). Using tracer studies, Durbin and Jenkinson (1961b) have shown that there is an increase in influx and efflux of ^{42}K, ^{36}Cl and ^{82}Br and also changes in ^{24}Na and ^{45}Ca fluxes on addition of carbachol to depolarized smooth muscle, suggesting an increase in membrane permeability to all these ions.

The changes in membrane activity following the administration of angiotensin or acetylcholine to the taenia in excess potassium are in agreement with the results of Burnstock (1968) and of Bülbring and Kuriyama (1963) using acetylcholine. It

should be pointed out that although the changes in membrane activity following the administration of angiotensin or acetylcholine to the taenia coli in excess potassium are in agreement with the results for acetylcholine of Burnstock (1958) and of Bülbring and Kuriyama (1963), the increase in tension of the taenia induced by angiotensin or acetylcholine in excess potassium was not maintained, even though the membrane remained depolarized. No adequate explanation could be given for this observation. Nevertheless, these results suggest that the mechanical response induced by angiotensin or acetylcholine does not solely depend upon membrane depolarization but also upon spike activity. This hypothesis is supported by the observation that large phasic contractions were superimposed on the relaxed tension, and are closely associated with spike discharge (see Fig. 26). It is known that excess potassium can reverse the excitatory effect of acetylcholine (Burnstock, 1958). However, such reversal occurs only with a potassium concentration some eight to ten times higher than normal and about three times higher than the concentration used in this study. Furthermore, the relaxation observed in this study was not associated with hyperpolarization.

The effects of angiotensin and acetylcholine were potentiated in excess calcium and spike amplitudes were enhanced. These observations strengthen the suggestion that angiotensin also increases the permeability to ions other than sodium, and are consistent with the hypothesis that calcium rather than sodium ions are the main carrier of the inward current for

spike generation in smooth muscle of the taenia (Brading et al 1969; Kuriyama and Tomita, 1970).

In the superfused rat uterus and dog carotid artery, angiotensin has been shown to increase sodium-efflux but not influx and the effect can be blocked by low concentrations of ouabain (Türker, Page and Khairallah, 1967). Because of the sensitivity to ouabain, these authors postulated that angiotensin stimulates the sodium pump. However, angiotensin has also been shown to cause an increase in sodium-influx in the epigastric vascular bed (Freidman, 1972) and rat uterus (Hamon and Worcel, 1973). These later authors postulated that angiotensin acts through an increase of sodium permeability of the membrane.

In normal physiological salt solution, acetylcholine and angiotensin depolarized the membrane and upon washing, the membrane repolarized and then hyperpolarized. This latter phase of repolarization after washing out of acetylcholine is thought to be related to active sodium extrusion (Bülbring and Burnstock, 1960 and Bülbring and Kuriyama, 1963) which involves an electrogenic sodium pump (Keynes, 1960). This hypothesis is supported by the recent observation that ouabain or potassium-free solution abolishes the hyperpolarization following stimulation of guinea-pig ileum by acetylcholine (Bolton, 1971,1973). Ouabain or removal of potassium is known to inhibit sodium-pump activity (Schatzmann, 1953; Skou, 1965). In this study, similar hyperpolarization was observed after the excitation by

angiotensin on the taenia. The experiments described in this chapter, however, were not designed to test whether a similar electrogenic extrusion of sodium due to the activation of the sodium pump was responsible for the after-hyperpolarization observed following angiotensin stimulation. Nevertheless the pattern of changes in membrane activity, such as depolarization, repolarization and spike discharge, induced by angiotensin is so similar to that by acetylcholine, that it is tempting to suggest an analogous ionic basis is involved during angiotensin action. This suggestion is favoured by the observation that, excess sodium or calcium enhanced the effects of angiotensin and acetylcholine. Moreover, both in sodium excess and calcium excess, the after-hyperpolarization was greater. This could be due to the increased activity of the sodium pump, caused by the increased intracellular sodium accumulation during prolonged excitation. The existence of an electrogenic sodium-pump has recently been demonstrated in the taenia coli (Casteels, Droogmans & Hendrickx, 1971). Therefore, an action of angiotensin on the sodium-pump as suggested by Turker _et al_ (1967) may not be the peptide's primary mode of action but rather as a consequence of the increase in sodium permeability induced by angiotensin. This suggested mechanism of angiotensin action is in accord with the general consensus that angiotensin causes a transient increase in the passive permeability of the cell membrane mainly to sodium, and to a lesser extent potassium and chloride and calcium ions. (Friedman and Friedman, 1964; Friedman, 1972; Hamon and Worcel, 1973).

In conclusion, the primary action of angiotensin on the cell membrane seems to be an increase in membrane permeability mainly to sodium and to a lesser extent to potassium, calcium and chloride ions. The increased intracellular concentration of sodium ions then accelerates the activity of the sodium-pump, resulting in a temporary increase in membrane potential. These electrical activities are closely associated with changes in muscle tension.

It should be emphasized that the conclusions arrived in this study are based on the well established ionic basis of acetylcholine action on smooth muscle cells. Because of the inherent difficulties of sucrose-gap recordings (see Introduction), these results should be supplemented with microelectrode studies. The application of the double sucrose-gap technique would be of advantage in elucidating the ionic currents involved and sodium pump activity in angiotensin action.

PART THREE

STUDIES TO DETERMINE THE POSSIBLE INVOLVEMENT OF PROSTAGLANDINS IN THE CONTRACTILE ACTION OF ANGIOTENSIN

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INTRODUCTION

The involvement of a neurogenic component in the contractile action of angiotensin in some intestinal smooth muscles has been fully discussed in Part One of this thesis. In recent years, the experimental evidence of a number of workers has indicated that the pharmacological actions of angiotensin both *in vivo* and *in vitro* may at least in part be mediated via a neurogenic component, involving the sympathetic nervous system. Bickerton and Buckley (1961) suggested that part of the pressor activity of angiotensin in the dog is a result of stimulation of centres having a controlling influence on the sympathetic nervous system within the hypothalamus. Furthermore, the vasoconstrictor action of angiotensin in isolated organs has been shown to be dependent upon an intact sympathetic innervation (Zimmerman, 1962; Laverty, 1963; Benelli *et al*, 1964; Owen, 1969). The precise mechanisms of interaction between angiotensin and the peripheral and central divisions of the sympathetic nervous system have not been fully ellucidated (see Owen, 1969; Gross, 1971).

Angiotensin will stimulate sympathetic ganglia and cause the release of catecholamines from the adrenal medulla (Lewis and Reit, 1966). The most recent evidence suggests that angiotensin may have yet other indirect actions involving the release of prostaglandins.

Angiotensin has been shown to cause a release of prostaglandin-like substances (mainly PGE_2) when infused through kidneys of the dog (McGiff, Crawshaw, Terragno and Lonigro, 1970; Aiken and Vane, 1971) and rabbit (Needleman, Kauffman, Douglas, Johnson and Marshall, 1973a)

and the spleen of the cat (Peskar and Hertting, 1973; Ferreira, Moncada and Vane, 1973) and rabbit (Douglas, Johnson, Marshall, Jaffe and Needleman, 1973). Furthermore, angiotensin has been shown to release PGE_2 from the splenic fat pad and mesenteric vascular bed of the rabbit (Needleman, Marshall and Douglas, 1973b). This release of prostaglandins can be blocked by prior treatment of these various tissues with indomethacin, which is a potent prostaglandin biosynthesis inhibitor (Vane, 1971).

However, the precise relationship between prostaglandin-release and angiotensin infusion is not clear. From his results on cat spleen, Hedqvist (1969, 1970) suggested that PGE_2 released during splenic contraction following nerve stimulation, had a homeostatic function, reducing both the amount of noradrenaline released from sympathetic neurones and its effects on the smooth muscle. This conclusion is in agreement with the suggestion of Bergström (1967) that prostaglandins may act as mediators of a local feedback mechanism. Both angiotensin and the catecholamines have profound vasoconstrictor effects and since the vasoconstrictor effect of angiotensin is dependent upon a functional sympathetic nervous system (see Introduction) a similar function could be ascribed to the prostaglandins released by angiotensin. However, it must be remembered that prostaglandins may be released by inert substances under conditions when there is no vasoconstrictor or contractile action (Gilmore, Vane and Wylie, 1969) or as the result of tissue damage or inflammation (Horton, 1969).

Prostaglandins of the E series are potent vasodilators (see Horton, 1971). PGE_1 has been shown to antagonize the pressor effect of noradrenaline, angiotensin and vasopressin in the rat (Holmes, Horton and Main, 1963). Prostaglandin release after infusion of vasoconstrictor drugs like angiotensin seems to agree with the general hypothetical role for prostaglandins as modulators of neurohumoral transmission and of hormonal action (see Hedqvist, 1970; Wenmalm and Stjarne, 1971 and McGiff and Hskovitz, 1973). This hypothesis is supported by the observation that inhibition of prostaglandin synthesis, augments the vascular reaction of the cat spleen to angiotensin (Peskar and Hertting, 1973; Ferreira et al, 1973) and vasopressin (Peskar and Hertting, 1973). Furthermore, the injection or topical application of prostaglandins is known to attenuate the change in activity of various smooth muscle preparations following nerve stimulation, noradrenaline or angiotensin (Holmes et al, 1963; Weiner and Kaley, 1969). McNeil and Sutherland (1973) continuously infused angiotensin into the superior mesenteric artery of the cat and observed an initial vasoconstriction followed by a partial recovery. The partial recovery was attributed to the release of prostaglandins from the vascular bed (Needleman et al, 1973b). Furthermore, Messina, Weina and Kaley (1973) observed that inhibition of prostaglandin synthesis, potentiated the arteriolar constrictor responses to angiotensin in the rat cremaster muscle. These authors suggested that the direct action of prostaglandins on smooth muscle is at least as important a homeostatic mechanism as is the reduction in noradrenaline release.

Angiotensin and noradrenaline are both able to constrict the blood vessels of the rabbit fat pad (with the spleen removed) and mesenteric vasculature but it is only angiotensin which causes a demonstrable release of prostaglandins (Needleman et al, 1973b). Furthermore, angiotensin and prostaglandin, but not noradrenaline, have been shown to cause an increase in dermal vasculature permeability in the rabbit (Robertson and Khairallah, 1972). From these and other findings, Needleman et al (1973b) suggested that angiotensin acted on precapillary vascular beds to release prostaglandins which in turn would alter capillary permeability, they also concluded that prostaglandins of the E series can contribute to the local adjustments in blood flow by direct relaxation of vascular smooth muscle.

It is apparent that the precise mode of action of angiotensin in vivo and in vitro may be more complex than realized hitherto. The evidence presented in this section adds support to the claim of an involvement of the prostaglandin system in angiotensin action.

Prostaglandins are released from various intestinal smooth muscles of different species and under different conditions. They are relased from the frog intestine (Vogt and Distelkotter, 1967) rat stomach (Bennett, Friedman and Vane, 1967) rabbit jejunum (Ferreira, Herman and Vane, 1972) and guinea-pig ileum (Botting and Salzman, 1974). An increase in prostaglandins output has also been reported in the guinea-pig ileum (Botting and Salzman, 1974) and rat stomach (Bennett et al, 1967) during electrical stimulation of nerves in the muscle wall. These observations seem to be in agreement with

the suggestion that prostaglandins are involved in the control of gastro-intestinal activity (Horton, 1969).

In view of the observations presented above, it seems pertinent to examine the possible involvement of prostaglandins or a prostaglandin-system in the angiotensin-induced contractile response in smooth muscles. The possibility that part of the angiotensin contraction in some smooth muscle may be mediated by yet some unknown mediators has been discussed in the preceding section. In subsequent chapters, experiments are described which attempt to test this hypothesis in the hope of bringing new evidence to light which may contribute to the present knowledge of interaction between angiotensin and prostaglandins.

CHAPTER I THE EFFECT OF PROSTAGLANDIN BIOSYNTHESIS INHIBITORS ON THE CONTRACTILE RESPONSES TO ANGIOTENSIN AND OTHER AGONISTS ON ISOLATED SMOOTH MUSCLE PREPARATIONS

The possible involvement of prostaglandins in some of the multiplicity of angiotensin actions has been discussed. The release of prostaglandins or prostaglandin-like substance (PLS) from several isolated organs following nerve stimulation or stimulation by drugs can be inhibited by prior administration of the non-steroid anti-inflammatory agents such as indomethacin and aspirin (Aiken and Vane, 1971; Ferreira, Moncada and Vane, 1971; Douglas et al, 1973) in concentrations which are known to inhibit prostaglandin biosynthesis (Vane, 1971). Indomethacin has also been shown to cause a direct relaxation of isolated rabbit jejunum, an action which appears related to its ability to inhibit prostaglandin biosynthesis (Ferreira, Herman and Vane, 1972).

In using indomethacin as a prostaglandin biosynthesis inhibitor for use with isolated smooth muscle preparations it should be remembered that it will antagonize the contractions of a number of smooth muscle preparations to a variety of agonists (Northover, 1967). This action which is seen with higher concentrations than those necessary to inhibit prostaglandin biosynthesis appears to be related to an inhibition of the entry of calcium ions into the muscle cells (Northover, 1971).

RESULTS

1.1. Effect of indomethacin and aspirin on the resting tone of intestinal and vascular smooth muscle preparations

(Guinea-pig ileum and taenia coli; rat stomach fundus strip and ileum; rabbit aortic strip and rat portal vein)

Concentrations of indomethacin (2.8 - 11.2 x 10^{-5}M) or aspirin (>20 x 10^{-5}M) added to the bath and left in contact with the preparations for 15 to 20 minutes caused a progressive reduction in resting tone and at the same time reduced the spontaneous activity of the rat portal vein and the guinea-pig taenia coli. The spontaneous activity of the guinea-pig ileum which is often observed in the resting period, was also occasionally suppressed. After removal of indomethacin and upon repeated rinsing of the tissues with normal physiological saline solution, the tension and spontaneous activity gradually returned and reached the initial state in 15 to 30 minutes.

1.2. Effect of indomethacin and aspirin on the contractile responses to angiotensin and other agonists on isolated smooth muscle preparations

Concentrations of indomethacin (2.8 - 11.2 x 10^{-5}M) kept in contact with the preparations for a minimum of 20 minutes caused a selective reduction of angiotensin contractions in all the tissues used with the exception of the rat colon, where no inhibitory effect was observed. Aspirin was about 5 to 10 times less effective in antagonizing the angiotensin-induced contractile responses. The relative potency of indomethacin and aspirin in reducing angiotensin contraction is in agreement with their

ability to inhibit prostaglandin synthesis (Vane, 1971). For these reasons, indomethacin was mainly used for subsequent studies.

Indomethacin (2.8 - 5.6 x 10^{-5}M) was most effective in reducing angiotensin-induced contractions on the guinea-pig ileum. Indomethacin (5.6 x 10^{-5}M) reduced by about 50% submaximal contractile responses to angiotensin in the guinea-pig ileum, rat stomach strip and rat ileum (for precise values see Table XII). The inhibition by indomethacin of the angiotensin-induced contractile responses in these preparations was progressive. Figure 31 illustrates the effect of indomethacin (5.6 x 10^{-5}M) to a submaximal contractile response to angiotensin and acetylcholine over a 2 hour period on the guinea-pig ileum.

Indomethacin (2.8 - 11.2 x 10^{-5}M) did not cause a significant reduction of submaximal contractions to angiotensin in the guinea-pig taenia coli (mean 20%, 4 preparations). The same concentrations of indomethacin showed either no inhibitory action (4 preparations) or a slight potentiation (3 preparations) of the contractile responses to angiotensin or acetylcholine on the rat colon.

Table XII summarizes the results of the effect of indomethacin on the contractile responses to angiotensin and other agonists on the intestinal and vascular smooth muscle preparations used. Detailed descriptions of the effects of indomethacin on angiotensin-induced contractions of the guinea-pig ileum, rabbit aortic strip and rat portal vein is given below.

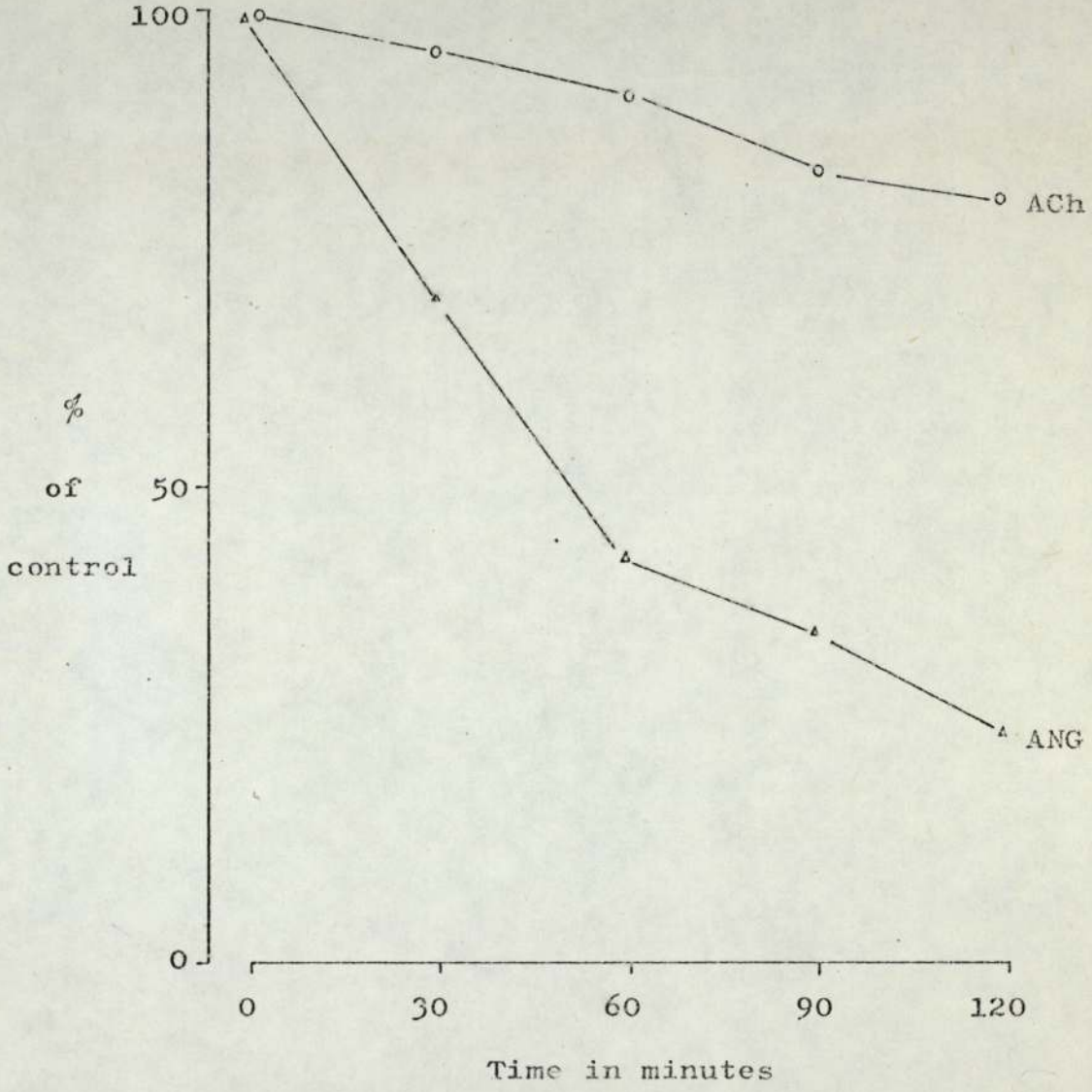

Fig. 31. The effect of indomethacin (5.6×10^{-5}M) on the contractile responses to angiotensin (△—△)(ANG) and acetylcholine (○—○)(ACh) over a 2 hour period, on the guinea-pig ileum. Each point is the mean of 4 observations. Control response corresponds to 75% of maximum response of each agonist. Values for angiotensin are the mean of the fast and slow component.

	Agonists					n
	Angiotensin	Acetycholine	Histamine	Bradykinin	Noradrenaline	
Guinea-pig ileum Fast Slow	49.7± 4.8 54.2± 6.1	16.8±3.5	19.3±4.7	31.7±2.3	-	8
Rat ileum Fast Slow	33.4± 2.0 49.2± 1.6	10.7+5.0	-	-	-	4
Rat stomach strip	38.1± 4.6	12.4±3.2	-	26.3±3.5	-	6
Rat colon	0.0 ± 7.2	0.0±6.3	-	-	-	5
Guinea-pig taenia coli	18.2± 2.8	13.1±4.2	-	-	-	7
Rabbit aortic strip	38.5± 3.3	-	-	-	6.3±4.1	4
Rat portal vein	48.4± 4.7	38.6±6.8	-	-	42.5±2.1	4

Table XII. Effect of indomethacin (5.6×10^{-5}M) on the myotropic action of various agonists on intestinal and vascular smooth muscle preparations. Each value is the % inhibition of a submaximal contraction (corresponding to about 75% of maximum response) to each of the agonist. ± standard error of mean. Further details see text.

1.2.1. Guinea-pig ileum

Indomethacin was about 5 times more effective in reducing contractions due to angiotensin than those due to acetylcholine, histamine or bradykinin (Fig.32). The concentration of indomethacin (10^{-4}M) effective in inhibiting histamine and bradykinin responses is about the same as that reported by Northover (1971) for the guinea-pig stomach strip and by Sorrentino, Capasso and Rosa (1972) for the guinea-pig ileum and rat uterus.

Indomethacin (5.6×10^{-5}M) caused a marked reduction of angiotensin contraction over the whole range of concentration used (Fig.33a). The only effect seen against acetylcholine was a small depression of the maximum response (Fig.33b).

Contractions to each of the agonists used, each causing about 75% of maximum response, were reduced by indomethacin (5.6×10^{-5}M) in the following way: angiotensin 52.0 $\pm$ 5.5% (mean of fast and slow components), bradykinin 31.7 $\pm$ 2.3% , histamine 19.3 $\pm$ 4.7% and acetylcholine 16.8 $\pm$ 3.5% (see Table XII , Fig.34 and Fig. 35).

1.2.2. Rabbit aortic strip and rat portal vein

The effect of indomethacin on various agonists on the rabbit aortic strip and rat portal vein is shown in Figs. 36 and 37respectively. The selectivity of indomethacin inhibition of angiotensin action on vascular tissues was less marked than that for intestinal tissues, for example on the rabbit aortic strip, indomethacin (5.6×10^{-5}M) caused a depression of the maximum

response to angiotensin by 38.5 $\pm$ 3.3% but was without effect on the responses to noradrenaline or adrenaline. Indomethacin was least selective on the rat portal vein preparation (Fig. 37). The submaximal responses to the various agonists being reduced by : angiotensin 48.4%, noradrenaline 42.5% and acetylcholine 38.6% (n = 4).

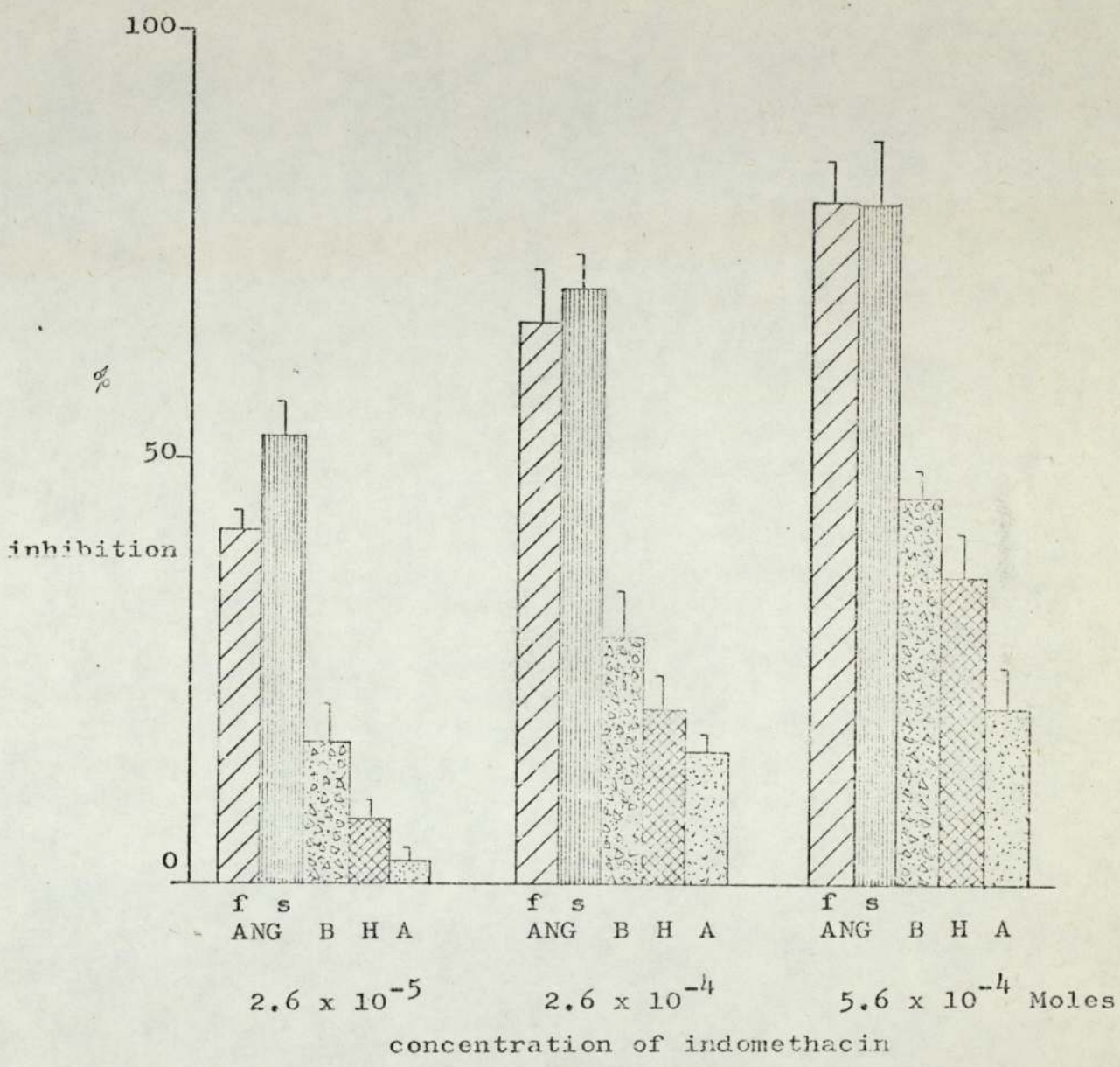

Fig. 32. Effect of indomethacin on the contractile responses of guinea-pig ileum to angiotensin (2×10^{-8}M)(ANG), bradykinin (4×10^{-8}M)(B), histamine (2×10^{-8}M)(H) and acetylcholine (4×10^{-8}M)(A). % inhibition was calculated on the preceding response without indomethacin. Responses to angiotensin indicated as fast (f) and slow (s) components. Vertical bar S.E.M. (n = 4).

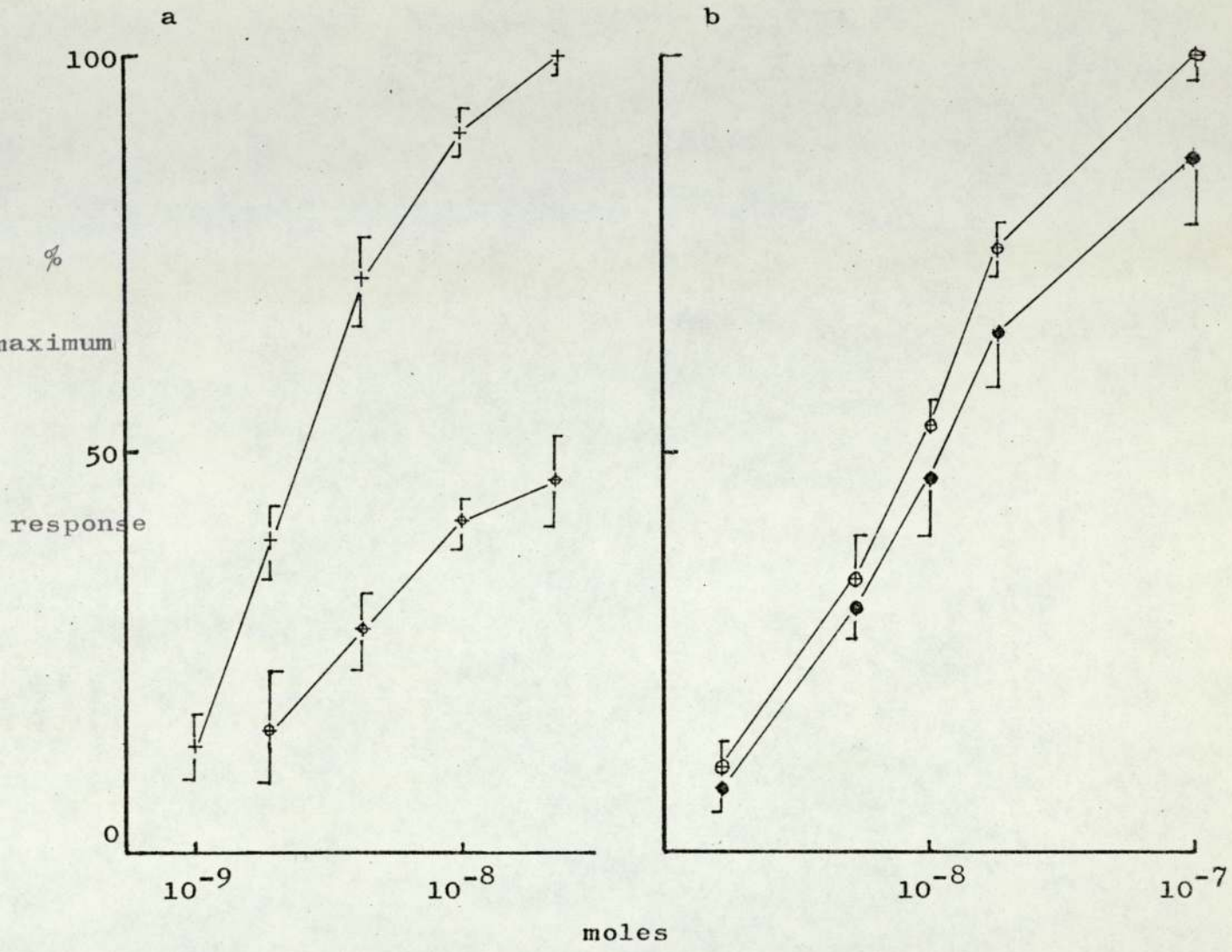

Fig. 33. Effect of indomethacin on the increase in tension of guinea-pig ileum preparation to a)angiotensin and b) acetylcholine. +—+ ; ⊕—⊕ ; control responses to angiotensin and acetylcholine respectively. ◆—◆ ; ●—● ; responses to angiotensin and acetylcholine in the presence of indomethacin (5.6×10^{-5}M) which had been left in contact with the tissue for a minimum period of 20 minutes.

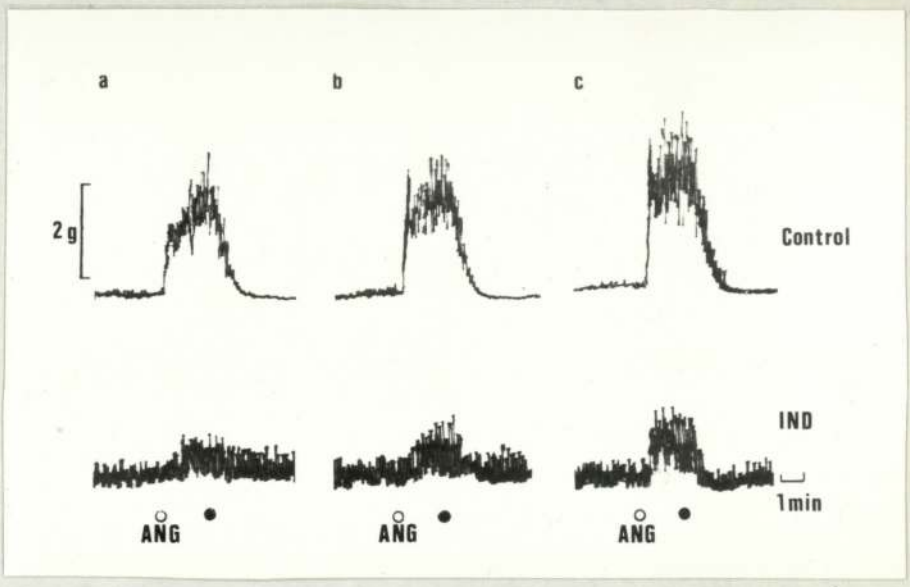

Fig. 34. Contractile responses of isolated guinea-pig ileum to angiotensin (ANG). Upper record = control responses to ANG, (a) 5×10^{-9}M, (b) 5×10^{-8}M and (c) 10^{-7}M. Lower record = responses repeated 30 minutes after indomethacin (5.6×10^{-5}M)(IND) was added to the bath. Angiotensin was added at open circles and removed at closed circles.

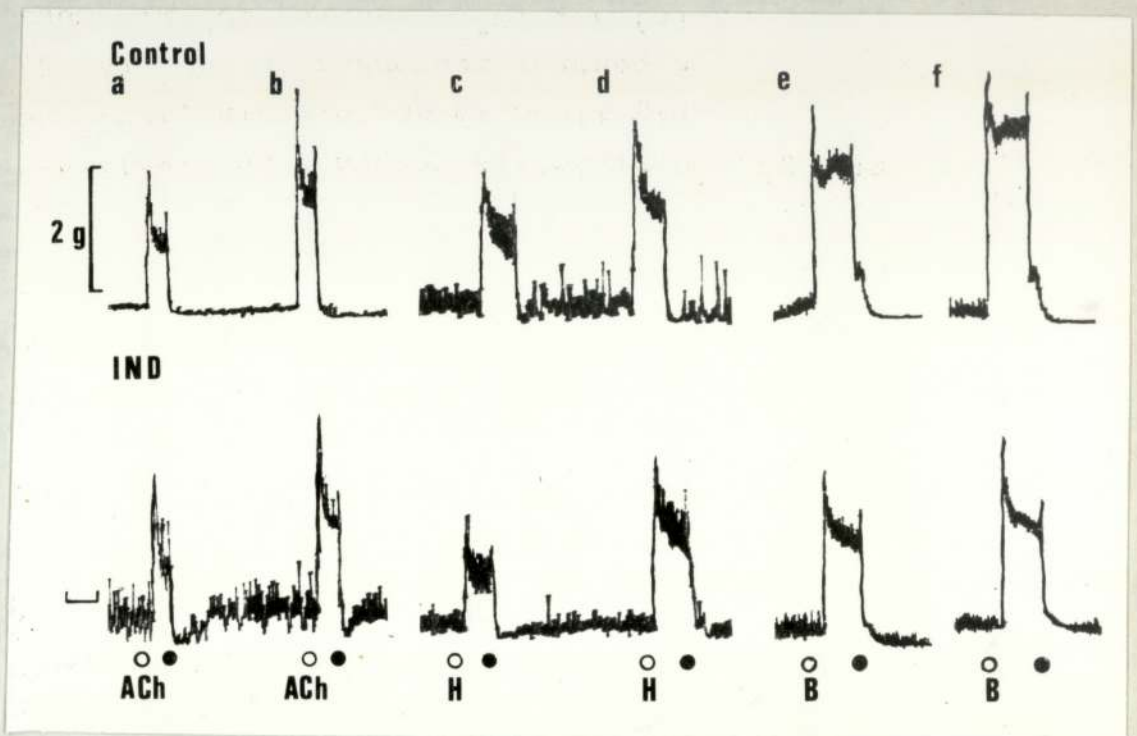

Fig. 35 . Contractile responses of guinea-pig ileum to acetylcholine (ACh), histamine (H) and bradykinin (B). Upper record = control responses to (a) 8 x 10^{-9}M, (b) 6 x 10^{-8}M of ACh; (c) 1.6 x 10^{-8}M, (d) 3.3 x 10^{-8}M of H and (e) 10^{-8}M, (f) 10^{-7}M of B. Lower record = responses repeated 30 minutes after indomethacin (5.6 x 10^{-5}M)(IND) was added to the bath. Drugs were added at open circles and removed at closed circles. Time marker = 30 s.

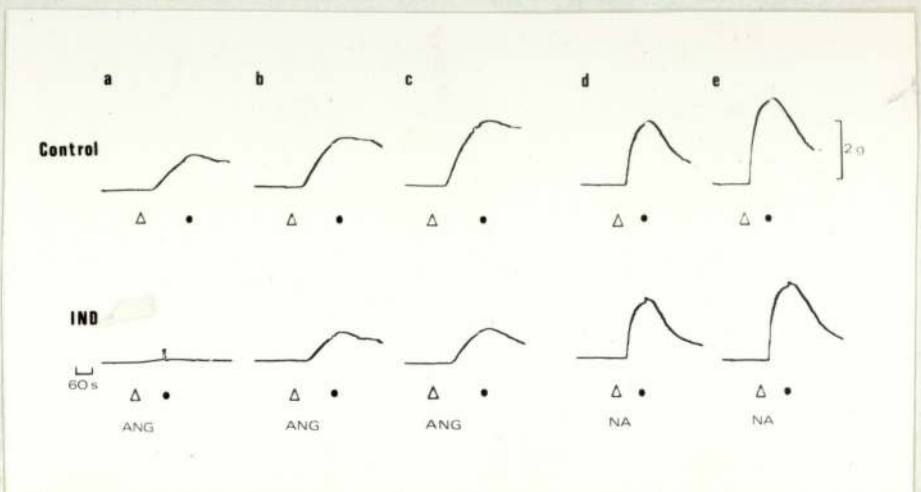

Fig. 36. Isometric contractile responses of rabbit aortic strip to angiotensin (ANG) and noradrenaline (NA). Upper record = control responses to (a) 5 x 10^{-9}M (b) 2 x 10^{-8}M (c) 2 x 10^{-7}M ANG, and (d) 5.6 x 10^{-8}M (e) 2.6 x 10^{-7} NA. Lower record = responses repeated 30 minutes after indomethacin (5.6 x 10^{-5}M)(IND) was added to the bath. Drugs were added at Δ and removed at ●.

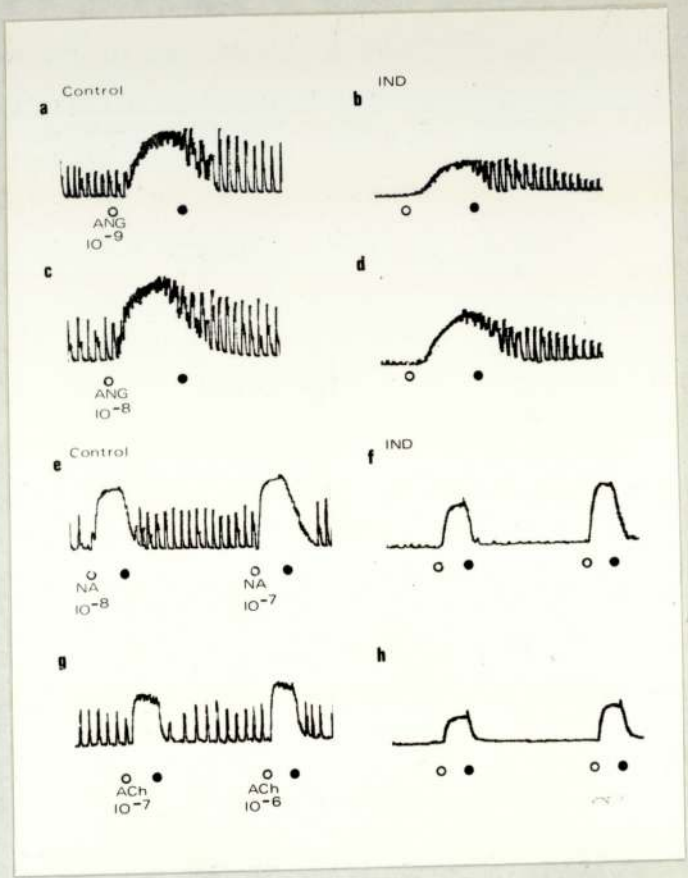

Fig. 37 . Isotonic contractile responses of isolated rat portal vein to angiotensin (ANG), noradrenaline (NA) and acetylcholine (ACh). Left panel = control responses to ANG (a & c), NA (e) and ACh (g). Right panel = responses repeated 20 minutes after indomethacin (2.8×10^{-5}M)(IND) was added to the bath. Drugs were added at open circles and removed at closed circles.

DISCUSSION

The results reported in this chapter show that indomethacin which is an aspirin-like anti-inflammatory drug has a selective inhibitory action on the contractile response to angiotensin on intestinal and vascular smooth muscle. Indomethacin will also antagonize the contractile responses of these smooth muscle preparations to a variety of agonists, but the concentration of indomethacin required is considerably higher than that required for angiotensin antagonism with the exception of rat portal vein. The relatively low concentration of indomethacin used in inhibiting angiotensin contractions suggests that it is acting here by a mechanism other than preventing the influx of calcium into cells (Northover, 1971).

Recently, it has been shown that indomethacin can inhibit the biosynthesis and thus the release of prostaglandins from isolated spleens and kidneys of various species (Vane, 1971; Ferreira et al, 1971; Douglas et al, 1973) an action which appears related to its ability to inhibit the enzyme dioxygenase necessary for prostaglandin-E_2 and $F_{2-\alpha}$ synthesis (Smith and Lands, 1971). The inhibition of this enzyme is effected by a concentration of indomethacin about 40 times less than those reported in this chapter to cause inhibition of angiotensin contractions. Similar low concentrations of indomethacin which inhibit the synthesis of prostaglandins in spleen and kidney (Douglas et al, 1973) are without effect on the responses to angiotensin and other agonists on the rabbit aortic strip and rat

stomach strip (Park, Regoli and Rioux, 1973). However, the concentrations of indomethacin used in this study are within the concentration range for plasma levels reported in man and rat after an oral dose of 200 mg and 10 mg respectively (Hucker, Zacchei, Cox, Brodie and Cantwell, 1966). This concentration of indomethacin is known to inhibit carrageenin-induced oedema in vivo in the rat (Di Rossa and Willoughby, 1971). Recently, various authors have reported that indomethacin, in similar concentrations used in the present study, effectively inhibit the synthesis of prostaglandins in isolated intestinal smooth muscle preparations (Ehrepreis, Greenberg and Belman, 1973; Botting and Salzman, 1974). This disparity in the concentrations of indomethacin used in intestinal smooth muscle studies and in perfused organs, probably reflects regional differences in prostaglandin-synthetase activity (Flower and Vane, 1972; Flower, Grygleswski, Herbaczynskacedro and Vane, 1972).

The reduction in angiotensin contractions coincident with indomethacin does not appear to be due to tachyphylaxis, as no significant changes in angiotensin contractions were observed in adjacent tissues dosed concurrently. The inhibitory effect of indomethacin on angiotensin contraction is dependent upon the duration of exposure and the concentration of indomethacin added to the bath. A minimum contact time of 20 minutes is necessary for any significant reduction of angiotensin-induced contractions. The degree of inhibition progresses with time. This contrasts strongly with its inhibitory effect on other agonists, where inhibition was observed 5 minutes after adding indomethacin to the bath.

The contact time required by indomethacin to reduce angiotensin contractions is of about the same order as that required to cause a significant reduction in prostaglandin-E_2 output induced by adrenaline in the dog spleen (Ferreira et al, 1971) and in the spontaneous release of prostaglandin-E_2 from rabbit jejunum (Ferreira et al , 1972).

The circumstantial evidence presented here together with the results of other workers cited, suggest that the selective inhibition of angiotensin by indomethacin is probably due to its ability to inhibit prostaglandin synthesis. In this chapter, aspirin has been shown to reduce angiotensin contraction but only with a concentration some 10 times that of indomethacin. This seems to agree with the relative potency of aspirin and indomethacin in inhibiting prostaglandin-E_2 and $F_{2-\alpha}$ synthesis (Ferreira et al, 1971; Vane, 1971).

Contractile responses to angiotensin on the rat colon and guinea-pig taenia coli were not significantly affected by indomethacin. These results suggest that prostaglandins of the E or F series may not be involved in angiotensin action in these tissues and would add support to the specificity of angiotensin receptors on the rat colon and guinea-pig taenia coli (see Part I). However, it must be considered that indomethacin is less effective against the synthesis of PGE_1 and $PGF_{1-\alpha}$ from dihomo-γ-linolenic acid than it is against PGE_2 and $PGF_{2-\alpha}$ from arachidonic acid (Vane, 1971; Ferreira et al, 1971 and Introduction).

Furthermore, the rat colon has been shown to be rather insensitive to PGE_1 and PGE_2, but is very responsive to $PGF_{1\alpha}$ and $PGF_{2-\alpha}$ (Vane, 1971). Therefore, the possible involvement of prostaglandins in the contractile response induced by angiotensin in these preparations remains. It is not possible to test this hypothesis until specific prostaglandin antagonists become available.

In summary, the results presented in this chapter, show that indomethacin selectively antagonizes the action of angiotensin on the smooth muscle preparations used with the exception of the rat colon, guinea-pig taenia coli and rat portal vein. The relatively low concentrations of indomethacin used, the necessity to use a long contact time and selectivity for angiotensin all suggest that indomethacin may be acting here by preventing prostaglandin synthesis.

CHAPTER II FURTHER STUDIES TO DETERMINE THE RELATIONSHIP BETWEEN ANGIOTENSIN ACTION AND PROSTAGLANDINS IN SMOOTH MUSCLE PREPARATIONS

In the preceding chapter, contractile responses of a number of smooth muscle preparations to angiotensin and other agonists were shown to be inhibited by indomethacin and aspirin. The most important aspect of these results is that indomethacin and aspirin can inhibit angiotensin-induced contractions at a concentration much less than that required to reduce contractions induced by other agonists. The underlying mechanism for the selective inhibition by aspirin-like drugs was discussed in the light of recent findings that these drugs can prevent release of prostaglandins from several perfused organs (Vane, 1971) presumably because of their ability to inhibit dioxygenase an enzyme necessary for prostaglandin E_2 and $F_{2-\alpha}$ synthesis (Smith and Lands, 1971; Flower, 1974).

If it is accepted that the inhibition of angiotensin contraction by indomethacin or aspirin is due to their effect on prostaglandin biosynthesis, then this suggests that part of the contractile response to angiotensin in these smooth muscle preparations is dependent upon the presence or the release of prostaglandins. This hypothesis was further examined on the isolated guinea-pig ileum and rat portal vein preparations.

RESULTS

2.1. The effect of prostaglandins on the guinea-pig ileum

Prostaglandin E_2 and $F_{2-\alpha}$ were implicated in the contractile response of angiotensin on the guinea-pig ileum (see preceding chapter). The effects of PGE_2 and $PGF_{2-\alpha}$ were therefore examined on this preparation. PGE_2 or $PGF_{2-\alpha}$ caused a dose-dependent contractile response. $PGF_{2-\alpha}$ was less active than PGE_2 on the guinea-pig ileum. The threshold concentrations of PGE_2 and $PGF_{2-\alpha}$ were 1.4×10^{-9}M and 2.8×10^{-8}M respectively.

The contractile responses induced by PGE_2 and $PGF_{2-\alpha}$ were characterized by a delay in onset of 15 - 30 seconds. This latent period progressively reduced with increasing dose but was not completely eliminated by higher concentrations of prostaglandins. The contraction was progressive, reaching a maximum in between 60 - 90 seconds (Fig. 38). In this respect, the contractile response of prostaglandin very much resembles the time course of angiotensin-induced contractions (see Part I).

2.2. The effect of indomethacin on prostaglandin-induced contractions

Sorrentino, Capasso and Di Rosa (1972) reported that indomethacin was about 4 times more effective in producing an inhibition of PGE_2 contractions than histamine responses on the

guinea-pig ileum. The effect of indomethacin on PGE_2 contractile response was therefore examined on this preparation.

Concentrations of indomethacin (2.8×10^{-5} - 11.2×10^{-4}M) which caused a significant reduction of angiotensin contractions also reduced PGE_2 - induced contractile responses (Fig. 38). However, the inhibition of prostaglandin contractions was not as great as the inhibition of angiotensin responses but more than the inhibition of acetylcholine responses. The actual values of inhibition of responses around 50% maximum on the tissue were angiotensin 52% PGE_2 32% and acetylcholine 16%. Following removal of the indomethacin responses to PGE_2 and acetylcholine returned to control levels within 10 minutes, whereas those of angiotensin often required 15 to 20 minutes to return to control levels.

2.3. The effect of exogenous PGE_2 on the responses to angiotensin and acetylcholine

The addition of PGE_2 to the bath in concentrations of between 5.6×10^{-10}M and 2.8×10^{-9}M caused an enhancement to the contractile responses induced by all effective concentrations of angiotensin (Fig. 39). The potentiation was more marked with contractions induced by lower concentrations of angiotensin and furthermore, the fast component was potentiated more than the slow one. PGE_2 (2.8×10^{-9}M) added to the bath and left for 3 - 5 minutes, potentiated the fast and slow component of the

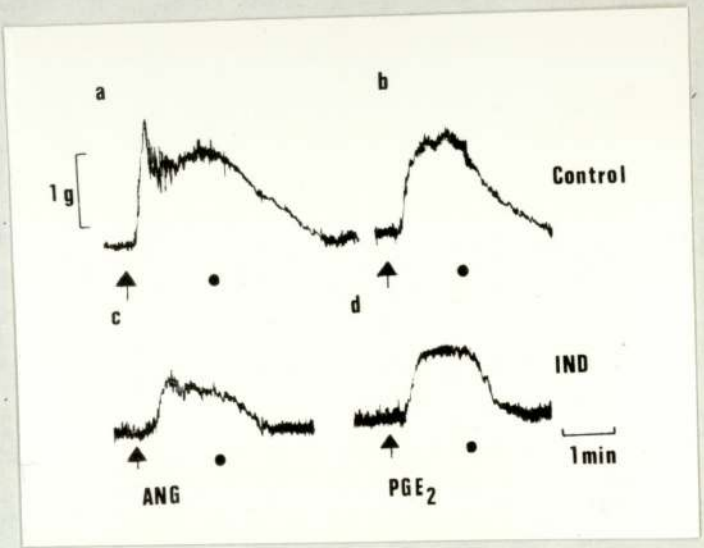

Fig. 38 . Contractile responses of isolated guinea-pig ileum to angiotensin (5×10^{-8}M)(ANG) and prostaglandin-E_2 (5.6×10^{-8}M)(PGE_2). Upper record = control responses (a) & (b). Lower record = responses repeated (c) & (d) 30 minutes after indomethacin (5.6×10^{-5}M) (IND) was added to the bath. Drugs were added at arrows and removed at closed circles.

angiotensin contraction of around 50% of maximum by 35.2 ± 9.6% and 26.7±3.6% respectively (n=5). The potentiating effect of PGE_2 on angiotensin responses decreases with time, such that if the same concentration of PGE_2 was left in the bath for a period longer than 10 minutes, the potentiation was virtually abolished. This observation suggests that there is a mechanism for inactivating PGE_2 in the tissue.

The same concentrations of PGE_2 (5.6×10^{-10}M - 2.8×10^{-9}M) which enhanced the contractile responses to angiotensin also caused a potentiation of contractions induced by acetylcholine (Fig.39). However, the degree of potentiation was less than that for the angiotensin contraction, causing only a 15.4 ±2.1% (n=5) potentiation of a submaximal contractile response to acetylcholine.

2.4. The effect of PGE_2 on the inhibition of angiotensin response by indomethacin

Low concentrations of PGE_2 (2.8×10^{-9}M - 2.8×10^{-8}M) added to the bath containing the guinea-pig ileum which had been exposed to indomethacin for periods of more than 30 minutes, invariably caused a small but sustained increase in tension of the tissue that lasted for about 5 minutes. During this period, the inhibition by indomethacin of angiotensin-induced contraction was reversed (Fig. 40).

For most experiments the concentration of PGE_2 used was 1.2×10^{-8}M, this restored the fast component from 46.1% to $95.4 \pm 5.5\%$ and the slow component from 52.8% to $88.2 \pm 5.7\%$ ($n = 6$) of the initial angiotensin response. Removal of PGE_2 resulted in the recurrence of the indomethacin inhibition. Exposure to indomethacin for more than 2 hours or increasing the concentration of indomethacin to 2.3×10^{-4}M, reduced this effect of exogenous PGE_2.

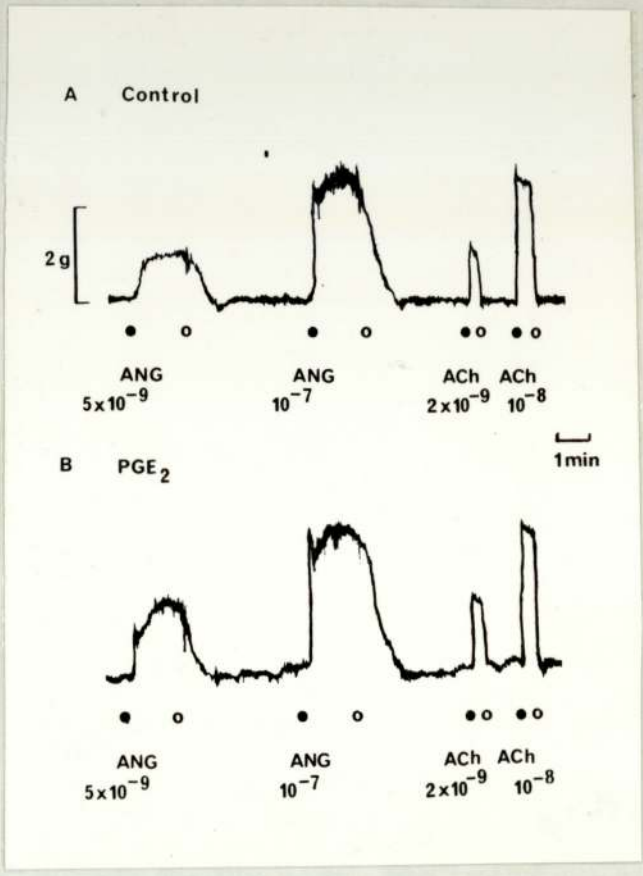

Fig. 39. Contractile responses to angiotensin (ANG) and acetylcholine (ACh) on isolated guinea-pig ileum: (A) control responses, (B) responses repeated in the presence of prostaglandin-E_2 (2.8×10^{-10} M)(PGE_2). Drugs added at closed circles and washed out at open circles.

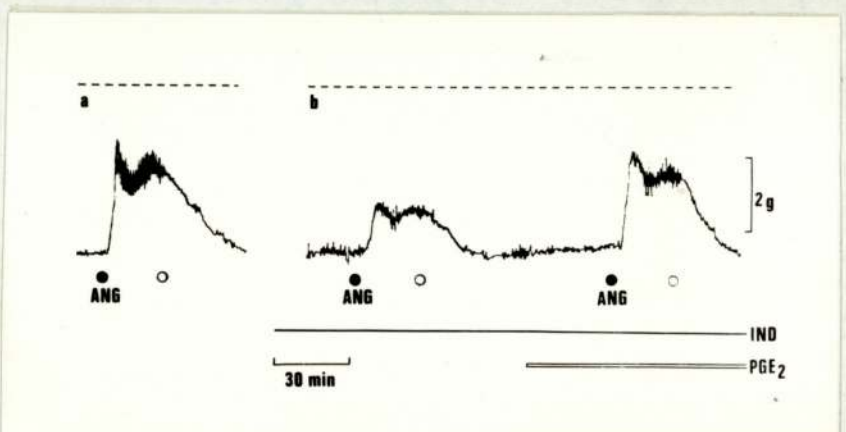

Fig. 40. Contractile responses of guinea-pig ileum to 5 x 10^{-8}M angiotensin (closed circles). Solid horizontal bar represents the presence of 5.6 x 10^{-5}M indomethacin (IND), the open horizontal bar represents the presence of 1.2 x 10^{-8}M prostaglandin-E_2 (PGE_2). Time marker at 10 s intervals.

DISCUSSION

One of the putative roles for prostaglandins is in the modulation of neurohumoral transmission and of hormonal action (Hedqvist, 1970; Bergstrom, 1967; Horton, 1969; Ferreira et al, 1973). It is therefore possible that some of the multiplicity of angiotensin actions could be due to the interaction of prostaglandins and angiotensin. An inter-relationship between angiotensin and prostaglandins has already been demonstrated in some smooth muscle preparations (Robertson and Khairallah, 1972; Needleman et al,1973a,b). The contractile response to angiotensin in some smooth muscle preparations has been shown to be inhibited by indomethacin (Chong and Downing, 1973) an action which appears related to the latter's ability to inhibit prostaglandin synthesis, (see preceding chapter).

The results reported here add further support to the suggestion that angiotensin-induced contraction may be partly mediated via prostaglandin release. These results were based upon the criteria for the indirect action of a drug involving the release of a secondary mediator (see Burn, 1968). These are 1) agents which inhibit the synthesis or procedures which cause a depletion of the proposed mediator should be able to reduce or abolish the effect of the drug, 2) the demonstration of an interaction between the drug and the proposed mediator, i.e. potentiation of one by the other and 3) the proposed mediator should be able to reverse the response which shows signs

of tachyphylaxis or which has been reduced due to depletion of the mediator. These criteria have been used by various workers in connection with their proposition of an indirect action of angiotensin in intestinal and vascular smooth muscle and also in whole animal studies.

Drugs which inhibit the synthesis of Ach such as hemicholium and drugs which deplete neuronal stores of noradrenaline like reserpine (Burn, 1968) are known to reduce the contractile and vasoconstrictor responses to angiotensin in some intestinal and vascular smooth respectively. (see Part I). The contractile response to angiotensin in the ileum of the rabbit and guinea-pig is enhanced in the presence of anticholinesterase agents (Khairallah and Page, 1961) and vasoconstrictor response to angiotensin in the perfused mesenteric blood vessels of the rat is increased by addition of noradrenaline or electrical stimulation of the sympathetic nerves (McGregor, 1965). Addition of noradrenaline can reverse angiotensin responses both in vivo and in vitro. Thus, the pressor response to angiotensin in aortic strips of the rat and guinea-pig (Liebau et al, 1966) are reduced by depletion of noradrenaline and restored when the stores are replenished. These observations led the respective authors to propose that the contractile action of angiotensin on intestinal smooth muscle is partly mediated via a release of ACh, and the constrictor or pressor response on vascular smooth muscle and cardiovascular system be mediated via a release of noradrenaline.

The experiments described in this and the preceding chapter show that the contractile response to angiotensin was selectively reduced after inhibition of prostaglandin biosynthesis by indomethacin. The concentration of indomethacin used in this study has recently been confirmed to inhibit prostaglandin E_2 synthesis in the isolated guinea-pig ileum (Botting and Salzman, 1974 and Ehrepreis et al, 1973). The contractile responses to angiotensin were enhanced by the addition of low concentration of prostaglandin E_2. The enhancement of angiotensin contraction by PGE_2 did not appear to be due to an increase in smooth muscle reactivity, since responses to ACh were not significantly potentiated. Furthermore, and perhaps the most significant was the observation that PGE_2 can reverse the inhibition by indomethacin of angiotensin-induced contraction in the guinea-pig ileum. These results are in accord with the criteria cited earlier, and indicate that angiotensin contractile response may be partly mediated via a release of prostaglandin-E_2, since $PGF_{2-\alpha}$ did not reverse the inhibition.

Similar interactions between angiotensin and prostaglandin system have been reported in some vascular smooth muscle preparations by various workers. Thus, Khairallah et al (1967) observed that PGE_1 enhanced the contractile response of cat carotid artery strips to angiotensin and 5-HT but not noradrenaline. An indirect action of 5-HT in vascular smooth

muscle has been reported (Born, 1970; Greenberg, Kadowitz, Diecke and Long, 1973). Recently, Greenberg et al (1973) reported that low concentrations of $PGF_{2-\alpha}$ potentiated contractile responses of canine vascular strips to angiotensin and 5-hydroxytryptamine (5HT), but had little effect on the responses to noradrenaline. These authors suggested that $PGF_{2-\alpha}$ may act to facilitate neurotransmitter release from sympathetic nerve endings by angiotensin and 5-HT, as well as enhancing vascular smooth muscle reactivity. However, these results can also be interpreted as prostaglandins having a direct action on smooth muscle ~~thus~~ such that the contractile actions of angiotensin and possibly 5-HT may be mediated via a release of prostaglandins, since $PGF_{2-\alpha}$ has a pressor effect in whole subject (see Horton, 1968).

The results presented here together with those of other authors suggest that a prostaglandin system is closely associated in the indirect action of drugs which involve the liberation of a secondary mediator, and that in some tissues, the mediator may be prostaglandin-E_2. It should be emphasized that indomethacin at the concentrations used had a slight inhibitory effect on the myotropic action of PGE_2 on the preparations studied. This inhibition would at least partially mask the inhibitory effect on prostaglandin synthesis of indomethacin. If angiotensin releases prostaglandins then indomethacin need not have a strong effect on synthesis in order to block angiotensin induced contractions.

Attempts to demonstrate potentiation of angiotensin contractile responses and reversal of indomethacin induced inhibition by PGE_2 and $PGF_{2-\alpha}$ in the rat portal vein proved negative. These results together with the lack of effect of indomethacin in reducing angiotensin contraction in the rat colon, might be attributed to a difference in angiotensin receptors in the rat.

In summary, since PGE_2 enhanced the angiotensin-induced contraction and reversed the inhibition by indomethacin, it was concluded that PGE_2 or a closely related prostaglandin was involved in angiotensin-induced contractile response in the guinea-pig ileum.

CHAPTER III ATTEMPTS TO DETERMINE THE SITES OF PROSTAGLANDIN ACTION AND RELEASE AND TO DETECT ITS PRESENCE FOLLOWING STIMULATION BY ANGIOTENSIN

In the preceding two chapters, contractile responses to angiotensin were shown to be reduced after blockade of prostaglandin biosynthesis and further evidence was presented which suggested a prostaglandin system was involved in angiotensin induced contractions of some smooth muscle preparations. It appears that part of the contractile response to angiotensin may in fact be mediated via a release of prostaglandin-E_2. In order to test this hypothesis further it is desirable to try and detect a release of prostaglandins from the tissues coincident with angiotensin action.

If one makes the assumption that part of the contractile component of angiotensin action is mediated by prostaglandin, then in order to understand more fully the mode of action of angiotensin, the site and mode of action of the prostaglandins need to be elucidated.

The results of previous experiments with indomethacin suggest that if prostaglandins are released they affect both the slow and fast components of angiotensin action. One of the possible sites for an involvement of prostaglandins in the fast component of action of angiotensin on the guinea-pig ileum is at

the intramural postganglionic cholinergic nerve endings, by facilitating the release of acetylcholine. It was therefore decided to examine the effect of inhibition of prostaglandin biosynthesis on the contractions of guinea-pig ileum induced by transmural electrical stimulation. Other experiments described in this chapter were further attempts to determine the possible sites of prostaglandin release and to detect their presence in the guinea-pig ileum following angiotensin stimulation.

RESULTS

3.1. The effect of indomethacin on responses induced by transmural electrical stimulation

The response of the isolated guinea-pig ileum to transmural electrical stimulation with the stimulus parameters applied (see Methods) consisted of 'spike' like contractions (see Fig. 15). These 'spike' like contractions were abolished in the presence of tetrodotoxin in a concentration of 10^{-7} M , which is known to abolish all nervous activity (Kao, 1966) indicating that with the parameters used these contractions were due entirely to a stimulation of intrinsic neuronal elements.

Indomethacin (5.6×10^{-5}M) added to the bath caused a progressive reduction in the amplitude of the electrically-induced contractile responses, such that after 30 minutes, the preparation failed to respond to subsequent electrical stimulation. Under these conditions, the preparation still contracted to exogenous acetylcholine (Fig.41c).

3.2. The effect of exogenous PGE_2 on the inhibition of transmural stimulation by indomethacin

In 4 experiments, attempts were made to reverse the blockade of transmural stimulation by indomethacin with exogenous PGE_2. When contractile responses to transmural stimulation were reduced to 40 - 25% of control, PGE_2 (2.2×10^{-10}M - 1.2×10^{-8}M) added to the bath gradually restored the contractions to about 85% of control responses (Fig. 41).

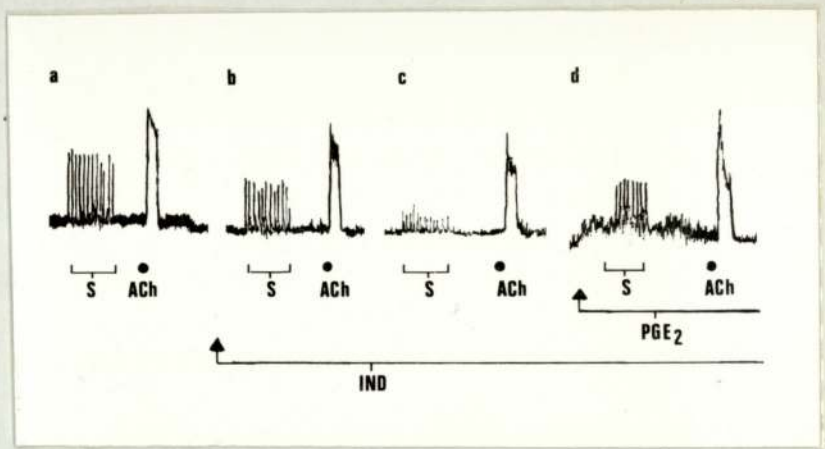

Fig. 41. Contractile responses of guinea-pig ileum to transmural stimulation (0.5Hz)(S) and acetylcholine (8×10^{-8}M)(ACh).
(a) control responses; (b) & (c) responses repeated 15 and 30 mins after indomethacin (5.6×10^{-5}M)(IND);(d) responses repeated in the presence of IND and prostaglandin-E_2 (1.2×10^{-8}M)(PGE_2).

3.3. The effect of indomethacin on angiotensin responses in the presence of tetrodotoxin

The basis for the use of tetrodotoxin has been discussed (see Part I). In order to determine whether the prostaglandin involved in angiotensin contraction was of neuronal origin, the contractile responses to angiotensin were examined in the presence of both tetrodotoxin and indomethacin. The results are shown in Fig. 42.

Tetrodotoxin (10^{-7} M) abolished the fast component and reduced the slow component by 35 - 51% of a submaximal contractile responses to angiotensin. This reduction in the slow component was significant ($P<0.005$). Addition of indomethacin (5.6×10^{-5}M) caused a further reduction in the slow component (Fig.42), such that in 20 - 30 minutes after the addition of indomethacin, the slow component of the response was further reduced by about 25%.

3.4. The effect of indomethacin on angiotensin responses in depolarized guinea-pig ileum preparation

It is known that several types of smooth muscle can still respond on exposure to agonists such as acetylcholine, carbachol, 5-hydroxytryptamine and adrenaline, even when depolarized by immersion in isotonic KCl or K_2SO_4 Ringer solutions (Evans, Schild and Thesleff, 1958; Edman and Schild, 1961; Durbin and Jenkinson, 1961a). This observation led to the suggestion that mechanical response can be dissociated from electrical events, and that drugs can stimulate smooth muscle without depolarization (Evans et al 1958; Robertson, 1960). The action of indomethacin

on angiotensin contractions was therefore examined on depolarized guinea-pig ileum preparation, in an attempt to determine if the component of action of angiotensin sensitive to indomethacin could be observed in depolarized smooth muscle.

An immediate increase in tension corresponding to the maximum increase in tension induced by supramaximal concentrations of acetylcholine was observed when the guinea-pig ileum was immersed in isotonic K_2SO_4 Ringer. Reducing the bath temperature to 25°C, caused the tension to decline gradually to a level well above the polarized resting tension. Under these conditions the tissue still contracted to angiotensin and acetylcholine. The contractions obtained were smaller in amplitude and of a slower time course, and required a higher concentration of each of the agonist. (Similar contractile response to acetylcholine and histamine have been reported by Evans et al (1958) on guinea-pig ileum depolarized with KCl or K_2SO_4).

When the guinea-pig ileum was depolarized, indomethacin (5.6×10^{-5}M) had no effect on the contractions produced by angiotensin or acetylcholine. However, in 3 preparations although there was a slight reduction in angiotensin contractions, it was proportionately less than the reduction in normal Ringer.

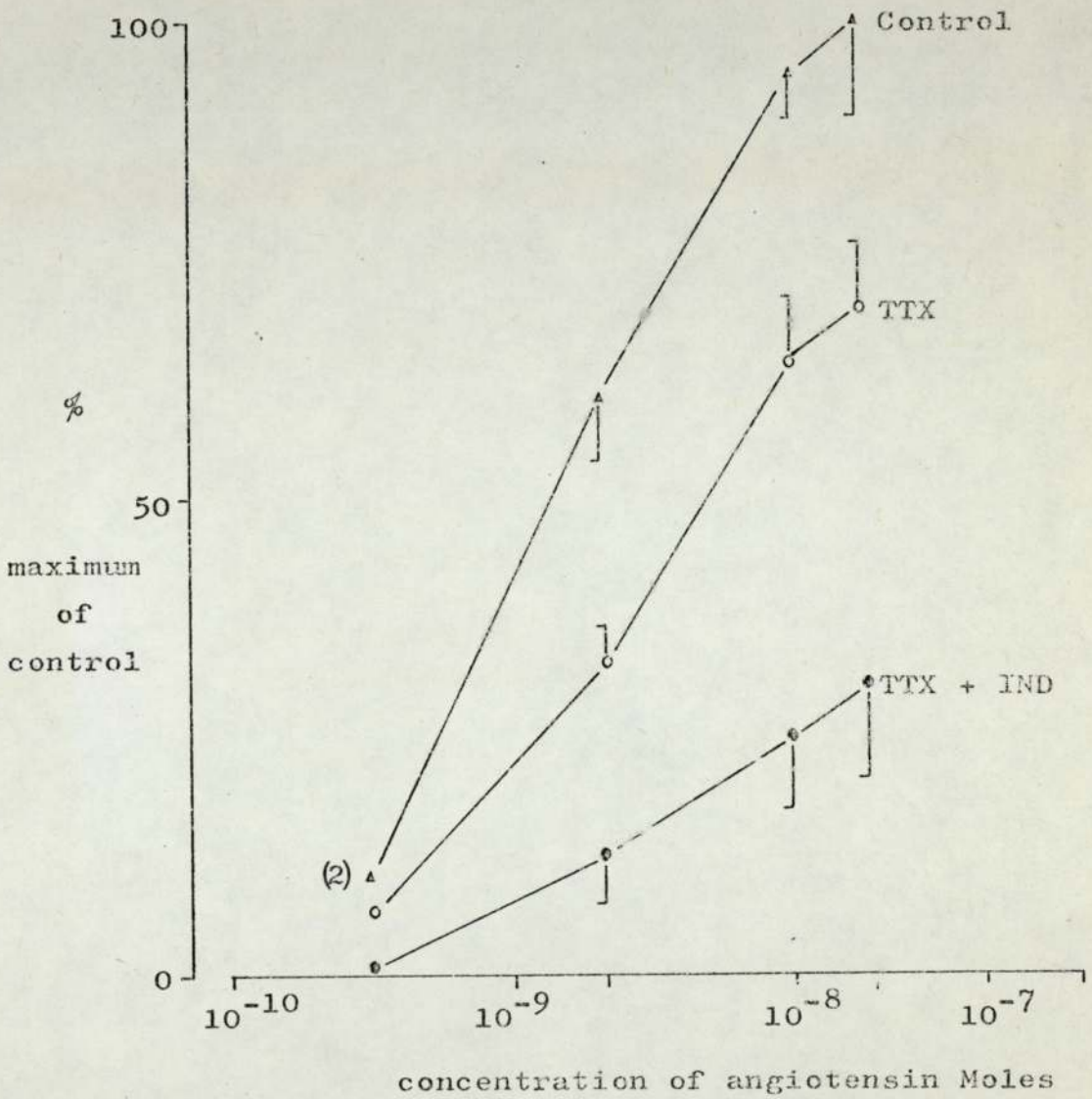

Fig. 42. Concentration effect curves of guinea-pig ileum to angiotensin (slow component); showing control responses (▵ —▵), responses in the presence of tetrodotoxin (TTX)(10^{-7}M)(∘ — ∘) and responses in the presence of both TTX and indomethacin (IND)(5.6×10^{-5}M)(• —•). Each point is the mean of at least 4 observations, unless otherwise indicated in brackets.

3.5. The detection of prostaglandins following stimulation by angiotensin

Experiments were set up to detect the release of prostaglandin-like substances (PLS) from the guinea-pig ileum following angiotensin stimulation. The results obtained were variable and no conclusive evidence could be drawn. Of the 3 tissues generally used for the detection of prostaglandins (Ferreira and Vane, 1967), i.e. the rat stomach strip, rat colon and chick rectum, it was found that only the chick rectum did not contract to angiotensin (Fig.43c). Therefore, for simplicity, in 6 of the total of 10 experiments performed, only the chick rectum was used as an assaying organ in the superfusion. Figure 43 shows the results of one of these experiments. Addition of angiotensin into the perfusion fluid caused a contraction in the guinea-pig ileum but not the chick rectum. However, about 90 seconds later (1 - 2 mins) an increase in spontaneous activity in the chick rectum was observed (4 out of 6 experiments) which persisted for the next minute or two. The increase in spontaneous activity was too small to make any further quantitative or qualitative studies.

In the remaining 4 experiments, the perfusion fluid from the guinea-pig ileum was allowed to superfuse in descending order, the chick rectum, rat stomach strip and rat colon. The tissues were so arranged because prostaglandins are spontaneously released from the rat stomach (Wolfe and Coceani, 1967; Bennett et al, 1967) and may blunt any possible release of prostaglandin from the guinea-

pig ileum induced by angiotensin. The results obtained in these cascade experiments were again not conclusive. Only in two experiments was a small contraction in the chick rectum observed following stimulation of the guinea-pig ileum with angiotensin. Figure 44 illustrates one of these recordings. The size of contraction in the chick rectum approximated to that induced by 1.2×10^{-8}M of PGE_2 applied directly to the tissue. Addition of indomethacin (5.6×10^{-5}M) into the perfusion fluid caused a siginificant reduction in the angiotensin-induced contractions in the guinea-pig ileum and rat stomach strip and abolished the small response in the chick rectum. The contraction in the rat colon was only slightly reduced.

The results obtained from this series of experiments did not appear to warrant further analysis. Because of the size and weight of the segments of guinea-pig ileum, further attempt to detect the release of prostaglandins was not made.

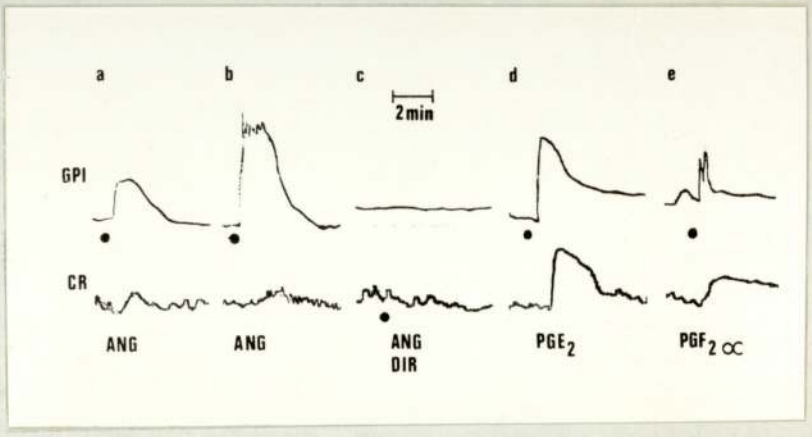

Fig. 43. Contractile responses of guinea-pig ileum (GPI) and chick rectum (CR) to angiotensin (ANG) and prostaglandin-E_2 (PGE_2) and $F_{2-\alpha}$ ($PGF_{2-\alpha}$) in the cascade experiment: (a) & (b), 4 x 10^{-8}M and 10^{-7}M angiotensin respectively; (c) 10^{-7}M angiotensin applied directly to chick rectum, (ANG DIR); (d) 1.2 x 10^{-7}M PGE_2 and (e) 2.1 x 10^{-6}M $PGF_{2-\alpha}$. Further details refer to text. Drugs were added at black dots.

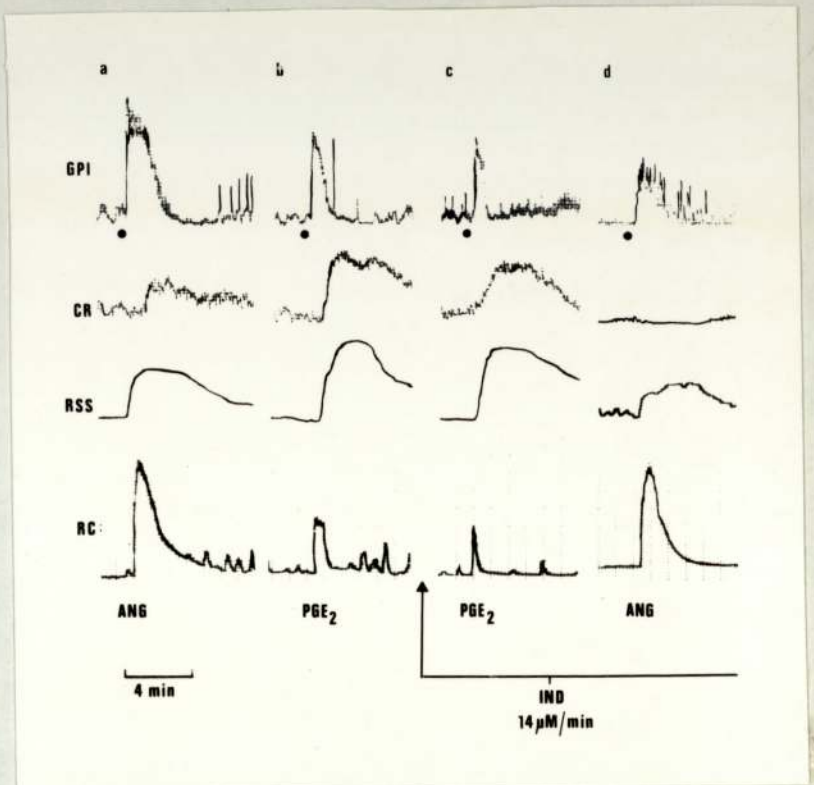

Fig. 44. Contractile responses of guinea-pig ileum (GPI), chick rectum (CR), rat stomach strip (RSS) and rat colon (RC) to angiotensin (ANG) and prostaglandin-E_2 (PGE_2), in the cascade experiments: (a) 10^{-7}M ANG, (b) 5.6 x 10^{-8}M PGE_2, (c) & (d) responses to ANG and PGE_2 repeated 20 minutes after indomethacin (IND) (14 uM/min) was introduced into the perfusion fluid at between (b) and (c). Drugs were added at black dots. Further details refer to text.

DISCUSSION

Two sites of action of prostaglandin can be postulated to account for its involvement in angiotensin contraction. These are 1) an action at nerve endings by facilitating or mediating acetylcholine released by angiotensin and 2) a direct action on the smooth muscle cell membrane in association with specific prostaglandin receptors. The latter postulate is evident by the facts that prostaglandins can stimulate smooth muscles at fairly low concentrations (Horton, 1968,1971) and prostaglandin-induced contractile responses in various smooth muscles are not antagonized by known pharmacological blocking agents (Paton and Daniel, 1967; Coceani and Wolfe, 1966).

Interactions between prostaglandins and transmitter release at autonomic nerve endings have been reported by various workers. Prostaglandins of the E series have been shown to inhibit the effect of sympathetic nerve stimulation by reducing the amount of noradrenaline released (Hedqvist and Brundin, 1969; Wennmalm and Stjarne, 1971) and a modulatory role in sympathetic nerve function has been postulated (see Introduction). Prostaglandins of the F series (mainly $F_{2-\alpha}$) however, have been suggested to facilitate the release of noradrenaline from sympathetic nerve endings induced by angiotensin and 5-hydroxy-tryptamine (Greenberg , Kadowitz, Diecke and Long, 1973), an action which has been discussed in the preceding chapter. Furthermore, PGE_2 and PGE_1 have been shown to reverse the inhibition by indomethacin of electrically induced contractions

of the guinea-pig ileum (Ehrepreis et al, 1973). These authors suggested that a prostaglandin system was directly involved in the mechanism by which acetylcholine is released. The system which consists of prostaglandin, the enzyme or enzymes which synthesize prostaglandin, a prostaglandin receptor site or sites, calcium and the enzyme or enzymes which destroy prostaglandin, is thought to couple cholinergic nerve terminal excitation with acetylcholine release.

Transmural electrical stimulation of guinea-pig ileum is known to cause a release of acetylcholine coincident with contraction (Paton, 1955). The evidence supporting the hypothesis that the fast component of angiotensin-induced contraction of guinea-pig ileum is due to the release of acetylcholine, is substantial (see Part I). Against this background, the finding that indomethacin had a profound inhibitory effect on contraction of guinea-pig ileum induced by transmural electrical stimulation and the fact that exogenous PGE_2 could partially restore the contractions to normal, would seem to add support to the hypothesis of Ehrepreis et al (1973) of an involvement of prostaglandins in the release of acetylcholine in this preparation. These observations also suggest that the cholinergic component of angiotensin contractile response may be similarly governed by prostaglandin or prostaglandins, since this component was reduced after prostaglandin synthesis blockade (see Part II, Chapter I). The finding that indomethacin caused a further reduction in angiotensin contraction even though the fast component was abolished

after hyoscine or tetrodotoxin, suggests that prostaglandin may also be involved in the slow component due to its direct myotropic action on the smooth muscle. However, these results do not distinguish the sig nificance of prostaglandin involved in these two components of angiotensin action. The availability of specific prostaglandin antagonists would be useful for further elucidation of the interaction between prostaglandin and angiotensin.

It has not been established where prostaglandins are released from tissues. Both neuronal and extra-neuronal sources have been suggested (see Bennett et al, 1967; Ferreira et al, 1973). The experiments described in this chapter do not distinguish between these possibilities for angiotensin. However, the demonstration that indomethacin caused a further reduction in the angiotensin contractile response of the guinea-pig ileum even though the preparation was functionally denervated with tetrodotoxin (Gershon, 1967) suggests that prostaglandin released by angiotensin arises mainly from an extra-neuronal source. The observations that indomethacin caused a greater reduction in the slow component of angiotensin response than did tetrodotoxin in the guinea-pig ileum and that indomethacin inhibited angiotensin contractions of preparations where tetrodotoxin had no effect, seem to favour such a view. But a neuronal origin of prostaglandin cannot be ruled out, in view of the facts that i) indomethacin causes some reduction of the fast component and ii) tetrodotoxin

has an inhibitory effect on the slow component of the angiotensin response on the guinea-pig ileum and rabbit jejunum (see Part I).

The results obtained with depolarized preparations, suggest that the release of PGE_2 is dependent upon a polarized cell membrane. Depolarization of excitable cells by angiotensin (see Part II) would then facilitate prostaglandin release. The findings that in depolarized guinea-pig ileum preparation, angiotensin-induced contractile response was smaller in amplitude than contraction induced by acetylcholine and that under such circumstances, indomethacin was ineffective against angiotensin, would favour the hypothesis.

Attempts to detect the release of prostaglandin-like substances from the isolated guinea-pig ileum following angiotensin stimulation did not give conclusive results. The experiments were based on the superfused organ system (Ferreira and Vane, 1967) used for the detection of prostaglandins released from isolated spleens and kidneys (Ferreira et al , 1973; Needleman et al , 1973 a). The detection being circumscribed by the limits of sensitivity of the method used. The finding that in two of the experiments described, a small contraction was observed on the chick rectum when superfused with angiotensin via the guinea-pig ileum, would tend to indicate a presence of prostaglandin. However, such an observation could not be confirmed with the rest of the preparations, where in most, only a slight increase in tension or spontaneous

activity was observed in the chick rectum. One of the possible explanations is that the amount of prostaglandin released is very small, perhaps less than the sensitivity of the chick rectum to prostaglandin, which was about 1.14×10^{-8}M. The findings that only low concentrations ($10^{-10} - 10^{-9}$M) of prostaglandin-E_2 potentiated normal responses to angiotensin and relatively small amounts (10^{-9}M) of prostaglandin-E_2 reversed the inhibition of angiotensin response due to blockade of prostaglandin synthesis (see Part III, Chapter II), suggest that even small amounts, less than 10^{-8}M , of prostaglandin released by angiotensin would be sufficient to participate in the contractile action of the peptide. Another possibility is that there is rapid degradation of the prostaglandin by tissue enzymes. The use of more sensitive biochemical estimation methods such as gas-liquid chromatography and radio-immunoassay in conjunction with standard biological assay procedure therefore seems desirable before any positive conclusion can be drawn.

CHAPTER IV. THE EFFECT OF INDOMETHACIN ON THE PRESSOR RESPONSES TO ANGIOTENSIN IN THE PITHED RAT PREPARATION

It is generally accepted that the pressor effect of angiotensin in whole animals is partly due to its interaction with the sympathetic nervous system, by facilitating adrenergic transmitter release (Benelli et al, 1964; Haefely et al, 1965). Thus, Day and Owen (1970) showed that in the conscious cat, reserpine pretreatment caused a 50% inhibition of pressor responses to angiotensin, and in the pithed rat angiotensin enhanced the responses to endogenous noradrenaline (Day and Owen, 1969). These authors suggested that the pressor action of angiotensin is dependent upon an intact sympathetic nervous system. However, Schmitt and Schmitt (1968) reported that the pressor effect of angiotensin in the pithed rat was independent of noradrenaline release from the adrenal medulla or from sympathetic nerve endings.

Recently, angiotensin has been shown to cause a release of prostaglandins from various perfused tissues of different species including the kidney of the rat (Danon and Chang, 1973). From their results on splenic fat pad of the rabbit, Needleman et al (1973b)suggested that the prostaglandin released by angiotensin has a direct action on the vascular bed and its effect may blunt the pharmacological action of angiotensin, i.e. that of vasoconstriction. Furthermore, angiotensin and

prostaglandin E_1, but not noradrenaline have been shown to cause an increase in dermal permeability in the rabbit (Robertson and Khairallah, 1972) suggesting that angiotensin action may be related to its release of prostaglandins. Similar inter-relationships between angiotensin and the prostaglandins have been indicated in the contractile action of angiotensin on some isolated smooth muscle preparations (Chong and Downing, 1973), and the results presented in the preceding chapters of this Part, have added further support to the hypothesis.

In view of these reports, it seems appropriate to suggest that the pressor action of angiotensin in whole animals may also be influenced by endogenous prostaglandins. It was therefore decided to examine the effect of indomethacin on the angiotensin-induced pressor responses in the pithed rat preparation (Gillespie & Muir, 1967). This preparation has the advantage of studying the effect of drugs on the cardiovascular system free from the influence of possible effects on the central nervous system. It is essentially a smooth muscle preparation '*in situ*'.

RESULTS

4.1. Reproducibility of the pressor responses to angiotensin, noradrenaline and sympathetic outflow stimulation

Pressor responses to angiotensin (50ng/Kg noradrenaline (100ng/Kg) and sympathetic outflow stimulation (0.5Hz) were studied for up to 4 hours. The responses to sympathetic outflow stimulation did not vary significantly ($\pm$6%, n=3) from the initial response throughout the test period. The responses to angiotensin and noradrenaline increased gradually by up to 40% of the initial responses during the first 90 minutes after pithing and thereafter the responses remained fairly constant throughout the rest of the test period. These findings are in agreement with those of Day, Hall and Owen (1972), although the reason for the progressive increase in the sensitivity to noradrenaline and angiotensin is not clear.

In subsequent experiments in which the effect of indomethacin on pressor responses was studied, indomethacin was not administered into the preparation until the responses to the pressor agents became steady, or until at least 90 minutes after pithing.

4.2. Effect of indomethacin on basal blood pressure

Indomethacin (0.5-5mg/kg,i.v.) caused a small increase (about 10mmHg) in the basal blood pressure which lasted for about 15 minutes. Similar increases in blood pressure were observed during an intravenous infusion of indomethacin (over an hour). In a few experiments, the slight increase in blood pressure gradually declined to the basal level after about 20 - 30 minutes even though the infusion continued. An increase in blood pressure following indomethacin has been reported by Davis and Horton (1972) in rabbits.

4.3. Effect of indomethacin on the pressor responses to angiotensin, noradrenaline, vasopressin and to sympathetic outflow stimulation

Indomethacin produced a dose-dependent effect on the pressor responses to angiotensin, noradrenaline and to sympathetic outflow stimulation. The responses to noradrenaline and sympathetic outflow stimulation remained unchanged or increased slightly by between 10 - 20% during the period in which indomethacin (2mg/kg) was infused. The responses to angiotensin remained unchanged during the first 20 minutes of indomethacin infusion, but showed a gradual reduction over the rest of the test period. Towards the end of 2 hours of indomethacin infusion, the responses to angiotensin were reduced by about 25% (mean of 2 experiments).

Similar patterns of responses to angiotensin, noradrenaline and sympathetic outflow stimulation were observed during an infusion of 5mg/kg of indomethacin. However, at this dose level, the inhibition of angiotensin responses was more pronounced, such that at 30 and 90 minutes after the start of indomethacin infusion, the pressor responses were reduced by 26 $\pm$ 6% and 65 $\pm$ 11% (n = 5) of the initial response respectively (Fig. 45). The responses to noradrenaline and sympathetic outflow stimulation remained virtually unchanged or (2 experiments) showed a slight reduction ($<$ 10%)(Fig. 45).

Abolition of angiotensin responses could not be achieved even with a dose level of 10mg/kg of indomethacin. However, the responses to angiotensin were markedly reduced with this dose level (by about 80%) while the responses to noradrenaline and sympathetic outflow stimulation were reduced by about 40% and 30% respectively (2 experiments).

The selective reduction of angiotensin pressor responses occured some 30-40 minutes following the start of indomethacin infusion, and maximum reduction was observed within 90 minutes (Fig. 46). The long time course of action of indomethacin in inhibiting angiotensin pressor responses is similar to its long latency of action in inhibiting angiotensin contraction of isolated smooth muscle preparations. It is a possibility therefore that indomethacin is acting by similar mechanism *in vivo* as it does *in vitro*, i.e. a possible inhibition of prostaglandin biosynthesis or a prevention of prostaglandin

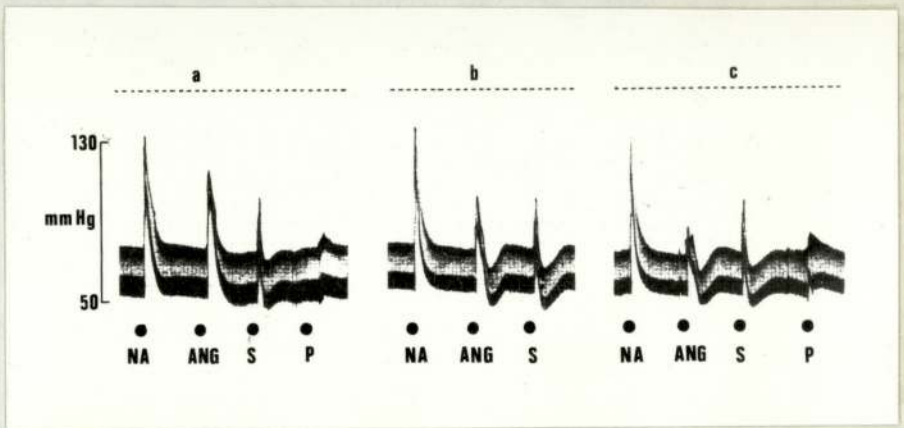

Fig. 45. Pithed rat blood pressure. a) control responses to intravenous noradrenaline (100ng/kg)(NA), intravenous angiotensin (50ng/kg)(ANG), intravenous prostaglandin-$F_{2-\alpha}$ (0.5ug/kg)(P) and to sympathetic outflow stimulation (0.5Hz)(S). b) responses to NA, ANG & S repeated 30 mins after indomethacin (5mg/kg); c) responses to NA, ANG, S & P repeated 90 mins after indomethacin. Time marker in minutes.

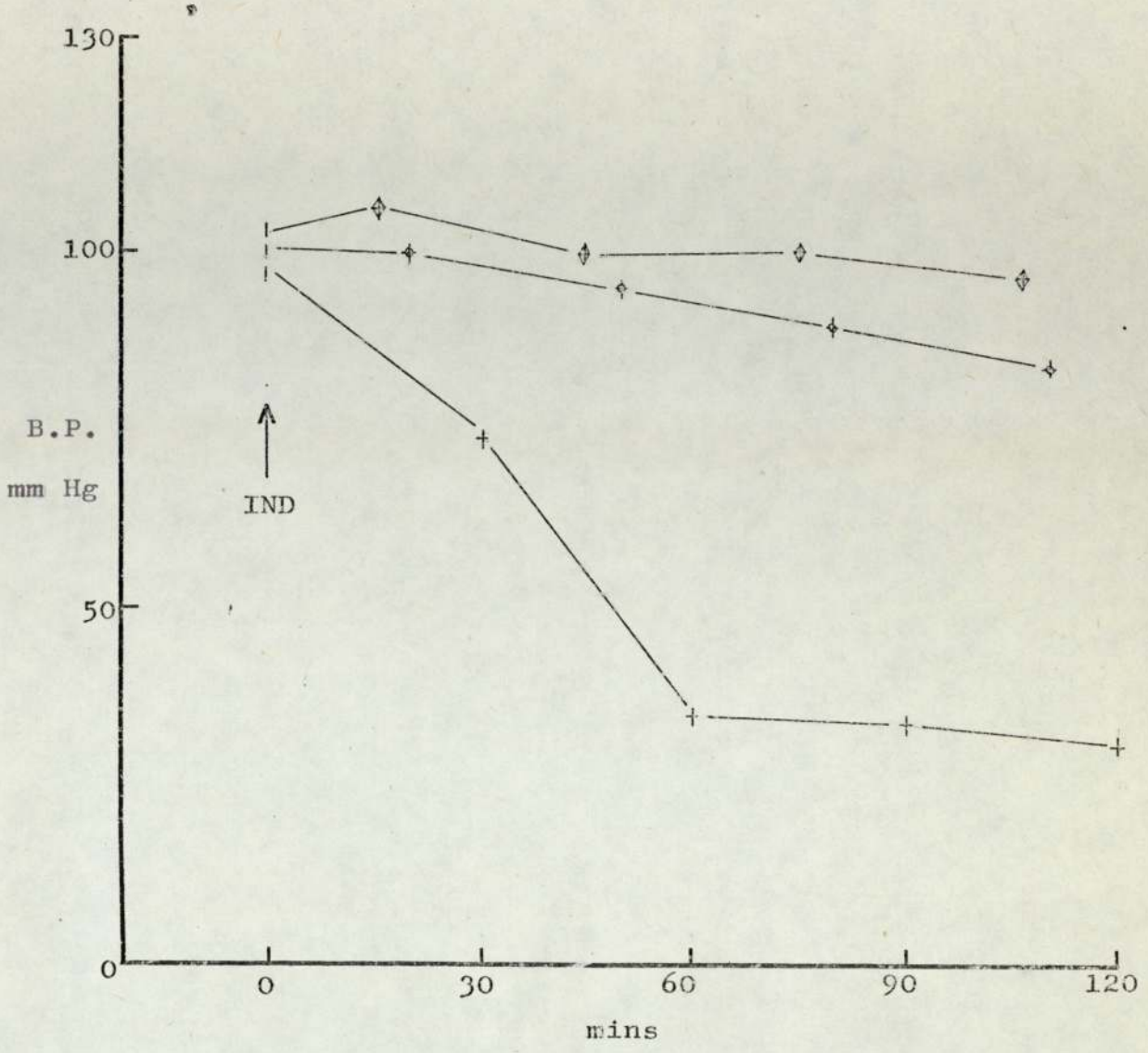

Fig. 46. Effect of indomethacin (5mg/kg)(arrow)(IND) on responses expressed as a percentage of mean control responses to 50 ng/kg angiotensin (+), sympathetic outflow stimulation (◆) and 100 ng/kg noradrenaline (◆), over a 2 hour period. Vertical bars indicate standard errors of the mean (n = 5) unless indicated.

action released by angiotensin.

The selectivity of indomethacin was tested further by comparing its action in depressing the responses of angiotensin, vasopressin and noradrenaline (2 experiments). Fig.47 shows the results of one of the experiments. One hour after the start of indomethacin infusion, the responses to vasopressin and noradrenaline remained fairly constant, whereas the responses to angiotensin were markedly reduced. Towards the end of the test period (2 hours), the responses to vasopressin and noradrenaline were reduced by about 15%, while those of angiotensin were reduced by 60% of the initial responses (Fig.47).

4.4. Effect of indomethacin on $PGF_{2-\alpha}$ pressor responses

In two experiments the effect of indomethacin (5mg/kg) on the pressor responses to $PGF_{2-\alpha}$ (0.5µg/kg) was tested. Indomethacin did not cause a reduction of the $PGF_{2-\alpha}$ response (Fig.45 a and c).

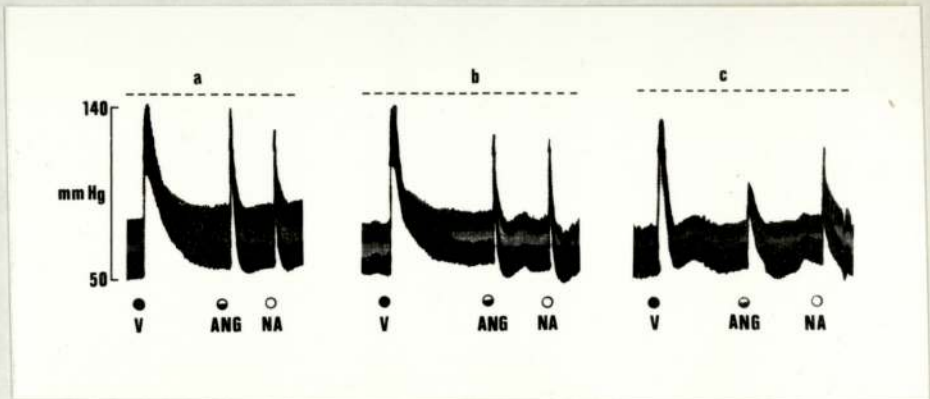

Fig. 47. Pithed rat blood pressure. a) control responses to vasopressin (5mu/kg)(V), angiotensin (50ng/kg)(ANG) and noradrenaline (100ng/kg)(NA). (b) and (c), responses to V, ANG & NA, repeated 30 and 60 mins after indomethacin (5mg/kg). Time marker in minutes.

DISCUSSION

The ability of indomethacin to inhibit prostaglandin synthetase activity *in vivo* and *in vitro* is now well established (see Flower, 1974). Grodzinska, Schoror, Wartner, Forster and Gryglewski (1974) reported that indomethacin caused a slight but prolonged increase in the resting blood pressure of the anaesthetized rat. Similar increases in resting blood pressure by indomethacin have been reported for the rabbit (Davis and Horton, 1972). These latter authors suggested that the small rise in blood pressure by indomethacin was produced by an inhibition of prostaglandin synthetase; the normally released prostaglandins acting to keep the blood pressure down. In this study, indomethacin was also found to cause a small increase in resting blood pressure of the pithed rat. The increase was less than 10% compared to the 16% reported by Grodzinska *et al* (1974). The greater increases in blood pressure reported by these workers may be related to the central actions of prostaglandins.

The most significant result presented in this chapter is that indomethacin caused a selective inhibition of angiotensin-induced pressor responses in the pithed rat. This finding is rather surprising, since prostaglandins have an antihypertensive action in several animal species (see Horton, 1968). However, in this study, $PGF_{2-\alpha}$ but not PGE_2 was shown to cause a rise in the systemic arterial blood pressure, although its action was much weaker than angiotensin or

noradrenaline, furthermore, the pressor action of $PGF_{2-\alpha}$ was not affected by indomethacin. A pressor action of $PGF_{2-\alpha}$ has similarly been reported in the rat, dog and spinal chick (Du Charne and Weeks, 1967). These results suggest that the pressor action of angiotensin in the pithed rat may in part be mediated by the release of $PGF_{2-\alpha}$.

The pressor action of $PGF_{2-\alpha}$ in the dog and rat is thought to be due to a venoconstrictor action and is dependent upon an intact sympathetic nerve supply to the veins (Du Charne and Weeks, 1967). The increased venous return increases cardiac output thus accounting for the rise in arterial blood pressure (see Horton, 1968). The venoconstriction in response to large bolus injections of $PGF_{2-\alpha}$ in the perfused hind paw of the dog, however, was thought to be independent of sympathetic nerve activity (Mark, Schmid, Eckstein and Wendling, 1971). $PGF_{2-\alpha}$ has also been reported to constrict both arterial and venous smooth muscle (Greenberg and Sparks, 1969). These results therefore strengthen the suggestion made here that the pressor action of angiotensin in the pithed rat could be due to a release of $PGF_{2-\alpha}$.

Recently, Greenberg, Kadowitz, Diecke and Long (1973) examined the effects of $PGF_{2-\alpha}$ on arterial and venous contractility to various stimuli and reported that low concentrations of $PGF_{2-\alpha}$ enhanced contractile responses of all vascular strips to tyramine and $BaCl_2$ but not to noradrenaline, while high concentrations of $PGF_{2-\alpha}$ potentiated the effects of all three stimulants, and

that reserpine pretreatment abolished $PGF_{2-\alpha}$-induced facilitation of the contractile response to tyramine but not $BaCl_2$. From these and other results on calcium uptake studies, these authors suggested that $PGF_{2-\alpha}$ may act to facilitate neurotransmitter release and vascular smooth muscle reactivity by modifying membrane permeability to calcium ions. The pressor action of angiotensin is generally believed to be due to an increase in sympathetic discharge, brought about by a stimulation of hypothalamic sympathetic centres in the central nervous system (Bickerton and Buckley, 1961) and its ability to facilitate noradrenaline release at peripheral sympathetic nerve endings (Benelli et al, 1964). The results presented in this chapter and those of other workers reviewed, suggest that the pressor action of angiotensin in whole animal, at least in the pithed rat, involves not only an interaction with the sympathetic nervous system but also with the prostaglandins. However, the results presented here should be interpreted with caution in view of the small number of experiments involved.

In this study, it was shown that the pressor responses to vasopressin were not reduced by indomethacin. This result indicates that the rise in systemic blood pressure induced by vasopressin is independent upon the release of $PGF_{2-\alpha}$. In this connection, it is interesting to note that, while angiotensin releases both PGE_2 and $PGF_{2-\alpha}$ from the cat spleen, vasopressin only releases PGE_2 and only in amounts much smaller than those induced by angiotensin (Peskar and Hertting, 1973).

Furthermore, the amount of PGE_2 released by angiotensin in the cat spleen is always more than $PGF_{2-\alpha}$ (Ferreira et al, 1973; Peskar and Hertting, 1973). From these results it is concluded that angiotensin can induce the release of either PGE_2 or $PGF_{2-\alpha}$, while vasopressin can induce the release of only PGE_2 . Since PGE and PGF compounds differ markedly in their vasoactivities (Nakano, 1972) PGE_2 formed may be involved in maintaining homeostasis (Bergström, 1967) and $PGF_{2-\alpha}$ formed may in fact portray some of the pressor action of angiotensin, either by its direct constrictor action on vascular smooth muscle or in conjunction with its ability to facilitate noradrenaline release (Greenberg et al, 1973), both actions have also been ascribed for angiotensin.

SECTION FOUR

GENERAL DISCUSSION

GENERAL DISCUSSION

The mode of action of angiotensin in the whole animal or on isolated organs is complex and involves direct actions of the peptide on various tissues together with indirect actions of modulating the release of, or causing the release of, other pharmacologically active substances.

Angiotensin is one of the most potent naturally occuring stimulants of smooth muscle known and its actions on smooth muscle appear to predominate in the generation of its many pharmacological actions. Isolated smooth muscle preparations are therefore relevant and convenient for the study of the mode of action of angiotensin at a cellular level.

The first part of this Thesis was concerned with the specificity of receptor sites on various intestinal and vascular smooth muscles to angiotensin. Khairallah and Page (1961) and Robertson and Rubin (1962) reported that the contractions induced by angiotensin on the isolated guinea-pig and rabbit ilea could be partially antagonised by atropine and potentiated by anti-cholinesterases. These authors suggested that part of the action of angiotensin on smooth muscle was indirect and due to the liberation of acetylcholine from an intra-mural nervous network. Khairallah and Page (1961, 1963) further suggested that angiotensin was able to stimulate intra-mural parasympathetic ganglia and cholinergic nerve endings. Godfraind and co-workers (1966a,b) analysed the contractile response to angiotensin on the isolated guinea-pig ileum which revealed fast, (atropine sensitive) and slow (atropine insensitive)

components. These workers concluded that the fast component was mediated via a cholinergic pathway and that the slow component was due to the peptide's direct action on the muscle cells. The results presented in the first part of this thesis are in agreement with the findings of the above workers. Biphasic contractile responses to angiotensin were demonstrated in guinea-pig, rabbit and rat ilea but not in the rat stomach strip, rat colon or the guinea-pig taenia coli. The studies with known selective pharmacological antagonists on the action of angiotensin on these smooth muscle preparations suggests that the release of acetylcholine to produce responses such as the fast component of the responses of the guinea-pig or rabbit ileum is not a wide spread phenomenon. The responses to angiotensin in the other preparations mentioned could not be antagonised by selective antagonists for acetylcholine, histamine or 5-hydroxytryptamine. Upon these findings, it is suggested that angiotensin has a direct action on these smooth muscles. However, the possibility that some yet unrecognised mediator substance could participate in the contractile response to angiotensin remains.

The contraction of vascular smooth muscle <u>in vitro</u> in response to angiotensin is due primarily to a direct action of the peptide on the vascular smooth muscle cell. Indirect neurogenic effects of angiotensin due to a release of noradrenaline (Liebau, Distler and Wolf, 1966) appear to contribute little to the contractile responses induced by angiotensin on the rabbit aortic strip and the rat portal vein. Nevertheless as suggested for intestinal smooth muscle, it is

possible that some mediators may evade screening with the usual pharmacological antagonists.

The sensitivity and specificity of the rat portal vein to angiotensin renders it a preparation suitable for the assay of angiotensin in addition to the rat colon (Regoli and Vane, 1964) and the rabbit aortic strip (Helmer, 1959). The guinea-pig portal vein was found to be very insensitive to angiotensin. This further illustrates the heterogeneity in sensitivity of vascular smooth muscles to angio tensin (Bohr and Uchida, 1967; Carruba, Mandelli and Mantegazza, 1973).

Taking all the above data into consideration, it is suggested that angiotensin receptors on smooth muscle cells are distinct from those of other known smooth muscle agonists. This hypothesis is substantiated by the reports of other workers using angiotensin derivatives and angiotensin analogues (Meyer, Papadimitriou and Worcel, 1970; Peach, 1972; Park,Regoli and Rioux, 1973). Meyer et al (1970) examined the potency of several angiotensin derivatives on the rat colon, rat uterus and rat aorta and concluded that the receptors in the three tissues are different. Peach (1972) after studying the order of potency of several angiotensin analogues on the cat papillary muscle in vitro, concluded that receptors for angiotensin in the heart are different from those found in smooth muscle such as rat uterus.

Contraction of smooth muscle is the mechanical end product of a series of events that can be initiated by the combination of a stimulant drug with its specific receptor on the smooth muscle cell. Irrespective of the agent having a direct or in-

direct action, the event that immediately precedes and is responsible for the contractile response is always an increase in the concentration of ionized calcium in the vicinity of the contractile protein (Bianchi, 1961; Bohr, 1964; 1973). However, the events leading to such an increase in activator calcium seems less universal and to vary dependent on the particular agent used to initiate the sequence of events (Somlyo, 1972). Action potentials and depolarization of the membrane are important but not essential links, since muscles depolarized with potassium rich solution still responds to drugs (Evans, Schild and Thesleff, 1958). There are conflicting reports regarding the importance of membrane depolarization and action potential discharge as an initial stage of angiotensin-induced contraction (for references, see Part II).

Angiotensin like acetylcholine, depolarized the guinea-pig taenia coli in a dose dependent manner (Fig. 18) with graded increases in spike frequency.

The effect of angiotensin on the cell membrane appears to cause an increase in sodium permeability. In a sodium-free medium, angiotensin was without effect, whereas ACh still evoked a small depolarization. The depolarizing action of angiotensin seems therefore to be more dependent upon the presence of extracellular sodium than does that of ACh. Angiotensin-induced depolarization seems also to be partly dependent upon the presence of external K, Ca and Cl ions, since a reduction in the concentration of any of these ions, reduced the depolarizing responses of the peptide. In addition to sodium, the

entry of calcium ions was of importance in mediating angiotensin-induced depolarizations as shown by the responses to angiotensin when the external calcium ion concentration was increased. The available data suggest that the sodium current normally predominates.

The depolarization induced by angiotensin and acetylcholine on the taenia coli was followed by hyperpolarization. The degree of 'after-hyperpolarization' was enhanced in high external sodium ion concentrations. Similar 'after-hyperpolarizations' have been described for acetylcholine on various smooth muscles (for references, see Part II: Chapter II) and for depolarizing drugs on isolated ganglia (Brown 1966; Kosterlitz, Lees and Wallis, 1970). The hyperpolarization is believed to be due to the operation of an electrogenic sodium pump (Burnstock and Straub, 1960; Brown, Brownstein and Schofield, 1969). This hypothesis has recently been supported for smooth muscle by Bolton (1973).

In summary, the action of angiotensin on the cell membrane is to cause an increase in permeability to mainly sodium ions, and to a lesser degree, potassium, calcium and possibly chloride ions. The resultant accumulation of intracellular sodium would further stimulate the electrogenic sodium pump (Thomas, 1965) and thus cause a brief period of hyperpolarization. An increase in sodium permeability may also partly explain some of the physiological effects of angiotensin, such as renal tubular sodium reabsorption and intestinal fluid transport.

The depolarizing action of angiotensin reported here, besides initiating a complex series of events that leads ultimately to muscle contraction, may also be involved in the peptide's ability to release endogenous mediators such as ACh and noradrenaline from neuronal stores. Although there is no electrophysiological evidence of angiotensin receptors on autonomic nerve terminals, a depolarizing action of angiotensin has indeed been described for the autonomic ganglion (see Haefely, 1970). Angiotensin is also known to release prostaglandin-like substances from several isolated organ systems, such as spleen, kidney and mesenteric-vascular bed (for references, see Part III). It is tempting to suggest that the release of prostaglandin-like (PGL) substances by angiotensin may be due to the peptide's depolarizing action. There is however, no evidence to substantiate this postulation.

Prostaglandins are widely distributed in animal tissues. Among the physiological roles that have been ascribed to them, the role as modulator of hormonal action and of neurotransmitter release (Bergstrom, 1968; Horton, 1970; Hedqvist, 1970) appeared pertinent to the study of the mode of action of angiotensin. Since angiotensin has been shown to cause a release of PGL-substances from isolated organs, prostaglandins could therefore be involved in the angiotensin-induced contractions of smooth muscle. This hypothesis was tested on the guinea-pig ileum and pithed rat preparations with the use of indomethacin, which is a potent prostaglandin synthesis inhibitor (Vane, 1971). The results presented in the last part of this Thesis

substantiate this hypothesis. Evidence for the participation of prostaglandins in angiotensin action have been discussed in length under the appropriate chapters. In recapitulation, the main points are 1) indomethacin has a selective inhibitory action for angiotensin; 2) the concentration of indomethacin used was within the range reported to inhibit prostaglandin synthetase enzyme system. Although indomethacin can inhibit other enzyme system such as phosphodiesterase (Floss and Sharp, 1972) the concentrations required are considerably higher (see Flower, 1974); 3) the long time-course required by indomethacin for effective inhibition of angiotensin response; 4) subthreshold concentrations of PGE_2 potentiated contractile responses to angiotensin and 5) exogenous PGE_2 could partially restore the inhibition by indomethacin of angiotensin contractions. To complement the above evidence, prostaglandin-like substance was detected in the perfusate of the guinea-pig ileum when exposed to angiotensin but only in 4 out of 10 experiments. The difficulty in detecting PGL-material in every experiment has been discussed under the appropriate section. Nevertheless, it must be emphasized that there is a rapid removal of prostaglandins by tissue enzymes, such as 15-hydroxy-dehydrogenase, which is responsible for the inactivation of PGE_{2-} and $PGF_{2-\alpha}$. These enzymes are present in guinea-pig tissues (see Bergstrom et al, 1968; Flower, 1974).

The site of prostaglandin release by angiotensin is not known. Although both neuronal (Ehrepreis et al, 1973) and non-nervous cellular sites (Piper and Vane, 1971) have been suggested.

In this study, the slow component of the contractile responses to angiotensin in the guinea-pig ileum was further reduced by indomethacin even though tetrodotoxin had reduced it by an initial 30%. If part of the contraction was due to the release of prostaglandin, then this result suggests that prostaglandins could be from a neuronal and extraneuronal source. In the depolarized guinea-pig ileum, angiotensin still caused a small contraction. The contraction, however, was not affected by inhibiting prostaglandin synthesis with indomethacin. This finding is in favour of the earlier postulation that angiotensin releases prostaglandin-like material from tissues by means of the peptide's depolarizing action. If this assumption is true, it is pertinent to suggest that the depolarizing action of angiotensin on the cell membrane activates prostaglandin synthetase activity, since cells do not store prostaglandins and release is in most cases equivalent to de novo synthesis (Piper and Vane, 1971). Further studies with the aid of intracellular microelectrodes or double sucrose-gap techniques and more sensitive biochemical prostaglandin assay procedures would help to clarify this situation.

Angiotensin has been implicated in the pathogenesis of hypertension in one way or another (see review, Page and McCubbin, 1968). Both prostaglandins and kidney extracts may lower blood pressure in animals and in humans. The pressor action of angiotensin in the pithed rat was reduced after

inhibition of prostaglandin synthesis by indomethacin. Responses to noradrenaline, sympathetic outflow stimulation and that of another peptide, vasopressin were not significantly affected. These results suggest that part of the pressor response induced by angiotensin could be mediated via a release of prostaglandin-$F_{2-\alpha}$. The observation that the pressor response to angiotensin could not be completely abolished after indomethacin treatment, either by increasing the dosage or prolonging the infusion time, suggests that prostaglandin may perhaps only exert a modulatory role in angiotensin action. Participation of prostaglandins in angiotensin's pressor effect may seem contradictory in view of the fact that prostaglandins are potent hypotensive agents in most species (see Horton, 1968). However, $PGF_{2-\alpha}$ is known to cause a rise in blood pressure in the rat and spinal chick (Du Charne and Weeks, 1967). In this respect, it is of interest to note that prostaglandins can increase the level of cyclic AMP, the postulated second messenger of hormone action, thus mimicking the effects of hormones such as adrenaline (Butcher and Baird, 1968).

Tachyphylaxis to angiotensin has been reported in various tissues (for references see Introduction). The mechanism for the production of tachyphylaxis to angiotensin cannot be resolved in this study. Tachyphylaxis to angiotensin was demonstrated in all the smooth muscle preparations examined with the exception of the rabbit aortic strip, rat portal vein, rat colon and rat fundus strip. The results available suggest that tachyphylaxis to angiotensin may be species specific. In the

guinea-pig ileum, taenia coli and rabbit ileum, tachyphylaxis could be avoided by increasing the dose interval or by frequent washings between successive application of the drug. These findings would favour the hypothesis that tachyphylaxis was due to receptor occupation and slow dissociation of the drug-receptor complex (Khairallah et al, 1966). The alternative that tachyphylaxis could be due to transmitter exhaustion (Distler, et al, 1966) cannot be excluded, in view of the results presented in this Thesis, that angiotensin action may involve the release of prostaglandins in addition to ACh. Recently, tachyphylaxis to angiotensin in the rabbit coeliac artery has been partly attributed to angiotensin-induced prostaglandin release (Aiken, 1974), although in this case, the prostaglandin is thought to exert an inhibitory action on the artery that attenuates the vasoconstrictor action of angiotensin. The effect of angiotensin on membrane activity would suggest that tachyphylaxis could be related to the rate of repolarization of cell membrane, for example, the rate of repolarization following stimulation by angiotensin was much greater than that of acetylcholine. It is apparent that none of the above hypotheses can satisfactorily account for the phenomenon of angiotensin tachyphylaxis.

In summary, the action of angiotensin on smooth muscle is complicated by its direct and indirect actions. The results presented suggest that angiotensin can release ACh from intrinsic nerve endings, which in turn accounts for part of its action

on intestinal smooth muscle. Evidence is also presented that part of the peptide's contractile and vasoconstrictor action may involve the release of endogenous prostaglandins such as PGE_2 and $PGF_{2-\alpha}$. The direct action of the peptide on smooth muscle appears to be related to its depolarizing action which is dependent upon sodium ions.

SECTION FIVE

REFERENCES

Adrian, R.H. & Slayman, C.L. (1966). Membrane potential and conductance during transport of sodium, potassium and rubidin in frog muscle. J. Physiol. (Lond). 184, 970-1014.

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

Aiken, J.W. & Vane, J.R. (1971). Blockade of angiotensin-induced prostaglandin release from dog kidney by indomethacin. Pharmacologist, 13, 293.

Alpert, J.S. & Hickler, R.B. (1971). In 'Kidney Hormones', Ed. Fisher, J.W. pp.525-563. Academic Press, Lon. N.Y.

Ambache, N., Brummer, H.C., Whiting, J. & Wood, M. (1966a). Atropine resistant substances in extracts of plexus-containing longitudinal muscle (PC-LM) from guinea-pig ileum. J. Physiol. 186, 32-33P.

Ambache, N., Brummer, H.C., Rose, J.G. & Whiting, J. (1966b). Thin-layer chromatography of spasmogenic unsaturated hydroxy-acids from various tissues. J. Physiol. 185, 77-78P.

Änggård, E. & Bergström, S. (1963). Biological effects of an unsaturated trihydroxy acid ($PGF_{2-\alpha}$) from normal swine lung. Acta Physiol. Scand. 58, 1-12.

Arawaka, K., Smeby, R.R. & Bumpus, F.M. (1962). Synthesis of succinyl-isoleucyl-5-angiotensin II and N-(-poly-O-acetyl-seryl)-isoleucyl-5-angiotensin II. J.Amer. Chem. Soc. 84, 1424-26.

Auerbach, J. (1864). Fernere vorläutige Mittheilung über den Nervenapparat des Darmes. Virchous Arch. path. Anat. Physiol. 30, 457-60.

Axelsson, J. (1961). Dissociation of electrical and mechanical activity in smooth muscle. J. Physiol. 158, 381-98.

Axelsson, J. (1970). Mechanical properties of smooth muscle and the relationship between mechanical and electrical activity. In 'Smooth Muscle', Ed. Bulbring, E., Brading, A., Jones, A. & Tomita, T. pp. 289-315. Edward Arnold Publ.Lond.

Axelsson, J., Johansson, B., Jonsson, O. & Wahlstrom, B. (1966). The effects of adrenergic drugs on electrical and mechanical activity of the portal vein. Symp. Elect. Activ. Innerv., Blood Vessels,(Cambridge, 1966) Biblphie anat. 8, 16-20.

Barr, L. & Berger, W. (1964). The role of current flow in the propagation of cardiac muscle action potentials. Pflugers Archiv. ges. Physiol. 279, 192-94.

Barr, L. & Dewey, M.M. (1968). Electrical transmisson at the nexes between smooth muscle cells. J. Gen. Physiol. 51, 347-69.

Beleslin, D.B. (1968). The effect of angiotensin on the peristaltic reflex of the isolated guinea-pig ileum. Br. J. Pharmacol., 32, 583-590.

Benelli, G., Della Bella, D. & Gandini, A. (1964). Angiotensin and peripheral sympathetic nerve activity. Br. J. Pharmacol. 22, 211-9.

Bennett,M.R.& Burnstock, G. (1968). Electrophysiology of transmission in the intestine. In 'Handbook of Physiology', Alimentary Canal IV. Amer. Physiol. Soc., Washington, D.C.

Bennett, A., Friedman, C.A. & Vane, J. (1967). The release of prostaglandin E_1 from the rat stomach. Nature (Lon). 216, 868-73.

Berger, W. & Barr, L. (1969). Use of rubber membranes to improve sucrose-gap and other electrical recording techniques. J. Applied Physiol. 26 (3), 378-382.

Bergström, S. (1967). Isolateion, structure and action of the prostaglandins. In 'Prostaglandins', Proc. 2nd. Nobel Symp., Stockholm, Ed. Bergström, S. & Sammuelsson, B. pp. 21-30. Almqvixt & Wiksell, Stockholm. Interscience,N.Y.

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

Bergström, S., Eliasson, R., Euler, von U.S. & Sjövall, J. (1959). Some biological effects of two crystalline prostaglandin factors. Acta Physiol. Scand. 45, 133-44.

Bergström, S., Ryhage, R., Samuelsson, B. & Sjövall, J. (1963). Degradation studies of prostaglandins. Acta Chem. Scand. 16, 501-2.

Bergström, S. & Samuelsson, B. (1965). Prostaglandins. Annu. Rev. Biochem. 34, 101-8.

Bernstein, J. (1902). Untersuchungen zur Thermodynamik der bioelektrischen Ströme. Pflugers Arch. ges. Physiol. 92, 521-62.

Bernstein, J. (1912). Elekrobiologie. Braunschweig: Vieweg.

Bianchi, C.P. (1961). Calcium movements in muscle. Circulation, 24, 518-22.

Bickerton, R.K. & Buckley, J.R. (1961). Evidence for a central mechanism in angiotensin induced hypertension. Proc. Soc. Exp. Biol., N.Y. 106, 834-6.

Bisset, G.W. & Lewis, G.P. (1962). A spectrum of pharmacological activity in some biologically active peptides. Brit. J. Pharmacol., 19, 168-82.

Blair-West, J.R. & McKenzie, J.S. (1966). Sodium concentration and the effect of angiotensin II on ileal smooth muscle. Experientia, 22, 291-2.

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. Pharmacol., 31, 229-43.

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

Blair-West, J.R., McKenzie, J.S. & McKinley, M.J. (1971). The actions of angiotensin II on the isolated portal vein of the rat. Europ. J. Pharmacol. 15, 221-30.

Bock, K.D. & Gross, F. (1961). Renin and angiotensin tachyphylaxis. Circulation Res. 9, 1044-50.

Bohr, D.F. (1964). Contraction of vascular smooth muscle. Canad. Med. Ass. J., 90, 174-9.

Bohr, D.F. (1967). Individualities of vascular smooth muscles in response to angiotensin. Circulation Res. 21, 135-43.

Bohr, D.F. (1973). Vascular Smooth Muscle Updated. Circulation Res. 32, 665-72.

Bolton, T.B. (1971). On the nature of the oscillations of the membrane potential (slow waves) produced by acetylcholine or carbachol in intestinal smooth muscle. J.Physiol. 216, 403-18.

Bolton, T.B. (1973). Effects of electrogenic sodium pumping on the membrane potential of longitudinal smooth muscle from terminal ileum of guinea-pig. J. Physiol. 228, 693-712.

Born, G.V.R. (1970). 5-Hydroxytryptamine receptors in smooth muscle, pp. 418. Williams and Wilkins, Baltimore,

Botting, J.H. & Salzmann, R. (1974). The effect of indomethacin on the release of prostaglandin E_2 and acetylcholine from guinea-pig ileum at rest and during field stimulation. Br. J. Pharmacol. 50, 119-24.

Boucek, R.J., Takashita, R. & Fojaco, E. (1963). Relationship between microanatomy and functional properties of the coronary arteries. (Dog). Anat. Rec. 147, 199-207.

Boudouin, M., Meyer, P., Worcel, M., Fermandjian, S. & Morgat, J.L. (1972). In 'Structure-activity relationships of proteins and polypeptide hormones'. Ed. Margoulies, M & Greenwood, F.C. 496-7.

Bowman, W.C., Rand, M.J. & West, G.B. (1968). In 'A text book of Pharmacology'. Blackwell Scientific Publishers, Oxford, 702-33.

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

Bozler, E. (1948). Conduction, automacity and tonus of visceral muscles. Experientia, 4, 213-8.

Brading, A.F., Bülbring, E. & Tomita, T. (1969). The effect of sodium and calcium on the action potential of the smooth muscle of the guinea-pig taenia coli. J. Physiol., 200, 637-54.

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

Braun-Menendez, E., Fasciolo, J.C., Leloir, L.F. & Munõz, J.M. (1940). The substance causing renal hypertension. J. Physiol., 98, 282-98.

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

Brown, J.J., Davies, D.L., Lever, A.F. & Robertson, J.I.S. (1964). Variations in plasma renin conentration in several physiological and pathological states. Canad. Med. Ass. J., 90, 201-6.

Brown, J.J., Davies, D.L., Lever, A.F. & Robertson, J.I.S. (1965). Plasma renin concentration in human hypertension. II. Renin in relation to aetiology. Brit. Med. J., 2, 1215-9.

Bülbring, E. (1954). Membrane potential of smooth muscle fibres of the taenia coli of the guinea-pig. J. Physiol., 125, 302-15.

Bülbring, E. (1955). The correlation between membrane potential, spike discharge and tension in smooth muscle. J. Physiol., 128, 200-21.

Bülbring, E. & Burnstock, G. (1960). Membrane potential changes associated with tachyphylaxis and potentiation of the responses to stimulating drugs in smooth muscle. Br. J. Pharmacol., 15, 611-24.

Bülbring, E., Burnstock, G. & Holman, M.E. (1958). Excitation and conduction in the smooth muscle of the isolated taenia coli of the guinea-pig. J. Physiol., 142, 420-37.

Bülbring, E. & Hooton, I.N. (1953). Smooth muscle potentials recorded with intracellular electrodes. J. Physiol., 120, 8-9P.

"Bulbring, E., Brading, A., Jones, A. & Tomita, T. (1970). In 'Smooth Muscle'. London; Edward Arnold Publishers Ltd.

"Bulbring, E. & Kuriyama, H. (1963). Effect of changes in ionic environment on the action of acetylcholine and adrenaline on the smooth muscle cells of guinea-pig taenia coli. J. Physiol., 166, 59-74.

"Bulbring, E. & Lullman, H. (1957). Theeffect of metabolic inhibitors on the electrical and mechanical activity of the smooth muscle of guinea-pig taenia coli. J. Physiol. 142, 420-37.

"Bulbring, E. & Tomita, T. (1969). Calcium and the action potential in smooth muscle. In 'Calcium and Cellular Function', ed. Cuthbert, A.W. London, MacMillan.

Bumpus, F.M., Smeby, R.R. & Page, I.H. (1961). Angiotensin, the renal pressor hormone. Cir. Res., 9, 762-7.

Bumpus, F.M., Smeby, R.R., Page, I.H. & Khairallah, P.A. (1964). Distribution and metabolic fate of angiotensin II and various derivatives. Canad. Med. Ass. J.,90, 190-3.

Bumpus, F.M., Smeby, R.R. & Khairallah, P.A. (1970). Synthesis and biological properties of angiotensin II analogues. In 'Peptides. Chemistry and biochemistry', ed. Weinstein, B & Lande, S., pp. 127-50, Marcel Decker, N.Y.

Bunag, R.D., Page, I.H. & McCubbin, J.W. (1965). Neurogenic stimulation of renin release. Pharmacologist, 7, 152.

Burgen, A.S.V., Dickens, F. & Zatman, L.J. (1949). The action of botulinum toxin on the neuro-muscular junction. J. PHysiol., 109, 10-24.

Burks, T.F., Whitacre, T.S. & Long, J.P. (1967). Effects of calcium-deficient perfusion on isolated mesenteric arteries. Arch. Int. Pharmacodyn. Ther., 168, 304-11.

Burn, J.H. (1968). In 'The autonomic nervous system'. 3rd. Edition, Blackwell Scientific Publications, Oxford.

Burnstock, G. (1958). The effects of acetylcholine on membrane potential, spike frequency, conduction velocity & excitability in the taenia coli of the guinea-pig. J. Physiol. 166, 59-74.

Burnstock, G. (1968). The autonomic neuromuscular junction. Proc. 24th Int. Congr. Physiol. Sci. (Washington) 6, 7-8.

Burnstock, G. (1970). In 'Smooth Muscle'. pp. 1-69. Ed. Bulbring, E., Brading, A., Jones, A. & Tomita, T. Edward Arnold, London.

Burnstock, G., Campbell, G. & Rand, M.J. (1966). The inhibitory innervation of the taenia coli of the guinea-pig caecum. J. Physiol., 182, 504-26.

Burnstock, G. & Holman, M.E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. J. Physiol., 155, 115-33.

Burnstock, G. & Holman, M.E. (1963). Smooth muscle: autonomic nerve transmission. Ann. Rev. Physiol., 25, 61-90.

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

Burnstock, G. & Robinson, P.M. (1967). Localization of catecholamine and acetylcholinesterase in autonomic nerves. Cir. Res., 21, 43-55.

Burnstock, G. & Straub, R.W. (1958). A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. J. Physiol., 140, 156-67.

Burgen, A.S.V. & Spero, L. (1968). The action of acetylcholine and other drugs on the efflux of potassium and rubidium from smooth muscle of the guinea-pig intestine. Br. J. Pharmacol., 34, 99-115.

Caesa, R., Edwards, G.A. & Ruska, H. (1957). Architecture and nerve supply of mammalian smooth muscle tissue. J. Biophys. Biochem. Cytol., 3, 867-78.

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

Campbell, G. (1970). Autonomic nerves supply to effector tissues. In'Smooth Muscle'. Ed. Bulbring, E., Brading, A., Jones, A. & Tomita, T. pp. 450-95. Edward Arnold.Lon.

Carruba, M., Mandelli, V. & Mantegazza, P. (1973). The effect of angiotensin II and other vasoactive drugs on isolated portal vein preparations. Arch. Int. Pharmacodyn. Ther., 201, 224-33.

Casteels, R. (1970). The relation between the membrane potential, spike discharge and tension in smooth muscle. J. Physiol., 128, 200-21.

Casteels, R., Droogmans, G. & Hendrickx, H. (1971a). Membrane potential of smooth muscle cells in potassium free solution. J. Physiol., 217, 281-95.

Casteels, R., Droogmans, G. & Hendrickx, H. (1971b). Electrogenic sodium pump in smooth muscle cells of the guinea-pig's taenia coli. J. Physiol., 217, 297-313.

Casteels, R. & Hendrickx, H. (1969). Pompe á sodium electrogene dans les fibres lisses du taenia coli de cobaye. J. Physiol., Paris. 61 (2), 240.

Chaturvedi, N.C., Park, W.K., Smeby, R.R. & Bumpus, F.M. (1970). Analysis of angiotensin II. I. Solid-phase synthesis. J. Med. Chem. 13, 177-81.

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

Coceani, F. & Wolfe, L.S. (1966). On the action of prostaglandin-E_1 and prostaglandins from brain on the isolated rat stomach. Can. J. Physiol. Pharmacol., 44, 933-50.

Connelly, C.M. (1959). Recovery processes and metabolism of nerve. Rev. Mod. Phys., 31, 475-84.

Cross, S.B., Keynes, R.D. & Rybova, R. (1965). The coupling of sodium efflux and potassium influx in frog muscle. J. Phsiol., 181, 865-80.

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

Daniel, E.E. (1963). Potassium movements in rat uterus studied in vitro. I. Effects of temperature. Can. J. Biochem. Physiol. 41, 2065-84.

Daniels, E.G. , Hineman, J.W., Leach, B.E. & Muirhead, E.E. (1967). Identification of prostaglandin-E_2 as the principal vasodepressor lipid of rabbit renal medulla. Nature, (Lon). 215, 1298-99.

Danon, A. & Chang, C.T. (1973). Fed. Proc., 32, 788.

Davies, N.T., Munday, K.A. & Parsons, B.J. (1969). The effects of protein synthesis inhibitor on angiotensin stimulated colonic fluid transfer. J. Physiol. 205, 17P.

Davies, N.T., Munday, K.A. & Parsons, B.J. (1970). The effect of angiotensin on the rat intestinal fluid transfer. J. Endocr., 48, 391.

Davignon, J. & Shepherd, J.T. (1964). Response of the human umbilical artery 'in vitro' to changes in transmural pressure. Fed. Proc. 23, 309.

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

Davis, J.O. (1974). The renin-angiotensin system in the control of aldosterone secretion. In 'Angiotensin', handbook of Exptl. Pharmacol. xxxvii. Springer-Verlag, Berlin, Heidelberg, New York. Ed., Page, I.H. & Bumpus, F.M. pp. 323-336.

Davis, H.A. & Horton, E.W. (1972). Output of prostaglandins from the rabbit kidney: Its increase on renal nerve stimulation and its inhibition by indomethacin. Br. J. Pharmacol. 46, 658-75.

Day, M.D., Hall, J. & Owen, D.A. (1972). Selective inhibition of angiotensin pressor responses in the pithed rat by Tetraethythiuram disulphide (Disulfiram) and sodium diethyldithiocarbamate (DDC). Br. J. Pharmacol. 44, 192-202.

Day, M.D. & Moore, A.F. (1973). Potentiation by angiotensin of noradrenaline-induced contractions of a rabbit isolated thoracic aorta. Br. J. Pharmacol., 48, 31P.

Day, M.D. & Owen, D.A.A. (1968). The interaction between angiotensin and sympathetic vasoconstriction in the isolated artery ofthe rabbit ear. Br. J. Pharmacol., 34, 499-507.

Day, M.D. & Owen, D.A.A. (1969). Inhibition of angiotensin pressor responses with diethyldithiocarbamate (DDC). Br. J. Pharmacol., 37, 517P.

Day, M.D. & Owen, D.A.A. (1970). The effect of reserpine on the pressor responses to angiotensin in the conscious cat. Br. J. Pharmacol., 39, 414-27.

Day, M. & Vane, J.R. (1962). An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum. Br. J. Pharmacol., 20, 150-70.

Dean, R.B. (1941). Theories of electrolyte equilibrium in muscle. Biol. Symp., 3, 331-48.

Del Castillo, J. & Katz, B. (1954). Statistical factors involved in neuromuscular facilitation and depression. J. Physiol., 124, 574-85.

Del Castillo, J. & Katz, B. (1955). On the localization of acetylcholine receptors. J. Physiol., 128, 157-81.

Dela Lande, I.S. & Rand, M.J. (1965). A simple isolated nerve-blood vessel preparation. Aust. J. Exp. Biol. Med. Sci., 43, 639-56.

Deuben, R.R. & Buckley, J.P. (1970). Identification of a central site of action of angiotensin II. J. Pharmac. exp. Ther., 175, 139.

Devynik, M.A., Pernollet, M.G., Meyer, P., Fermadjian, S. & Fromageat, P. (1973). Angiotensin receptors in smooth muscle cells membranes. Nature, New Biology. 245, 55-8.

Devynik, M.A., Pernollet, M.G., Meyer, P., Fermadjian, S., Fromageat, P. & Bumpus, F.M. (1974). Solubilization of angiotensin II receptors in rabbit aortae membrane. Nature, New Biology, 249, 67-9.

Dewey, N.M. & Barr, L. (1962). Intercellular connection between smooth muscle cell : The nexus. Science, N.Y. 137, 670-72.

Dewey, N.M. & Barr, L. (1965). The structure and function of the nexus in smooth muscle. Anat. Record., 151, 343.

Dewey, N.M. & Barr, L. (1968). Structure of vertebrate intestinal smooth muscle. In 'Alimentary Canal', vol. IV. Motility. Handbook of Physiology. Amer. Physiol. Soc. Washington, D.C. pp. 1629-54.

Di Rosa, M. & Willoughby, D.A. (1971). Screens for anti-inflammatory drugs. J. Pharm. Pharmacol., 23, 297-8.

Distler, A., Liebau, H. & Wolfe, H.P. (1965). Action of angiotensin on sympathetic nerve endings in isolated blood vessels. Nature, (Lon). 207, 764-5.

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

Dorp, D.A. van (1966). The biosynthesis of prostaglandins. Mem. Soc. Endocrinol., 14, 39-47.

Du Charne, D.W. & Weeks, J.R. (1967). In 'Prostaglandins', Proc. 2nd. Nobel Symp., Stockholm, pp. 173-82. Almqvist & Wiksell, Stockholm, New York.

Dupont, J.R. & Sprinz, H. (1964). The neurovegetative periphery of the gut. A revaluation with conventional techniques in the light of modern knowledge. Amer. J. Anat., 114, 393-402.

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

Durbin, R.S. & Jenkinson, D.H. (1961b). The calcium dependence of tension development in depolarized smooth muscle. J. Physiol., 157, 90-96.

Edman, K.A.P. & Schild, H.O. (1961). Interactions of acetylcholine, adrenaline and magnesium with calcium in the contraction of depolarized rat uterus. J. Physiol., 155, 10-11P.

Edman, K.A.P. & Schild, H.O. (1962). The need for calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus. J. Physiol., 161, 424-41.

Ehinger, B., Falck, B. & Sporrong, B. (1966). Adrenergic fibres to the heart and to peripheral vessels. Biblphie anat., 8, 35-45.

Ehrepreis, S., Greenberg, J. & Belman, S. (1973). Prostaglandins reverse inhibition of electrically-induced contractions of guinea-pig ileum by morphine, indomethacin and acetylsalicylic acid. Nature, New Biology, 245, 280-82.

Eliasson, R. (1959). Studies on prostaglandins. Occurence, formation and biological actions. Acta. Physiol. Scand. 46, (158), 1-73.

Ellis, D.E. & Reit, E. (1969). Inhibition by lidoflazine of the contractile response of the rat isolated colon to angiotensin. Br. J. Pharmacol., 35, 132-40.

Euler, U.S.von. (1934). Zur Kenntuis der pharmkologischen Wirkungen var Nativsekreten und Extrackten mannlicher accessorischer Gescheechtsdrüsen. Arch. exp. Pathol. Pharmacol., 175, 78-84.

Euler, U.S.von. (1935). A depressor substance in the vesicular gland. J. Physiol., 84, 21-22P.

Euler, U.S.von. (1936). On specific vasodilating and plain muscle stimulating substances from accessory genital glands in man and certain animals, (prostaglandin and vesiglandin). J. Physiol., 88, 213-34.

Evans, D.H.L., Schild, H.O. & Thelseff, S. (1958). Effects of drugs on depolarized plain muscle. J. Physiol. 143, 474-85.

Falck, B. (1962). Observations on the permeabilities of the cellular lpcalization of monoamines by a fluorescence method. Acta Phsiol. Scand., 56, 1-25.

Fatt, P. & Katz, B. (1951). An analysis of the endplate potential recorded with an intracellular electrode. J. Physiol., 115, 320-70.

Feldberg, W. & Lewis, G.P. (1963). Release of adrenaline from cat's suprarenals by bradykinin and angiotensin. J. Physiol., 167, 46-47P.

Feldberg, W. & Lewis, G.P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. J. Physiol., 171, 98-108.

Feldberg, W. & Lewis, G.P. (1965). Further studies on the effects of peptides on the suprarenal medulla of cats. J. Physiol., 178, 239-51.

Ferreira, S.H., Herman, A & Vane, J.R. (1972). Prostaglandin generation maintain the smooth muscle tone of the rabbit isolated jejunum. Br. J. Pharmacol., 44, 328P.

Ferreira, S.H., Moncada, S. & Vane, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature, New Biology. 231, 237-9.

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. Pharmacol., 47, 48-58.

Ferreira, S.H. & Vane, J.R. (1967). Prostaglandins. Their disappearance from and release into the circulation. Nature, (Lon). 216, 868-73.

Fishlock, D.J. & Gunn, A. (1970). The action of angiotensin on the human colon in vitro. Br. J. Pharmacol., 39, 34-39.

Flores, A.G.A. & Sharp G.W.G. (1972). Exogenous prostaglandins and osmotic water flow in the toad bladder. Amer. J. Physiol. 223, 1392-97.

Flower, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev., 26, 33-67.

Flower, R.J., Grygleswski, R., Herbaczynskencedro, K. & Vane, J.R. (1972). The effects of anti-inflammatory drugs on prostaglandin biosynthesis. Nature, New Biology, 238, 104-6.

Flower, R.J. & Vane, J.R. (1972). Inhibition of prostaglandin synthetase in brain explains the antipyretic activity of paracetamol (4-acetaindophenol). Nature, (Lon). 240, 410-1.

Folkow, B., Johansson, B. & Mellander, S. (1961). The comparative effects of angiotensin and noradrenaline on consecutive vascular sections. Acta Physiol. Scand., 53, 99-104.

Freer, R.J. & Stewart, J.M. (1972). In 'Structure-activity relationships of proteins and polypeptide hormones'. ed. Margoulies, M. & Greenwood, F.C., Exerpta Medica, Amsterdam. pp. 490-5.

Friedman, S.M. (1972). Angiotensin and sodium in blood vessels. Ann. N.Y. Acad. of Sci.

Friedman, S.M. & Friedman, C.L. (1964). Ionic basis of vascular response to vasoactive substances. Canad. Med. Ass. J., 90, 167-73.

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 diseases', ed. Bajusz, E. pp.323-41. Basel: S. Karger.

Furchgott, R.F. & Bhadrakom, J. (1953). Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J. Pharmcol. exp. Ther., 108,129-43.

Furness, J.B. & Costa, M. (1973). The nervous release and the action of substances which affect intestinal muscle through neither adrenoceptors nor cholinoreceptors. Phil. Trans. R. Soc. (Lon).(B). 265, 123-34.

Gaddum, J.H., Peart, W.S. & Vogt, M. (1949). The estimation of adrenaline and allied substances in blood. J. Physiol. 108, 467-81.

Gansler, H. (1960). Phasenkontrast-und electronenmikroskopische Untersuchungen zur Morphologie und Funklim der glatter Muskulatun. Z. Zellforsch. Mikrosk. Anat., 52, 60-92.

Gascon, A.L. & Walaszek, E.J. (1966). Inhibition of valyl^5-angiotensinamide II by osajin. J. Pharm. (Lon). 18, 478-9.

Gershon, H. (1960). Effects of tetrodotoxin on innervated smooth muscle preparations. Br. J. Pharmacol., 29, 259-79.

Gillespie, J.S. & Muir, T.C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. Br. J. Pharmacol., 30,78-87.

Gilmore, N., Vane, J.R. & Wyllie, J.H. (1969). Prostaglandin release in response to infusion of particles. In 'Prostaglandins, peptides and amines', ed. Mantegazza, P. & Horton, E.W. pp. 21-29. Academic Press, Lon. N.Y.

Godfraind, T., Kaba, A. & Polster, P. (1966a). Dissociation in two contractile components of the isolated guinea-pig ileum response to angiotensin. Arch. Int. Pharmacodyn. 163, 227-9.

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

Gokhale, J.D., Gulati, O.D., Kelka, L.V. & Kelkar, V.V. (k966). Effect of some drugs on human umbilical artery in vitro. Br. J. Pharmacol., 27, 332-46.

Goldblatt, M.W. (1933). A depressor substance in seminal plasma. J. Soc. Chem. Ind. (Lon). 52, 1056-57.

Goldblatt, M.W. (1935). Properties of human seminal plasma. J. Physiol. 84, 208-18.

Goldblatt, H., Lynch, J., Hanzal, R.F. & Summerville, W.W. (1934). Studies on experimental hypertension. I. J. exp. Med. 59, 347-79.

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

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

Goodman, L.S. & Gilman, A. (1970). In 'A pharmacological basis of therapeutics'. 4th ed. The MacMillan Co., London, Toronto, pp. 549-84.

Greenberg, S., Kadowitz, P.J., Diecke, F.P.J. & Long, J.P. (1973). Effect of prostaglandin $F_{2-\alpha}$ on responses of vascular smooth muscle to serotonin, angiotensin and epinephrine. Arch. Int. Pharmacodyn., 206, 5-18.

Greenberg, R.A. & Sparks, H.V. (1969). Prostaglandins and consecutive vascular segments of canine hindlimb. Amer. J. Physiol., 216, 567-71.

Grodzinska, L., Schror, K., Wartner, U., Forster, W & Gryglewski, R. (1974). Prostaglandin synthetase activity in the renal medulla of normal, hypertensive rats. Pol. J. Pharmacol. Pharm., 26, 229-35.

Gross, F. (1971). Angiotensin. In 'Pharmacology of naturally occuring polypeptides and lipid-soluble acids! vol. I. pp. 73-287. Int. Encyl. Pharmacol. Ther. Ed. Walker, J.M. Pergamon Press. Oxford, N.Y. Toronto.

Gross, F., Brunner, H. & Ziegler, M. (1965). Renin-angiotensin system, aldosterone and sodium balance. Recent Progr. Hormone Res., 21, 119-77.

Gross, F., Schaechtelin, G., Brunner, H. & Peters, G. (1964). The role of the renin-angiotensin system in blood-pressure regulation and kidney function. Canad. Med. Ass. J., 90, 258-62.

Gross, F. & Turrian, H. (1960). In 'Polypeptides which affect smooth muscles and blood vessels ', Ed. Schachter, M. Pergamon Press. London.

Haefely, W. (1972). In 'Handbook of experimental Pharmacology'. XXXIII. Catecholamines. Ed. Blaschko, H. & Muscholl, E. pp. 661-725. Springer-Verlag, Berlin, N.Y., Heidelberg.

Haefely, W. (1970). Some actions of bradykinin and related peptides on autonomic ganglion cells. In 'Bradykinin and related kinins'. pp. 591-99. Plenum Press, New York, London.

Haefely, W., Hurlimann, A. & Thoenen, H. (1965). Effect of bradykinin and angiotensin on ganglionic transmission. Biochem. Pharmacol., 14, 1393.

Halliday, R.D. & Buckly, J.P. (1962). Central hypertensive effects of angiotensin II. Int. J. Neuropharmac. 1, 43-7.

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

Hange, A., Lunde, P.K.M. & Waaler, B.A. (1967). Effects of prostaglandin E_1 and adrenaline on the pulmonary vascular resistance (PVR) in isolated rabbit lungs. Life Sci. 6,673-80.

Hedqvist, P. (1969). Modulating effect of prostaglandin-E_2 on noradrenaline release from the isolated cat spleen. Acta Physiol. Scand., 75, 511-512.

Hedqvist, P. (1970). Studies on the effect of prostaglandin E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissues. Acta Physiol. Scand., 79 (345), 1-40.

Hedqvist, P. & Brundin, J. (1969). Inhibition by prostaglandin E_1 of noradrenaline release and of effector response to nerve stimulation in the cat spleen. Life Sci. 8, 389-395.

Helmer, O.M. (1957). Differentiation between two forms of angiotensin by means of spirally cut strip of rabbit aorta. Amer. J. Physiol., 188, 571-7.

Helmer, O.M. (1959). Studies in renin antibodies. Circulation, 17, 648-652.

Helmer, O.M. (1961). Presence of renin in plasma of patients with arterial hypertension. Circulation, 25, 169-73.

Helmer, O.M. (1964). Action of natural angiotensin II and synthetic analogues on strips of rabbit aorta. Amer. J. Physiol., 207, 368-70.

Hillarp, N.A. (1960). Peripheral autonomic mechanisms, In 'Handbook of physiology' Sect. I. Vol. II. Amer. Physiol. Soc. pp. 979-1006. Washington, D.C.

Hodgkin, A.L. & Huxley, A.F. (1939). Action potentials recorded from inside a nerve fibre. Nature, (Lon). 144, 710-11.

Hodgkin, A.L. & Huxley, A.F. (1945). Resting and action potentials in single nerve fibres. J. Physiol., 104, 176-95.

Hodgkin, A.L. & Keynes, R.D. (1955). Active transport of cations in giant squid axons from Sepia and Loligo. J. Physiol., 128, 28-60.

Hodgkin, A.L. & Rushton, W.A.H. (1946). The electrical constants of a crustacean nerve fibre. Proc. R. Soc. (B). 133, 444-79.

Holman, M.E. (1957). The effect of changes in sodium chloride concentration on the smooth muscle of the guinea-pig's taenia coli. J. Physiol., 136, 569-84.

Holman, M.E. (1958). Membrane potentials recorded with high-resistance microelectrodes and the effects of changes in ionic environment on the electrical and mechanical activity of the smooth muscle of thetaenia coli of the guinea-pig. J. Physiol., 141, 464-88.

Holman, M.E. (1968). An introduction to the electrophysical aspects of smooth muscle. In 'Handbook of Physiology', Alimentary Canal, IV. pp.1165-78. Amer. Physiol. Soc. Washington, D.C.

Holmes, S.W., Horton, E.W. & Main, I.H.M. (1963). The effect of prostaglandin E_1 on responses of smooth muscle to catecholamines, angiotensin and vasopressin. Br. J. Pharmacol., 21, 538-43.

Horton, E.W. (1963). Action of prostaglandin E_1 on tissues which respond to bradykinin. Nature, (Lon). 200, 892-3.

Horton, E.W. (1965). Biological activities of pure prostaglandins. Experientia. 21, 113-8.

Horton, E.W. (1968). The prostaglandins. In 'Recent advances in pharmacology'. 4th ed. Ed. Robson, J.M. & Stacey, R.S. Churchill, London. pp. 185-212.

Horton, E.W. (1969). Hypothesis on physiological roles of prostaglandins. Physiol. Rev., 49, 122-61.

Horton, E.W. & Main, I.H.M. (1965). Effects of prostaglandin on unanesthetised animals. J. Physiol., 177, 34P.

Horton, E.W. & Main, I.H.M. (1967). Central nervous action of the prostaglandins and their identification in the brain and spinal cord. In 'Prostaglandins'. Proc. 2nd. Nobel Symp. Stockholm. Ed. Bergstrom, S. & Samuelsson, B. pp. 253-60. Almqvist & Wiksell, Stockholm, Interscience, N.Y.

Housay, B.A. & Braun-Menendez, E. (1942). The role of renin in experimental hypertension. Br. Med. J., 2, 179-81.

Hrdina, P., Bonaccorsi, A. & Garattini, S. (1967). Pharmacological studies on isolated and perfused rat renal arteries. Eur. J. Pharmacol. 1, 99-108.

Hucker, H.B., Zacchei, A.G., Cox, S.V., Brodie, D.A. & Cantwell, N.H.R. (1966). Studies on the absorption, distribution and excretion of indomethacin in various species. J. Pharmacol. exp. Ther., 153, 237-49.

Hughes, J. & Roth, R.H. (1971). Evidence that angiotensin enhances transmitter release during sympathetic nerve stimulation. Br. J. Pharmacol. 41, 239-55.

Jenkinson, D.H. & Morton, I.K.M. (1967). The effect of noradrenaline on the permeability of depolarized intestinal smooth muscle to inorganic ions. J. Physiol. 188, 373-86.

Johansson, B. (1971). Electromechanical and mechanoelectrical coupling in vascular smooth muscle. Angiologica. 8, 129-43.

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

Julian, F.J., Moore, J.W. & Goldman, D.E. (1962a). Membrane potential of the lobster giant axon obtained by use of the sucrose-gap technique. J. Gen. Physiol., 45, 1195-6.

Julian, F.J., Moore, J.W. & Goldman, D.E. (1962b). Current voltage relations in the lobster giant axon membrane under voltage clamp conditions. J. Gen. Physiol. 45, 1217-22.

Kadowitz, P.J., Sweet, C.S. & Brody, M.J. (1971). Differential effects of prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ in the dog hindpaw. J. Pharmacol. exp. Ther. 177, 641-9.

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

Katz, B. (1966). Nerve, Muscle and Synapse. New York, McGraw-Hill.

Keatinge, W.R. & Richardson, D.W. (1963). Measurement of electrical activity in arterial smooth muscle by a sucrose-gap method. J. Physiol. 167, 57-58P.

Keatinge, W.R. (1966). Electrical and mechanical response of vascular smooth muscle to vasodilator agents and vasoactive peptides. Cir. Res., 18, 641-9.

Keech, M.K. (1960). Electronmicroscope study of the normal rat aorta. J. Biophys. Biochem. Cytol., 7, 533-8.

Kerkut, G.A. & Thomas, R.C. (1965). An electrogenic sodium pump in snail nerve cells. Comp. Biochem. Physiol. 14, 167-83.

Kernan, R.P. (1962). Membrane potential changes during sodium transport in frog sartorius muscle. Nature, (Lon). 193, 986-7.

Khairallah, P.A. (1971). Pharmacology of angiotensin. In 'Kidney Hormones'. Ed. Fisher, J.W. pp. 130-72. Academic Press, London, New York.

Khairallah, P.A. & Page, I.H. (1961). Mechanism of action of angiotensin and bradykinin on smooth muscle *in situ*. Amer. J. Physiol., 200, 51-4.

Khairallah, P.A. & Page, I.H. (1962). Effect of adrenergic agents on responses of smooth muscle to angiotensin. Amer. J. Physiol. 202, 841-4.

Khairallah, P.A. & Page, I.H. (1963). Effects of bradykinin and angiotensin on smooth muscle. Ann. N.Y. Acad. Sci., 104, 212-20.

Khairallah, P.A., Bumpus, F.M. & Turker, R.K. (1966). Angiotensin tachyphylaxis and its reversal. Cir. Res., 19, 247-54.

Khairallah, P.A., Page, I.H. & Turker, R.K. (1967). Potentiation of vascular myotropic responses by metanephrine and other noncatecholamines. Arch. int. Pharmacodyn. Ther. 169, 328-41.

Khairallah, P.A., Vadaparampil, G.J. & Page, I.H. (1965). Effect of ions on angiotensin interaction with smooth muscle. Arch. int. Pharmacodyn. Ther., 158, 155-64.

Kiran, B.K. & Khairallah, P.A. (1969). Angiotensin and norepinephrine efflux. Eur. J. Pharmacol., 6, 102-8.

Klinge, E., Mattila, M.J., Penttila, O. & Jukarainen, E. (1966). Influence of drugs on vasoactive peptides and amines in perfused human placenta. Ann. Med. Exp., Fenn., 44, 369-75.

Kobayashi, M. (1971). Relationship between membrane potential and spike configuration recorded by sucrose-gap method in the ureter smooth muscle. Comp. Biochem.Physiol., 38, 301-8.

Koketsu, K. (1971). The electrogenic sodiumpump. Adv. Biophys. 2, 77-112.

Kosterlitz, H.W., Lees, G.M. & Wallis,D.I.(1970). Further evidence for an electrogenic sodium pump in a mammalian sympathetic ganglion. Br. J. Pharmacol., 38, 464-5P.

Kuriyama, H. (1961). Recent studies on the electrophysiology of the uterus. In Ciba Foundation Study Group 9. pp. 51. London, Churchill.

Kuriyama, H. (1970). Effects of ions and drugs on the electrical activity of smooth muscle. In 'Smooth Muscle'. pp.366. Ed. Bulbring, E., Jones, A., Brading, A. & Tomita, T. Edward Arnold, London.

Kuriyama, H., Ito, Y. & Sakatomo, Y. (1970). Effects of tetraethylammonium chloride on the membrane activity of guinea-pig stomach smooth muscle. J. Physiol., 211, 445-60.

Kuriyama, H. & Tomita, T. (1970). The action potential in the smooth muscle of the guinea-pig taenia coli and ureter by the double sucrose-gap method. J. Gen. Physiol. 55, 147-62.

Kurzok, R. & Lieb, C.C. (1930). Biochemical studies of human semen. II. The action of semen on the human uterus. Proc. Soc. Exp. Biol. Med., 28, 268-272.

Laverty, R. (1963). A nervously-mediated action of angiotensin in anaesthetised rats. J. Pharm. Pharmacol., 15,63-8.

Lee, J.B. (1967). In 'Prostaglandins II'. Nobel Symp. Ed. Bergstrom, S. & Samuelsson, B., Almqvist & Wiksell, New York, Interscience, Amsterdam.

Lee, J.B., Corino, B.G., Takman, B.H. & Smith, E.R. (1965). Renomedullary vasodepressor substances, medullin; isolation, chemical characterization and physiological properties. Cir. Res., 17, 57-7.

Lee, J.B., Crowshaw, K., Takman, B.H., Attrep, K.A. & Gougoutas, J.Z. (1967). The identification of prostaglandins E_2, $F_{2-\alpha}$ and A from rabbit kidney medulla. Biochem. J., 105, 1251-60.

Lefer, A.M. (1967). Influence of mineralocorticoids and cations in the inotropic effect of angiotensin and norepinephrine in isolated cardiac muscle. Amer. Heart J. 73, 674-80.

Lewis, G.P. (1964). A comparison of angiotensin and bradykinin. Canad. Med. Ass. J., 90, 302-7.

Lewis, G.P. (1970). In 'Bradykinin and related kinins'. Ed. Sicuteri, F., Rocha e Silva, M & Back, N. pp. 571-90. Plenum Press, N.Y. London.

Lewis, G.P. & Reit, E. (1966). Further studies on the actions of peptides on the superior cervical ganglion and suprarenal medulla. Br. J. Pharmacol., 26, 444-60.

Liebau, H., Distler, A. & Wolfe, H.P. (1965). The noradrenaline releasing effect of angiotensin II in isolated blood vessels. Acta Endocr. Copenh., 50 (100), 138.

Liebau, H., Distler, A. & Wolfe, H.P. (1966). Untersuchengen zur indirekten sympathomimetischen wirkung von angiotensin an isolierten blutzefaben. Klin. Wschr., 44, 322-26.

Ling, G.N. (1966). Elecktriche Potentiale lebender zeller. In 'Die Zelle'. Ed. Metzuer, H., Stuttgert, H. wissenschaftliche verlagsgesellschafte.

Ling, G.N. & Gerard, R.W. (1949). The normal membrane potential of frog sartorius fibres. J. Cell Comp. Physiol., 34, 383-96.

Mann, M. & West, G.B. (1950). The nature of hepatic and splenic sympathin. Br. J. Pharmacol., 5, 173-7.

Margouilies, M. & Greenwood, F.C. (1972). In 'Structure-activity relationships of proteins and polypeptides hormones! Excerpta Medica, Amsterdam.

Mark, A.L., Schmid, P.G., Eckstein, J.W. & Wendling, M.G. (1971). Venous responses to prostaglandin $F_{2-\alpha}$. Amer. J. Physiol., 220, 222-6.

Marshall, J.M. (1962). Regulation of activity in uterine smooth muscle. Physiol. Rev., 42, 213-27.

Marshall, G.R., Vine, W. & Needleman, P.E. (1970). A specific competitive inhibitor of angiotensin II. Proc. Nat. Acad. Sci., U.S.A. 67, 1624-30.

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

McGiff, J.C., Crawshow, K., Terragno, N.A. & Lonigro, A.J. (1970). Release of prostaglandin-like substance into renal venous blood in response to angiotensin II. Cir. Res. 27, 1121-30.

McGiff, J.C. & Hskovitz, H.D. (1973). Prostaglandins and the kidney'. Cir. Res., 33, 479-88.

McGiff, J.C., Terragno, N.A., Strand, J.C., Lee, J.B., Lonigro, A.J. & Ng, K.K.F. (1969). Selective passage of prostaglandins across the lung. Nature, (Lon). 223, 742-45.

McGregor, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. J. Physiol. 177, 21.

McLean, J.R. & Burnstock, G. (1967). Innervation of the lungs of the toad (Bufo marinus) II. Fluorescent histochemistry of catecholamines. Comp. Biochem. Physiol., 22, 767-73.

McNeil, J.R. & Sutherland, G.B. (1973). Recovery response of intestinal resistance vessels during infusions of angiotensin. Fed. Proc., 32, 788.

Merrillees, N.C.R. (1968). The nervous environment of individual smooth muscle cells of the guineapig vas deferens. J. Cell. Biol., 37, 794-817.

Merrillees, N.C.R , Burnstock, G. & Holman, M.E. (1963). Correlation of fine structure and physiology of the innervation of smooth muscle in the guinea-pig vas deferens. J. Cell Biol., 19, 529-50.

Messina, E.J., Weina, R. & Kaley, G. (1973). Effect of inhibition of prostaglandin synthesis on arteriolar responsiveness. Fed. Proc., 32, 788.

Meyer, P., Papadimitriou, A. & Worcel, M. (1970). Possible existence of different types of angiotensin II receptors. Br. J. Pharmacol., 40, 541-2P.

Miyazaki, E., Ishizawa, M., Sunano, S., Syuto, B. & Sakagami, I. (1967). Stimulating action of prostaglandin on the rabbit duodenal muscle. In 'Prostaglandins', Proc. 2nd. Nobel Symp. Stockholm. pp. 277-82. Almqvist & Wiksell, Interscience, Stockholm, N.Y.

Mimran, A., Hinrichs, K.J. & Hollenberg, N.K. (1974). Characterization of smooth muscle receptors for angiotensin: Studies with an antagonist. Amer. J. Physiol., 226, 185-90.

Muirhead, E.E., Daniels, E.E., Pike, J.E. & Hinman, J.W. (1967). Renomedullary antihypertensive lipids and the prostaglandins. In 'Prostaglandins(, Proc. 2nd. Nobel Symp. pp. 183-196. Almqvist & Wiksell, Stockholm, N.Y.

Muirhead, E.E., Leach, B.E., Nyers, L.W., Brooks, B., Daniels, E.E. & Hinman, J.W. (1971). In 'Kidney Hormones'. pp. 485-506. Ed. Fisher, J.W. Academic Press, New York.

Nagatsu, I., Gillespie, L. & Glenner, G.G. (1965). Serum aminopeptidases, 'angiotensinase' and hypertension. I. Degradation of angiotensin II by human serum. Biochem. Pharmacol., 14, 721-8.

Nakano, J. (1972). Relationship between the chemical structure of prostaglandins and their vasoactivities in dogs. Br. J. Pharmacol., 44, 63-70.

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

Needleman, P., Kauffman, A.H., Douglas, J.R., Johnson, E.M. & Marshall, G.R. (1973a). Specific stimulation and inhibition of renal prostaglandin release by angiotensin analogs. Amer. J. Physiol., 224, 1415-9.

Needleman, P., Marshall, G.R. & Douglas, J.R. (1973b). Prostaglandin release from vasculature by angiotensin II: Dissociation from lipolysis. Eur. J. Pharmacol., 66, 316-9.

Ng, K.K.F. & Vane, J.R. (1968). Fate of angiotensin I in the circulation. Nature, (Lon). 218, 144-50.

Noble, D. (1966). Application of Hodgkin-Huxley equation to excitable tissues. Physiol. Rev., 46, 1-50.

Northover, B.J. (1967). The antagonism between anti-inflammatory drugs and substances that constrict veins. J. Path. Bact., 94, 206-12.

Northover, B.J. (1971). Mechanism of the inhibitory action of indomethacin on smooth muscle. Br. J. Pharmacol., 41, 540-51.

Nugteren, D.H. & Dorp, D.A. van. (1965). The participation of molecular oxygen in the biosynthesis of prostaglandins. Biochem. Biophys. Acta. 98, 654-56.

Ohashi, H., Nonomura, Y. & Ohga, A. (1967). The effect of angiotensin and bradykinin on the guinea-pig taenia coli. Jap. J. Pharmacol., 17, 247-57.

Orlov, R.S. & Plekanov (1968). Change of membrane potential of cells of vascular smooth muscles in response to excitation. Dokl. Akad. Nauk SSSR. 175 (1), 254-55.

Owen, D.A.A. (1969). Studies on the interaction between angiotensin and the sympathetic nervous system. PH. D. thesis. University of Aston in Birmingham, England.

Ogura, Y., Mori, Y. & Watanabe, Y. (1966). Inhibition of the release of acetylcholine from isolated guinea-pig ileum by crystalline tetrodotoxin. J. Pharmac. exp. Ther., 154, 456-62.

Paiva, T.B. & Paiva, A.C.M. (1960). The oxytocic activity of synthetic angiotensin-amide. J. Pharmacol., 130, 177-82.

Paiva, T.B., Juliano, L., Nouilhetas, V.L.A. & Paiva, A.C.M. (1973). The effect of pH on tachyphylaxis to angiotensin peptides in the isolated guinea-pig ileum and rat uterus. Eur. J. Pharmacol., 25, 191-6.

Panisset, J.C. (1967). Effect of angiotensin on the release of acetylcholine from preganglionic and postganglionic nerve endings. Canad. J. Physiol. Pharmacol., 45, 313-7.

Panisset, J.C. & Bourdois, P. (1967). Effect of angiotensin on sympathetic nerve endings: inhibition of the uptake of norepinephrine. Fed. Proc., 26, 466.

Pals, D.T., Masucci, F.D., Sipos, F. & Denning, G.S. (1971). A specific competitive antagonist of the vascular action of angiotensin II. Cir. Res., 29, 664-72.

Page, I.H. (1940). Difference in the activating effect of normal and hypertensive plasma on intestinal segments treated with renin. Amer. J. Physiol., 130, 29-33.

Page, I.H. & Bumpus, F.M. (1961). Angiotensin. Physiol. Rev., 41, 33-90.

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.

Page, I.H.& McCubbin, J.W. (1968). 'Renal Hypertension'. Year book Med. Publishers, Inc., Illinois.

Page, I.H., McCubbin, J.W., Schwarz, R.R. & Bumpus, F.M. (1957). Pharmacological aspects of synthetic angiotonin. Cir. Res., 5, 552.

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

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

Paton, W.D.M. & Daniel, E.E. (1967). On the contractile response of theisolated rat uterus to prostaglandin E_1. Canad. J. Physiol. Pharmacol., 45, 795-804.

Peach, M.J. (1972). Physiological roles of angiotensin. In 'Chemistry and biology of Peptides'. Ed., Meienhofer, J. pp. 471-93. Ann Arbor Sc. Publishers Inc., Ann Arbor.

Peach, M.J., Bumpus, F.M. & Khairallah, P.A. (1969). Inhibition of norepinephrine uptake in hearts by angiotensin II and analogs. J. Pharmacol. exp. Ther., 167, 291-99.

Pease, D.C. & Paule, W.J. (1960). Electron microscopy of elastic arteries: the thoracic aorta of the rat. J. Ultrastructure Res., 3, 469-83.

Peiper, U., Greibel, L. & Wende, W. (1971). Activation of vascular smooth muscle of rat aorta by noradrenaline and depolarization: two different mechanisms. Pfluegers Arch. 330, 74-89.

Peskar, B. & Hertting, G. (1973). Release of prostaglandins from isolated cat spleen by angiotensin and vasopressin. Naunyn-Schwiedeberg's Arch. Pharmacol., 279, 227-34.

Pickles, V.R. (1967). The myometrial actions of six prostaglandins: Consideration of a receptor hypothesis. In 'Prostaglandins', ed., Bergstrom, S. & Samuelsson, B. pp 79-84. Almqvist & Wiksell, Stockholm, Interscience, N.Y.

Piper, P.J. & Vane, J.R. (1971). The release of prostaglandins from lung and other tissues. Ann. N.Y. Acad. Sci., 180, 363-85.

Prosser, C.L. (1962). Conduction in unstriated muscles. Physiol. Rev. 42, 193-260.

Prosser, C.L. & Bortoff, A. (1968). Electrical activity of intestinal muscle under in vitro conditions. In 'Handbook of Physiology', Amer. Physiol. Soc. VI. pp. 2025-50. Washington.

Prosser, C.L., Burnstock, G. & Kahn, J. (1960). Conduction in smooth muscle : Comparative structural properties. Amer. J. Physiol., 199, 545-52.

Ramwell, P.W. & Shaw, J.E. (1970). Biological significance of the prostaglandins. Recent. Progr. Hormone Res., 26, 139-73.

Ramwell, P.W., Shaw, J.E., Douglas, W.W. & Poisner, A.M. (1966). Efflux of prostaglandin from adrenal glands stimulated with acetylcholine. Nature, 210, 273-4.

Read, J.B. & Burnstock, G. (1969). Adrenergic innervation of the gut musculature in vertebrates. Histochemie. 17,263-77.

Regoli, D. & Park, W.K. (1972). The pressor and myotropic effects and the antagonistic properties of several analogues of angiotensin II. Canad. J. Physiol. Pharmacol., 50, 99-112.

Regoli, D. & Vane, J.R. (1964a). A sensitive method for the assay of angiotensin. Br. J. Pharmacol., 23, 351-9.

Regoli, D. & Vane, J.R. (1964b). The release and detection of angiotensin and of catecholamines in the circulation of the dog. J. Physiol., 172, 34P.

Regoli, D. & Vane, J.R. (1966). The continuous estimation of angiotensin formed in the circulation of the dog. J. Physiol. 183, 513-31.

Renson, J., Barac, G. & Bacq, Z.M. (1959). Angiotenines, calcium et effect ocytocique. C.R. Soc. Biol. Paris. 153, 706-8.

Rhodin, J.A.G. (1962). Fine structure of vascular walls in mammals, with special reference to smooth muscle component. Physiol. Rev., 42(5), 48-81.

Rhodin, J.A.G. (1967). The ultrastructure of mammalian arterioles and precapillary sphincters. J. Ultrastruct. Res. 18, 181-223.

Richardson, K.C. (1958). Electron microscopic observations on Auerbach's plexus in the rabbit with special reference to the problem of smooth muscle innervation. Am.J. Anat. 103, 99-135.

Richardson, K.C. (1962). The fine structure of autonomic nerve endings in smooth muscle of the rat vas deferens. J. Anat., 96, 427-42.

Rious, F., Park, W.K. & Regoli, D. (1973). Pharmacology of angiotensin antagonists. Canad. J. Physiol. Pharmacol., 51, 108-113.

Robertson, P.A. (1960). Calcium and contractility in depolarized smooth muscle. Nature, (Lon)., 186, 316-7.

Robertson, A.L. & Khairallah, P.A. (1972). Effects of angiotensin II and some analogs on vascular permeability in the rabbit. Cir. Res., 31, 923.

Robertson, P.A. & Rubin, D. (1958). An indirect action of angiotensin I on smooth muscle. Nature, (Lon). 182, 867-8.

Robertson, P.A. & Rubin, D. (1962). Stimulation of intestinal nervous elements by angiotensin. Br. J. Pharmacol., 19, 5-12.

Robinson, R.L. (1967). Simulation of the catecholamine output of the isolated perfused adrenal gland of the dog by angiotensin and bradykinin. J. Pharmacol. exp. Ther., 156, 252.

Rocha e Silva, M. (1970). In 'Bradykinin and related Kinins', ed., Sicuiteri, F., Rocha e Silva, M & Back, N. pp. 507-24. Plenum Press, New York, London.

Rogers, D.C. & Burnstock, G. (1966). The intestitial cell and its place in the concept of the autonomic ground plexus. J. Comp. Neurol., 126, 255-84.

Rorive, G. & Hagemeijer, F. (1966). Influence de la noradrenalin et de l'angiotensine sur la composition ionique de la fibre musculaire de l'aorte de rat. Ann. Endocr. Paris, 27, 521-3.

Ross, C.A., Ludden, C.T. & Stone, L.A. (1960). Action of angiotensin on isolated guinea-pig ileum. Proc. Soc. Exp. Biol. N.Y. 105, 558-9.

Ryhage, R. & Samuelsson, B. (1965). The origin of oxygen incorporated during the biosynthesis of prostaglandin E_1. Biochem. Biophys. Res. Commun., 19, 279-82.

Sakurai, T. & Hashimoto, Y. (1965). The vasoconstrictor action of angiotensin in relation to catecholamines. Jap. J. Pharmacol., 15, 223.

Samuelsson, B. (1964). Identification of a smooth muscle-stimulating factor in bovine brain. Biochim. Biophys. Acta, 84, 218-9.

Samuelsson, B. (1965). On the incorporation of oxygen in the conversion of 8,11,14-eicosatrienoic acid to prostaglandin E_1. J. Amer. Chem. Soc., 87, 3011-3.

Samuelsson, B. (1972). Biosynthesis of prostaglandins. Fed. Proc., 31, 1442-50.

Schachter, M. (1970). In 'Bradykinin and related kinins'. Ed., Sicuiteri, F., Rocha e Silva, M. & Back, N. Plenum Press, New York, London.

Schatzman, H.J. (1953). Helv. physiol. Pharmac., Acta, 11, 346-54.

Schild, H,O. (1964). Calcium and the effects of drugs on depolarized smooth muscle. In 'Pharmacology of smooth muscle' ed., Bulbring, E. pp. 95-104. Oxford, Pergamon Press.

Schmitt, H. & Schmitt, H. (1968). Modifications des effets hypertenseurs de l'angiotensine par les agents adrenegiques et antiadrenegiques. Arch. Int. Pharmacodyn. Ther., 171, 31-46.

Schofield, G.C. (1968). The enteric plexus of mammals. In 'Int. Rev. Gen. Exptl. Zoology', ed. Fets W.J.L. & Hurrison, R.J. Academic Press, N.Y.

Schumann, H.J. & Güther, W. (1967). Untersuchungen zum wirkungsmechanismus von Angiotensin am isolierten Aortenpräparat und am Blutdruck von Ratten und Meerschweinchen. Nannyn-Schmiedbergs. Arch. Pharmak. exp. Path., 256, 169.

Schwyzer, R., Iselin, B., Kappeler, H., Riniker, B., Rittel, W. & Zuber, H. (1958). Synthese hochwirksamen oktopeptide wit der vermetliden Aminosauresequenz des moch unbekanten Hypertensin II aus Rinderserum. Helv. Chim. Acta. 41, 1287-95.

Scornik, O.A. & Paladini, A.C. (1961). Angiotensin blood levels in dogs with experimental renal hypertension. Amer. J. Physiol., 201, 526.

Scroop, G.C. & Lowe, R.D. (1968). Central pressor effect of angiotensin mediated by the parasympathetic nervous system. Nature, (Lon). 220, 1331-2.

Scroop, G.C. & Whelan, R.F. (1968). A central vasomotor action of angiotensin in man. Clin. Sci., 30, 79-90.

Shibata, S. & Briggs, A.H. (1966). The relationships between electrical and mechanical events in rabbit aortic strips. J. Pharmacol. exp. Ther., 153, 466-70.

Shipley, R.E. & Tilden, J.H. (1947). A pithed rat preparation suitable for assaying pressor substances. Proc. Soc. Exptl. Biol. N.Y., 64, 453-455.

Sih, C.J. & Takeguchi, C.A. (1973). Biosynthesis of prostaglandins. In 'The Prostaglandins', ed. Ramwell, P.W. vol.I. pp. 83-100. Plenum Press, N.Y. London.

Skeggs, L.T., Kahn, J.R. & Shumway, N.P. (1956). The preparation and formation of the hypertensin-converting enzyme. J. Exp. Med., 103, 295-9.

Skeggs, L.T., Lentz, K.E., Kahn, J.R., Woods, K.R. & Shumway, N.P. (1956). The amino acid sequence of hypertensin II. J. Exp. Med., 104, 193-7.

Skinner, S.L., McCubbin, J.W. & Page, I.H. (1964a). Control of renin secretion. Cir. Res., 15, 64-76.

Skinner, S.L., McCubbin, J.W. & Page, I.H. (1964b). Renal baroceptor control of acute renin release in normotensive nephrogenic and neurogenic hypertensive dogs. Cir. Res., 15, 522-31.

Skou, J.C. (1965). Enzymatic means for active transport of sodium and potassium across cell membrane. Physiol. Rev., 45, 596-617.

Smith, W.L. & Lands, W.E.M. (1971). Stimulation and blockade of prostaglandin biosynthesis. J. Biol. Chem. 246, 6700-2.

Somlyo, A.P. (1972). Excitation-contraction coupling in vertebrate smooth muscle: Correlation of ultrastructure with function. The Physiologist. 15, 338-48.

Somlyo, A.P. & Somlyo, A.V. (1964). Venomotor effects of angiotensin and isoproterenol. Clin. Res., 12, 193.

Somlyo, A.P. & Somlyo, A.V. (1968). Vascular smooth muscle. I. Normal structure, pathology, biochemistry and biophysics. Pharmacol. Rev. 20, 197-272.

Somlyo, A.P. & Somlyo, A.V. (1970). Vascular Smooth muscle. II. Pharmaoclogy of normal and hypertensive vessels. Pharmacol. Rev. 22, 249-353.

Somlyo, A.V. & Somlyo, A.P. (1966). Effect of angiotensin and beta-adrenergic stimulation on venous smooth muscle. Amer. Heart J., 71, 569-70.

Somlyo, A.V., Woo, C.Y. & Somlyo, A.P. (1965). Responses of nerve-free vessels to vasoactive amines and polypeptides. Amer. J. Physiol. 208, 748-53.

Sorrentino, L., Capasso, F. & Di Rosa, M. (1972). Indomethacin and prostaglandins. Eur. J. Pharmaol., 17, 306-8.

Stampfli, R. (1954). A new method for measuring membrane potential with external electrodes. Experientia, 10, 508-9.

Strong, K.C., Pease, D.C. & Paule, W.J. (1960). Electron microscopy of elastic arteries: the thoracic aorta of the rat. J. Ultrastruct. Res., 3, 469-83.

Su, C. (1965). Angiotensin receptor site and effect on sympathetic transmission in the isolated pulmonary artery. Fed. Proc. Fed. Amer. Soc. exp. Biol. 24, 489.

Sullivan, L.J. & Briggs, A.H. (1968). Effects of manganese on the response of aortic strips to angiotensin and norepinephrine contraction. J. Pharmacol. exp. Ther. 161, 205.

Sutter, M.C. (1965). The pharmacology of isolated veins. Br. J. Pharmacol. 24, 742.

Suzuki, A & Matsumoto, H. (1965). The mechanism of the contracting action of angiotensin on the excised small intestine of guinea-pigs analysed by the concentration-action curve. Kobe J. Med. Sci., 11, 1111-3.

Suzuki, A. & Matsumoto, H. (1966). The mechanism of contracting action of angiotensin on the isolated aortic strip of rabbit. Kobe J. Med. Sci., 12, 167-78.

Takeuchi, A. & Takeuchi, N. (1959). Active phase of frog's endplate potential. J. Neurophysiol. 22, 395-411.

Takeuchi, A. & Takeuchi, N. (1960). On the permeability of endplate membrane during the action of transmitter. J.. Physiol., 154, 52-67.

Taxi, J. (1965). Contribution a l'etude des connexions des neurones moteurs du systeme nerveux autonome. Naturelles Zoolgie,, 12, serie VII, 413-674.

Taylor, G.S., Paton, D.M. & Daniel, E.E. (1969). Characteristics of electrogenic sodium pumping in rat myometrium. J. Gen. Physiol., 56, 360-75.

Thaemert, J.C. (1966). Ultrastructural interrelationships of nerve processes and smooth muscle cells in three dimensions. J. Cell Biol., 28, 37-49.

Thomas, R.C. (1972). Electrogenic sodium pump in nerve and muscle cells. Physiol. Rev., 52, 563-94.

Thompson, J.L. (1970). Effect of angiotensin on the cardioaccelerator response to sympathetic nerve stimulation in isolated rabbit heart. Proc. Soc. exp. Biol. 135, 825.

Thurau, K., Valtin, H. & Schnermann, J. (1968). Kidney. Ann. Rev. Physiol. 30, 441-524.

Tigerstedt, T. & Bergman, P.G. (1898). Nieve und kreishauf. Skand. Arch. Physiol. 8, 223-71.

Trendelenberg, U. (1966). Observations of the ganglion stimulating action of angiotensin and bradykinin. J. Pharmac. exp. Ther., 154, 418-25.

Troshin, A.S. (1962). Problems of cell permeability. Oxford, Pergamon Press.

Turker, R.K. & Karahneyinoglu, E. (1968). Interaction of cocaine with angiotensin and tyramine on the isolated rabbit aortic strips. Experientia. 24, 921-2.

Turker, R.K., Page, I.H. & Khairallah, P.A. (1967). Angiotensin alteration of sodium fluxes in smooth muscle. Arch, Int. Pharmacodyn. Ther., 165, 394-404.

Ursillo, R.C. (1961). Electrical activity of the isolated nerve-urinary bladder strip preparation of the rabbit. Amer. J. Physiol., 201, 408-412.

Vander, A.J. (1965). Effect of catecholamines and the renal nerves on renin secretion in anesthetised dogs. Amer. J. Physiol., 209, 659-62.

Vander, A.J. (1967). Control of renin secretion. Physiol. Rev., 47, 359-82.

Vander, A.J. & Miller, R. (1964a). Control of renin secretion in the anesthetised dog. Amer. J. Physiol., 207, 537-46.

Vander, A.J. & Miller, R. (1964b). Control of renin secretion in anesthetised dogs. Fed. Proc., 23, 467.

Van Nueten, J.M., Dresse, A. & Dony, J. (1964). Action antagoniste de la cinnarizine vis-a-vis l'angiotensin in vitro. C.R. Soc. Biol., 158, 1750-4.

Vane, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. Br. J. Pharmacol. 12, 344-9.

Vane, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature, (Lon). New Biology. 231, 232-5.

Verity, M.A. (1971). Morphologic studies of the vascular neuroeffector apparatus. In 'Physiology and pharmacology of vascular neuroeffector systems'. Ed. Bevan, J.A., Furchgott, R.F., Maxwell, R.A. & Somlyo, A.P. pp. 2-12. S. Karger-Basel.

Verity, M.A. & Bevan, J.A. (1968). Fine structural study of the terminal effector plexus, neuromuscular and intervascular relationships in the pulmonary artery. J. Anat. Lond., 103, 49-63.

Vigneaud, v.Du. (1963). Discussion of paper by Nicolaides, Dewald and Croft. Ann. N.Y. Acad. Sci. 104, Art. I.,22-23.

Walaszek, E.J., Huggins, C.G. & Smith, C.M. (1963). Drugs that modify actions of pharmacologically active polypeptides. Ann. N.Y. Acad. Sci. 104, 281-9.

Walter, P. & Bassenge, E. (1969). Effect of angiotensin on vascular smooth muscles. Pflugers Arch. 307, 70-82.

Wathen, R.L., Kingsbury, W.S., Stouder, D.A., Schneider, E.G. Rostorfer, H.H. (1965). Effects of infusion of catecholamines and angiotensin II on renin release in anaesthetised dogs. Amer. J. Physiol., 209, 1012-4.

Waugh, W.H. (1962). Role of calcium in contractile excitation of vascular smooth muscle by epinephrine and potassium. Cir. Res. 11, 927-40.

Weatherall, M. (1962). Location of fraction of potassium in rabbit auricles. Proc. Roy. Soc. Lond.(B). 156, 83-95.

Weeks, J.R. (1972). Prostaglandins. Physiol. Rev., 52, 317-336.

Weeks, J.R. & Wingerson, F. (1964). Cardiovascular action of prostaglandin E_1 evaluated using anaesthetised relatively unrestrained rats. Fed. Proc. 23, 327.

Weiner, R. & Kaley, G. (1969). Influence of prostaglandin E_1 on the terminal vascular bed. Amer. J. Physiol. 217, 563-6.

Wennmalm, A. & Stjarne, L. (1971). Inhibition of the release of adrenergic transmitter by a fatty acid in the perfusate from sympathetically stimulated rabbit heart. Life Sci. 10,(1). 471-79.

Wolfe, L.S. & Coceani, F. (1967). Brain prostaglandins and studies of the action of prostaglandins on the isolated rat stomach. In 'Prostaglandins', pp. 265-75. Ed. Bergstrom, S. & Samuelsson, B. Almqvist and Wiksell, Stockholm, N.Y.

Woodbury, J.W. & Brady, A.J. (1956). Intracellular recording from moving tissues with a flexibly mounted ultra-micro-electrode. Science, 123, 100-1.

Yamachi, A. (1964). Electron microscopic studies on the autonomic neuromuscular junction in the taenia coli of the guinea-pig. Acta Anat. Nippon. 39, 22-38.

Zimmerman, B.G. (1962). Effect of acute sympathetomy on responses to angiotensin and norepinephrine. Cir. Res., 11, 780.

Zimmerman, B.G. & Gisslen, J. (1968). Pattern of renal vaso-constriction and transmitter release during sympathetic stimulation in presence of angiotensin and cocaine. J. Pharmac. exp. Ther. 163, 320-9.

Zimmerman, B.G. & Whitmore, L. (1966). Effect of angiotensin and phenoxybenzamine on release of norepinephrine during sympathetic nerve stimulation. Fed. Proc. 25, 383.

Zimmerman, B.G. & Whitmore, L. (1967). Effect of angiotensin and phenoxybenzamine on release of norepinephrine in vessels during sympathetic nerve stimulation. Int. J. Neuropharmacol. 6, 27-38.

ADDENDUM TO REFERENCES

Blaustein, M.P. & Goldman, D.E. (1966). Action of anionic and cationic nerve-blocking agents : experiments and interpretation. Science, 153, 429-32.

Brown, D.A. (1966). Depolarization of normal and pre-ganglionically-denervated superior cervical ganglia by stimulant drugs. Br. J. Pharmacol., 26, 511-20.

Brown, D.A., Brownstein, M.J. & Schofield, C.N. (1969). On the nature of the drug induced after-hyperpolarization in isolated ganglia. Br. J. Pharmacol., 37, 511P.

Koenig, K. (1962). Membrane potential measurements in the skeletal muscle with the 'saccharose-gap' method. Pflueger Arch. ges. Physiol., 275, 452-60.

Vogt, W. & Distelkotter, B. (1967). Release of prostaglandin from frog intestine. In ' Prostaglandins'. 2nd. Nobel Symposium, Stockholm. Almqvist & Wiksell, Stockholm, Interscience, New York.

Boadle-Biber, M.C., Hughes, J. and Roth, R.H. (1969). Angiotensin accelerates catecholamine biosynthesis in sympathetically innervated tissues. Nature (Lon). 222, 987-988.