THE MODE OF ACTION OF BRADYKININ

BY

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SUMMARY

The action of bradykinin on transepithelial transfer of sodium and water in isolated rat jejunum and on smooth muscle contraction of rat terminal ileum has been investigated.

(1) Bradykinin was shown to stimulate transfer at low control transfer, inhibit transfer at high control transfer and have no effect at intermediate transfer in rat jejunal sacs. Stimulation of transfer occurred only when bradykinin was in the serosal solution while inhibition of transfer occurred whether bradykinin was in the serosal or mucosal solution.

Bradykinin-induced stimulation of transfer was not affected by adrenalectomy, nephrectomy, combined adrenalectomy-nephrectomy, nor maintenance on 1% saline drinking solution or low sodium diet pretreatment.

Meclofenamic acid abolished the bradykinin-induced inhibition of water transfer while prostaglandins A_1, E_1 and $F_{2\alpha}$ all potentiated this action.

Theophylline inhibited water transfer and potentiated the bradykinin-induced inhibition of water transfer. Cyclic AMP and dibutyryl cyclic AMP both inhibited water transfer and the bradykinin-induced inhibition of water transfer was potentiated by the latter.

(2) Bradykinin-induced contractions of rat terminal ileum were little affected by hyoscine while those of acetylcholine were abolished.

Anoxia reduced markedly responses to bradykinin while those of acetylcholine were little affected.

Theophylline reduced the responses of rat terminal ileum to bradykinin significantly more than those to acetylcholine.

Aspirin and indomethacin reduced markedly the responses to bradykinin while not affecting those to acetylcholine and PGI_2. Meclofenamic acid at a concentration of 3.4 μM blocked bradykinin-induced contractions but

had no effect on those to acetylcholine, PGE_2 or $PGF_{2\alpha}$ and at a concentration of 17.0 μM drastically reduced bradykinin responses but also reduced those to acetylcholine, PGE_2 and $PGF_{2\alpha}$. Flufenamic acid drastically reduced responses to bradykinin while not affecting those to acetylcholine and PGE_2 and slightly affecting those to $PGF_{2\alpha}$.

Polyphloretin phosphate reduced responses to bradykinin, $PGF_{2\alpha}$ and PGE_2 but not acetylcholine. Diphloretin phosphate reduced responses to bradykinin, $PGF_{2\alpha}$ and PGE_2 in a dose dependent manner but not those to acetylcholine. SC 19220, in a dose dependent manner, inhibited responses to bradykinin and PGE_2 but not to acetylcholine and $PGF_{2\alpha}$. 7 oxa - 13 - prostynoic acid non specifically reduced responses to acetylcholine, bradykinin and PGE_2.

Bradykinin, in the presence of SQ 20881, increased the release of prostaglandin-like activity from rat terminal ileum and this was reduced or abolished in the presence of indomethacin, aspirin, meclofenamic acid or flufenamic acid.

The extract of PG-like activity did not appear as PGE, PGA or PGF on TLC, but included a substance with similar mobility as 15-Keto-prostaglandin E_2.

A C K N O W L E D G E M E N T S

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CONTENTS

GENERAL INTRODUCTION

The present study has involved an investigation of the action of bradykinin on transepithelial sodium and water transfer and on the contractile ability of rat terminal ileum. Thus it is considered relevant to briefly review the biochemistry and possibly physiological actions of this peptide.

The study of the action of bradykinin on sodium and water transfer in isolated rat jejunum was stimulated by suggestions that the kallikrein-kinin system might fulfill the role of a natriuretic hormone (Adetuyibi & Mills, 1972; Marin Grez, Cottone & Carretero, 1972). The literature regarding the possible renal significance of the kallikrein-kinin system is reviewed although fuller discussions concerning the existence of a natriuretic hormone can be found elsewhere (Klahr & Rodriguez, 1975; de Wardener, 1974). It is also considered relevant to review the literature regarding the action of the kallikrein-kinin system on transepithelial movement of electrolytes and water.

The second part of this study is concerned with the contractile action of bradykinin on rat terminal ileum and so a review is made of the action of bradykinin on isolated smooth muscle preparations as well as previous investigations into the contractile action of bradykinin.

The experiments performed demonstrated relationships between bradykinin and both prostaglandins and cyclic AMP. Therefore a brief review of the biochemistry of the prostaglandins and modification of the synthesis of prostaglandins by non steroidal antiinflammatory drugs is included. The possible physiological significance of the prostaglandins is covered fully elsewhere (Andersen & Ramwell, 1974; Horton, 1969, 1972a,b; Ramwell, 1973; Weeks, 1972) and it was considered pertinent to restrict the present work to a review of the actions of prostaglandins on intestinal smooth muscle contractility and to the action of

prostaglandins - cyclic AMP systems on sodium and water movement in the mammalian intestine. In order to adequately cover the latter action of prostaglandins and cyclic AMP a brief review of the mechanisms operating in the movement of water and electrolytes in the mammalian intestine are included although more comprehensive accounts are available (Schultz & Curran, 1968, 1970; Schultz & Frizzell, 1972; Schultz, Frizzell & Nellans, 1974).

Finally it is considered relevant to review the data regarding observed interactions between bradykinin and the prostaglandins.

1. THE KALLIKREIN-KININ SYSTEM

(a) Biochemistry

Abelous & Bardier (1909) and Pribram & Herrnheiser (1920) described a substance in human urine which produced a prolonged hypotension on intravenous injection into the dog and Frey and his colleagues (Frey, 1926; Kraut, Frey & Bauer, 1928) showed that it was a large molecular weight, non-dialyzable, thermolabile substance.

While attempting to find the source of this vasodepressor substance, a similar substance was found in blood (Frey & Kraut, 1928) and pancreas (Kraut, Frey & Werle, 1930) and assuming that the active substance in urine was identical with that in blood and pancreas, it was called kallikrein (Gk; Kallikreas=pancreas). Blood and pancreatic kallikrein was shown to be present in an inactive form (prekallikrein) which could be activated by change in pH and organic solvents (Werle, 1934; 1936a) and by enzymes such as pepsin and trypsin (Kraut, Frey & Werle, 1933; Werle, Forrell & Maier, 1955). Since Frey and his associates considered kallikrein as an activatable circulating hormone with pronounced vascular actions, they also referred to it as 'Kreislaufhormon'.

Werle, Gotze & Keppler (1937) found that a potent smooth muscle contractile substance was released enzymically by all the known kallikreins from an inactive precursor in plasma. This substance was called Substanz Dk to indicate its pharmacological property - darmkontrahierende Substanz - Werle & Hambuechen (1943) drew attention to the parallel between the indirect pharmacological action of renin and kallikrein, although in the former instance the angiotensin which is released is hypertensive whereas Substanz Dk is hypotensive. In 1948 the name of Substanz Dk was changed to Kallidin and its precursor was called kallidinogen (Werle & Berek, 1948).

Rocha e Silva, Beraldo & Rosenfeld (1949) described the release from a substrate in the globulin fraction of plasma by trypsin and certain snake venoms of an active peptide, which they called bradykinin because of the slow contraction of smooth muscle which it produced. It is now clear that

the trypsin-bradykininogen-bradykinin system is closely related to the kallikrein-kallidinogen-kallidin system.

Later the generic term kinin (Schacter & Thain, 1954) led to the use of the term kininogenase for any enzyme (kallikreins, trypsin, snake venoms) that liberates a kinin from an inactive protein substrate called kininogen. The term kallikrein has been retained however for those endogenous kininogenases discovered many years ago (eg kallikrein in urine, pancreas, blood and submaxillary gland). Similarly the names kallidin and bradykinin have been retained for the deca- and nona-peptide kinins respectively (see below) and related kinins, thus far, have been named in terms of either of these substances. The literature relating to the kallikrein-kinin systems has been reviewed by Schacter (1969) and Erdos (1970).

Subsequent investigations have modified the earlier views described above. Kallikreins from different sources differ in their susceptibilities to various protease inhibitors (Webster & Pierce, 1961; 1963; Werle & Maier, 1952) in their electrophoretic mobilities (Moriya, Pierce & Webster, 1963), in their adsorption on diethylaminoethyl (DEAE) cellulose columns (Webster & Pierce, 1961), in their estimated molecular weights (Frey, Kraut & Werle, 1968), in their immunological specificities (Habermann, 1962; Webster, Emmart, Turner, Moriya & Pierce, 1963) and in the relative proportions of their amino acid and carbohydrate contents (Frey, Kraut & Werle, 1968). Thus it would appear that kallikrein from different sources are not identical molecules, as originally assumed, nor is it likely that they are derived from a parent molecule.

Kallikreins are highly specific enzymes and their only natural substrate is kininogen, an $\alpha 2$ globulin of plasma. The kallikreins have little or no non-specific proteolytic activity (Van Arman, 1952), any non-specific proteolytic activity decreases with purification whereas the kinin-releasing activity, pharmacological potency and the rate of hydrolysis all increase (Werle & Trautschold, 1963).

Fritz, Eckert & Werle (1967) found that the molecular weights of kallikreins from pancreas, urine and submaxillary gland were relatively similar, ranging from 33,000 to 36,000 but plasma kallikrein has been estimated to have a molecular weight of 97,000 (Habermann, & Klett, 1966). This enzyme appears to differ significantly from other kallikreins in its non-specific proteolytic activity (Habermann & Klett, 1966) and in its behaviour towards inhibitors (Webster & Pierce, 1961; 1963).

Plasma kininogen have been separated by heating (Werle, Gotze & Keppler, 1937), precipitation with ammonium sulphate (Rocha e Silva, Beraldo & Rosenfeld, 1949), heating and precipitation with $(NH_4)_2SO_4$ (Holdstock, Mathias & Schacter, 1957) and lowering of pH to 2 (Horton, 1959) and from experiments on activation of the kininogenase-kininogen-kinin system of plasma by glass contact (Armstrong, Jepson, Keele & Stewart, 1957) Margolis & Bishop (1963), Eisen (1964) and Vogt (1964) inferred that more than one kininogen exists in plasma. More recently several kininogens have been isolated from human plasma (Pierce, 1968; Pierce & Webster, 1966) and bovine plasma (Habermann, 1963; Habermann, Klett & Rosenbuch, 1963; Kato, Nagasawa & Suzuki, 1967)

Small kininogen fragments containing 13–16 amino acid residues have been prepared which are capable of yielding kinins on incubation with trypsin, kallikrein and other kininogenases (Habermann, 1966; Habermann, Blennenmann & Muller, 1966; Habermann & Helbig, 1967; Hochstrasser & Werle, 1964; 1966; 1967). Habermann and his associates obtained a tetradecapeptide and a hexadecapeptide while Hochstrasser & Werle obtained two tridecapeptides.

Pierce (1968) suggested the involvement of an ester linkage in the attachment of bradykinin to its native kininogen molecule and that kininogenase cleaved this ester bond rather than a peptide bond.

The generic term kinin was first introduced in 1954 by Schacter & Thain after finding several substances in a free form in wasp venom which closely resembled bradykinin and kallidin. Similar compounds were soon seen

in various organs and tissues, occurring in either a free state or released by an enzyme from an inactive precursor in plasma or serum. The figure below shows the degree of structural similarity of the five natural kinins whose structures have been established.

i)	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Bradykinin
ii)	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Kallidin
iii)	Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Met-Kallidin
iv)	Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Wasp kinin
v)	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Tyr	Phyllokinin

There is a common nonapeptide sequence and the kinins differ only in having additional N or C terminal amino acid residues. Kinins i, ii and iii are called plasma kinins and are derived from plasma kininogen by the action of various kininogenases.

The naturally occurring kinins have a number of pharmacological actions in common. All are hypotensive and contract most isolated smooth muscle preparations but relax rat duodenum; they increase capillary permeability, produce pain when applied to a blister base on human skin, and cause in the guinea pig a broncho-constriction which is specifically antagonised by salicylates and related compounds (Schacter, 1969). Kinins produce a characteristic delayed and relatively slow contraction of smooth muscle, compared with histamine and acetylcholine (Rocha e Silva; Beraldo & Rosenfeld, 1949) and in producing an initial inhibition of the rhythmic contractions of the isolated rabbit ileum that precedes the contraction they cause (Holdstock, Mathias & Schacter, 1957; Schacter & Thain, 1954).

There are only quantitative differences between the effects of bradykinin, kallidin and met-kallidin in their potencies in different test preparations. Thus while kallidin is only half as active as bradykinin on the isolated guinea pig ileum, rat uterus and duodenum, it is two to three times more active as a hypotensive agent (Schacter, 1969). In general it appears that trypsin and most snake venoms release bradykinin from plasma

kininogen, and that kallikreins (except plasma kallikrein which releases bradykinin) release kallidin (Habermann, 1963; Kato, Nagasawa & Suzuki, 1967; Webster & Pierce, 1963). This, however, would appear too simplistic an idea of the situation since it is likely that when impure preparations of kininogens and kininogenase are employed to produce kinins serial conversion of split products may be produced by different enzymes and may result in the accumulation of several kinins (Schacter, 1969). A possible scheme has been suggested by Webster & Pierce, (1963):-

Glandular kallikrein + kininogen ⟶ kallidin ⟶ bradykinin

Plasma kallikrein + kininogen ⟶ bradykinin

Trypsin + kininogen ⟶ kallidin ⟶ bradykinin

Snake venom + kininogen ⟶ kallidin

⟶ bradykinin

Werle and his associates demonstrated the presence of a kinin-inactivating enzyme in plasma (Werle, Gotze & Keppler, 1937) and that kinin was destroyed at varying rates by sera of different animals (Werle & Hambuechen, 1943). This enzyme has also been found in lymph (Schacter, 1960). Erdos & Sloane (1962) characterised a carboxypeptidase which cleaves the C-terminal arginine residue of bradykinin and kallidin, naming it carboxypeptidase N or plasma kininase I. Human plasma contains a second plasma kininase which attacks the Pro^{7}—Phe^{8} site (Yang & Erdos, 1967). An aminopeptidase, in human plasma, which converts kallidin to bradykinin has been described by Webster & Pierce (1963), and Erdos, Renfrew, Sloane & Wohler (1963). Chymotrypsin is also an effective kininase, attacking the Phe^{8}—Arg^{9} residue rapidly and the Phe^{5}—Ser^{6} more slowly (Boissonas, St Guttman & Jaquenod, 1960; Elliott, Lewis & Horton, 1960). The kinin-inactivating system is reviewed more thoroughly by Frey, Kraut & Werle, (1968) and Erdos (1966).

(b) Possible Physiological Significance of the Kallikrein-Kinin Systems

The potential amounts of prekallikreins and kininogens in plasma and lymph are so vast and so readily activatable (Armstrong, Jepson, Keele & Stewart, 1957; Schacter, 1956) that is is not surprising suggestions have been made implicating this system in a number of physiological processes. The kallikrein-kinin system has been implicated in blood flow control (Hilton & Lewis, 1955 a,b; 1956; 1958), mediation of the complex vascular changes occurring at birth (Melmon, Cline, Hughes & Nies, 1968) and more recently in the control of sodium and water homeostasis (Adetuyibi & Mills, 1972; Marin Grez & Carretero, 1971; Marin Grez, Cottone & Carretero, 1972) and in the aetiology of hypertension (Croxatto & San Martin, 1970; Marin Grez, Cottone & Carretero, 1972; Margolius, Geller, de Jong, Pisano & Sjoerdsma, 1972).

The evidence regarding the possible involvement of the kallikrein-kinin system in sodium and water homeostasis is reviewed below. Kallikrein-kinin involvement in blood flow control has been reviewed previously (Schacter, 1969, 1970; Hilton, 1970)

(c) Role of Kallikrein-Kinin System in Sodium and Water Homeostasis

(i) Evidence for the involvement of the Kallikrein-Kinin System in the control of Sodium and Water homeostasis

de Wardner, Mills, Clapham & Hayter (1961) suggested the existence of a natriuretic hormone after showing that there must be factors other than glomerular filtration rate (GFR) and aldosterone which control sodium homeostasis. The evidence regarding such a natriuretic hormone has been reviewed recently (de Wardener, 1974; Klahr & Rodriguez, 1975). The substances implicated most strongly as natriuretic hormones are the kallikrein-kinin system (Adetuyibi & Mills, 1972; Marin Grez, Cottone & Carretero, 1972) and prostaglandins (Bulgraff & Meiforth, 1971; Lee, 1974). It is of interest that prostaglandins have been implicated in the renal action of bradykinin (McGiff, Terragno, Malik & Lonigro, 1972; Miller, McGiff &

Nasjletti, 1973; McGiff, Itskovitz & Terragno 1975).

A physiological role for the kallikrein-kinin system in the intrarenal control of sodium and water homeostasis was first proposed by Webster & Gilmore (1964), Barraclough & Mills (1965) and Gill, Melmon, Gillespie & Bartter (1965).

Saline loading has been shown to produce a decrease in rat plasma kininogen (Nasjletti & Azzam 1970), an increase in urinary kallikrein in normal and essential hypertensive man (Adetuyibi & Mills, 1972), rats (Marin Grez & Carretero, 1973) and dogs (de Bono & Mills, 1974; Godon & Damas, 1974; Marin Grez, Cottone & Carretero 1972), and an increase in renal venous kinins (Marin Grez, Cottone & Carretero, 1972) although Margolius and his colleagues (Margolius, Geller, de Jong, Pisano & Sjoerdsma, 1972; Margolius, Horwitz, Celler, Alexander, Gill, Pisano & Keiser, 1974) did not observe these changes in the kallikrein-kinin system after saline loading in the rat or man. Marin Grez, Cottone & Carretero (1972) reported that binephrectomy abolished the saline-induced increases in urinary kallikrein and renal venous kinins suggesting a renal origin for these substances which is of interest since Mills (1970) has argued for a renal origin for the natriuretic hormone.

Marin Grez (1974) reported that rats injected with normal rabbit serum responded to a saline load with a brisk natriuresis and diuresis whereas a significantly smaller increase in urine flow and sodium excretion occurred in rats treated with bradykinin antibodies, which had previously been shown to selectively inhibit the oxytocic and hypotensive actions of bradykinin (Marin Grez, Marin Grez & Peters, 1974), suggesting that the kallikrein-kinin system is necessary for the usual natriuretic response to saline loading. It was suggested that the lower fractional excretion of sodium seen in antibody-treated animals was due to greater tubular reabsorption rather than decreased GFR.

Simultaneous increases in kallikrein excretion and natriuresis in rat and dog have been observed after increased renal artery pressure (Bevan,

MacFarlane & Mills, 1974), intrarenal substance P (Macfarlane, Mills & Ward, 1974), intrarenal angiotensin II (Macfarlane, Adetuyibi & Mills, 1974) and furosemide (Croxatto, Roblero, Garcia, Corthorn & San Martin, 1973). Nasjletti, Colina-Chourio & McGiff (1974; 1975) showed in the dog that the peptide SQ 20881, which inhibits kininase enzyme (Greene, Camargo, Krieger, Stewart & Ferreira, 1972), augmented urine flow, sodium excretion and concentration and excretion of urinary kinins. The excretion of kinins was positively correlated with sodium excretion and urine flow. It is also of interest that very high kallikrein excretion has been observed in patients with chronic renal failure in which there is a tendency to enter a sodium-losing state (Adetuyibi & Mills, 1972) and who have been likened to normal patients excreting a large saline load (Bricker, Saulo, Purkerson, Schultze, Avioli & Birge, 1968).

Adetuyibi & Mills (1972) suggested that there might be two mechanisms related to kallikrein excretion, one concerned with sodium excretion and the other with water excretion, after they noted a significant increase in kallikrein excretion and water excretion, without any change in sodium excretion, in man following an oral water load. However, this observation was not confirmed in dogs (Marin Grez, Cottone & Carretero, 1972) or man (Margolius, Horwitz, Pisano & Keiser, 1974a).

Mills & Ward (1975) confirmed in rabbits on a free salt and water diet that there were significant correlations between urinary kallikrein and sodium excretion and between urinary kallikrein and urine volume. However, in rabbits on either a high or low sodium diet there was no significant correlation between sodium excretion and urine volume or between sodium excretion and urinary kallikrein, while the correlation between urinary kallikrein and urine volume remained significant. In other experiments rabbits were given 2% saline for 5 days, followed by 1% saline for 6 days and then tap water for 6 days with fluid intake held constant at 100ml per day throughout. Urinary kallikrein was not correlated with sodium excretion either within or between the three stages. Nevertheless kallikrein

excretion showed a positive correlation with urine volume. It was concluded that in animals on a free salt and water intake the positive correlation between kallikrein excretion and sodium excretion is secondary to the positive correlation of urinary kallikrein with urine volume and that under these conditions kallikrein might not be physiologically involved in the control of sodium excretion. It was suggested alternatively that kallikrein may be involved in the control of sodium excretion only when sufficient fluid intake is available to allow for expansion of some critical fluid volume, an idea supported by Marin Grez and his colleagues (Marin Grez, Cottone & Carretero 1972; Marin Grez & Carretero 1973).

In contrast to the results of Marin Grez & Carretero (1973) and Nasjletti & Azzam (1969) it has been reported that low sodium diet in rats, which is known to increase aldosterone activity (Cade & Parenich, 1965; Marieb & Mulrow, 1965) caused an increase in kallikrein excretion which was unrelated to urine volume, sodium excretion and blood pressure (Margolius, Geller, de Jong, Pisano & Sjoerdsma, 1972; Margolius, Horwitz, Geller, Alexander, Gill, Pisano & Keiser, 1974; Margolius, Horwitz; Pisano & Keiser, 1974 a,b) and that the increased kallikrein excretion appeared to be linked to aldosterone concentration rather than renin activity (Wong, Talamo, Williams & Colman, 1975). Carretero, Oza, Scigli & Schork (1974) however were unable to demonstrate any correlation between either plasma renin or aldosterone and renal kallikrein in the rat.

It has been reported that significantly less kallikrein is excreted by adrenalectomised rats (Geller, Margolius, Pisano & Keiser, 1972) and spironolactone-treated normal patients (Margolius, Horwitz, Geller, Alexander, Gill, Pisano & Keiser, 1974) than their respective controls, whereas patients with primary aldosteronism excreted large amounts of kallikrein which fell on tumour removal or spironolactone treatment (Margolius, Geller, Pisano, & Sjoerdsma, 1971; Margolius, Geller, de Jong, Pisano & Sjoerdsma, 1972; Margolius, Horwitz, Pisano & Keiser, 1974b; Miyashita, 1971). The

mineralocorticoids deoxycorticosterone acetate (DOCA) and fludrocortisone have been shown to increase kallikrein excretion during the 'escape' phase from their sodium-retaining effects in rats, dogs and humans (Marin Grez & Carretero, 1973; Marin Grez, Oza & Carretero, 1973; Adetuyibi & Mills, 1972; Edwards, Adetuyibi & Mills, 1973) and Marin Grez, Oza & Carretero (1973) suggested that the gradual increase in kallikrein excretion could be a reflection of the gradual expansion of extracellular fluid (ECF) volume. However, it has been reported that DOCA and fludrocortisone increase kallikrein excretion in rats and man without any changes in urine volume, body weight or sodium excretion (Geller, Margolius, Pisano & Keiser, 1972: Margolius, Horwitz, Geller, Alexander, Gill, Pisano & Keiser, 1974; Margolius, Horwitz, Pisano & Keiser, 1974b).

In summary, it is difficult to reconcile all the experimental data regarding the possible involvement of the kallikrein-kinin system in the control of sodium and water homeostasis. It is the belief of Margolius and his colleagues that kallikrein excretion is dependent on the circulating levels of mineralocorticoids while Marin Grez and his associates consider that the activation of the kallikrein-kinin system depends on the ECF volume. More recently Mills & Ward (1975) have proposed that kallikrein is primarily concerned with water homeostasis and that any observed correlations between kallikrein excretion and sodium excretion are secondary to changes in urine volume.

Finally, it is interesting that Marin Grez, Cottone & Carretero (1972) commented that the chemical characteristics of the natriuretic substances found by other investigators were quite similar to components of the kallikrein-kinin system. The urinary diuretic factor of Little, Angell & Brooks (1962) is similar to kallikrein and the natriuretic factor of Cort, Pliska & Dousa (1968) and Sedlakova, Lichardus & Cort (1969) is similar to kinins. The substance characterised by Scaley, Kirshman & Laragh (1969) although having a molecular weight similar to that of kallikrein is probably different since it resists boiling at pH 5.5 whereas kallikrein does not.

Although it would appear that the kallikrein-kinin system has a part to play in the control of sodium and water excretion additional experimental evidence to clarify its actual role is necessary.

(ii) Occurrence in Kidney and Urine

Large amounts of Kallikrein (Kraut, Frey & Werle, 1930) and kinins (Frey & Kraut, 1928; Gomes 1955; Miura, Erdos & Seki, 1968) have been observed in urine. It was initially thought that urinary kallikrein originated in the pancreas (Frey, 1929) but Beraldo, Feldberg & Hilton (1956) showed that pancreatectomy did not reduce urinary kallikrein and suggested a renal or plasma origin for the enzyme. Most urinary enzymes are thought to be of renal origin although several originate from the plasma (Rabb, 1968). Plasma kallikrein is different from urinary kallikrein in molecular dimension, electrophoretic mobility, inhibition pattern (Frey, Kraut, Werle, Vogel, Zickgraf-Rudel & Trautschold (1968) and urinary kallikrein produces kallidin while plasma kallikrein produces bradykinin (Webster & Pierce, 1963). Renal extracts were shown to contain prekallikrein (Schacter, 1956; Carvalho & Diniz, 1966), kallikrein (Kraut, Frey & Werle, 1930; Werle & Vogel, 1960), kinin (Abe, 1965; Werle, Leysath & Schmal, 1968) and kininase (Nustad, 1970a,b; Nasjletti, Colina-Chourio & McGiff, 1974, 1975) activity. Renal and urinary kallikrein were shown to be similar with respect to pH optimum, effect of inhibitors and ability to form kinins from dog and rat plasma kininogen (Carvalho & Diniz, 1966; Nustad, 1970a), suggesting that urinary kallikrein originates in the kidney. Nustad (1970a) suggested that the kidney might actively secrete kallikrein into the urine after observing the high urine:kidney ratio of kallikrein, an analogous situation to the pancreas and salivary gland. The majority of renal kallikrein activity (80-90%) was shown to be present in the cortex while kininogenase activity was equally distributed between renal cortex and medulla (Nustad, 1970b). Werle & Vogel (1960) showed that drugs which destroy proximal tubular cells reduced excretion of urinary kallikrein and Frey, Kraut, Werle, Vogel, Zickgraf-Rudel & Trautschold (1968)

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Large amounts of Kallikrein (Kraut, Frey & Werle, 1930) and kinins (Frey & Kraut, 1928; Gomes 1955; Miura, Erdos & Seki, 1968) have been observed in urine. It was initially thought that urinary kallikrein originated in the pancreas (Frey, 1929) but Beraldo, Feldberg & Hilton (1956) showed that pancreatectomy did not reduce urinary kallikrein and suggested a renal or plasma origin for the enzyme. Most urinary enzymes are thought to be of renal origin although several originate from the plasma (Rabb, 1968). Plasma kallikrein is different from urinary kallikrein in molecular dimension, electrophoretic mobility, inhibition pattern (Frey, Kraut, Werle, Vogel, Zickgraf-Rudel & Trautschold (1968) and urinary kallikrein produces kallidin while plasma kallikrein produces bradykinin (Webster & Pierce, 1963). Renal extracts were shown to contain prekallikrein (Schacter, 1956; Carvalho & Diniz, 1966), kallikrein (Kraut, Frey & Werle, 1930; Werle & Vogel, 1960), kinin (Abe, 1965; Werle, Leysath & Schmal, 1968) and kininase (Nustad, 1970a,b; Nasjletti, Colina-Chourio & McGiff, 1974, 1975) activity. Renal and urinary kallikrein were shown to be similar with respect to pH optimum, effect of inhibitors and ability to form kinins from dog and rat plasma kininogen (Carvalho & Diniz, 1966; Nustad, 1970a), suggesting that urinary kallikrein originates in the kidney. Nustad (1970a) suggested that the kidney might actively secrete kallikrein into the urine after observing the high urine:kidney ratio of kallikrein, an analogous situation to the pancreas and salivary gland. The majority of renal kallikrein activity (80-90%) was shown to be present in the cortex while kininogenase activity was equally distributed between renal cortex and medulla (Nustad, 1970b). Werle & Vogel (1960) showed that drugs which destroy proximal tubular cells reduced excretion of urinary kallikrein and Frey, Kraut, Werle, Vogel, Zickgraf-Rudel & Trautschold (1968) suggested

that renal kallikrein might reside in these cells. Nustad & Rubin (1970) did not observe any correlation between the subcellular localisations of kallikrein and renin, which has been observed in renal mitochondrial (Dengler & Reichel, 1960) and lysosomal (Robertson, Smeby, Bumpus and Page 1966) fractions. Renal kallikrein has been reported to occur in the lysosomal fraction (Carvalho & Diniz, 1964; 1966) but Nustad (1970b) found the greatest activity in endoplasmic reticulum membrane and plasma membrane. Nustad, Pierce & Vaage (1975) showed that kallikrein could be synthesised from radioactive amino acids by rat kidney slices and that this was indistinguishable from kallikrein isolated from rat kidney and urine.

Nasjletti, Colina-Chourio & McGiff (1974; 1975) showed that the average survival of bradykinin on intrarenal infusion was about 13% and this was increased by pretreatment with SQ 20881 which inhibits kininase activity (Greene, Camargo, Krieger, Stewart & Ferreira, 1972). It was also shown that SQ 20881 caused an increase in kinins in renal venous blood and urine, thus supporting the idea that kinins originate intrarenally.

(iii) Effect of the Kallikrein-Kinin System on Kidney Function

Bradykinin increases urine flow and sodium excretion when infused into the kidney of dogs (Barraclough & Mills, 1965; Kover, Szocs & Temler, 1968; Heidenreich, Keller & Kook, 1964; Webster & Gilmore, 1964), rats (Heller & Novakova, 1969) and humans (Gill, Melmon, Gillespie & Bartter, 1965). Barer (1963) showed that bradykinin increases renal blood flow (RBF) of cats without affecting systemic blood pressure or the function of the contralateral kidney and subsequent studies have confirmed these observations in dogs (Heidenreich, Keller & Kook, 1964; Kover, Szocs & Temler, 1968; Webster & Gilmore, 1964) and man (Goldberg, Dollery & Pentecost, 1965), although there are contrary reports (Barraclough & Mills, 1965; Kontos, Magee, Shapiro & Patterson, 1964; Gill, Melmon, Gillespie & Bartter, 1965). The effect of bradykinin on glomerular filtration rate (GFR) is confused with workers observing increases (Furuyama, Suzuki, Saito,

Onozawa, Shioji, Rikimaru, Abe & Yoshinaga, 1966), decreases (Gill, Melmon, Gillespie & Bartter, 1965), no effect (Dirks & Seely, 1967; Heller & Novakova, 1969) or variable effects (Barraclough & Mills, 1965).

It is possible that the diuretic and natriuretic actions of bradykinin could be due to renal 'physical' factors, direct inhibition of tubular function or a combination of both these factors.

Heidenreich, Keller & Kook, (1964) concluded that bradykinin-induced natriuresis and diuresis were due to a direct action on tubular cell reabsorption. Several workers have attempted to measure the effect of bradykinin on sodium and water reabsorption in the proximal tubules of superficial nephrons using the micropuncture method. Heller & Novakova (1969) took the view that bradykinin does not act chiefly by a 'physical' mechanism since it produced the same degree of natriuresis and diuresis as acetylcholine but had less effect on the clearance of PAH (C_{PAH}, a measure of RBF) and no effect on the clearance of inulin (C_{IN}, a measure of GFR) Alzamora & Capelo (1973) observed decreased proximal tubular reabsorption of sodium and water by bradykinin while Stein, Congbalay, Karsh, Osgood & Ferris(1972) did not.

Furuyama, Suzuki, Saito, Onozawa, Shioji, Rikimaru, Abe & Yoshinaga (1966) expalined the renal actions of bradykinin solely in terms of increased GFR and medullary blood flow. Kover, Szocs & Temler (1968) and Mertz (1963) both ooncluded that increased medullary blood flow was involved in this action and supporting evidence has been presented by Willis, Ludens, Hook & Williamson (1969) and Stein, Congbalay, Karsh, Osgood & Ferris (1972). Willis, Ludens, Hook & Williamson (1969) showed that in animals undergoing water diuresis bradykinin increased free water clearance suggesting reduction of proximal tubular reabsorption resulting in an increased delivery of sodium and water to distal diluting segments where, during water diuresis, most of the additional water is excreted but much of the sodium is reabsorbed. The same authors, using the autoperfused dog kidney,

showed that when blood flow was held constant bradykinin prodcued a fall in perfusion pressure, urine flow and sodium excretion which was thought to be due to decreased GFR. However, when perfusion pressure was held constant bradykinin did not alter C_{PAH} but urine flow and sodium excretion increased which was interpreted to suggest that increased blood flow due to bradykinin could be to those areas which do not extract PAH. When the RBF to normal dog kidneys was held constant bradykinin did not cause natriuresis and it was concluded that the renal actions of bradykinin could not occur without an increase in RBF. More recently Stein, Congbalay, Karsh, Osgood & Ferris (1972) offered evidence that bradykinin redistributed blood flow to inner cortical nephrons.

McGiff and his colleagues (McGiff, Terragno, Malik & Lonigro, 1972; Miller, McGiff & Nasjletti, 1973; McGiff, Itskovitz & Terragno, 1975) have reported that the renal effects of bradykinin are due to the involvement of a prostaglandin E-like substance.

It can be concluded that there is confusion concerning the nature of bradykinin action on the kidney and this led to the present investigation, the object of which was to study the effects of bradykinin on an isolated transepithelial transporting system when haemodynamic considerations were not involved. Therefore in the next section the effects of bradykinin on membrane transport are reviewed.

(iv) Effect of the Kallikrein-Kinin System on Transfer Across Membranes

Rasmussen, Schwartz, Young & Marc-Aurele (1963) showed that bradykinin did not alter the basal water transfer across the toad urinary bladder. This observation was confirmed in the toad bladder (Furtado & Machado, 1966; Furtado, 1967a) and in the toad skin with respect to sodium (Furtado & Machado, 1966).

Vasopressin stimulates water flow in the toad bladder (Bentley, 1958) and sodium movement in the toad skin (Ussing & Zerahn, 1951) and it was shown that bradykinin will prevent these stimulations of water and sodium

movements (Furtado & Machado, 1966; Furtado, 1967a). Furtado (1967a) also showed that in toad bladder oxytocin-induced water transfer (Heller, 1963) is also inhibited by bradykinin, and that the inhibition could be observed when bradykinin was incubated in either mucosal or serosal solution. The dose-response curves for vasopressin and oxytocin undergo a parallel shift to the right in the presence of bradykinin without suppression of the maximum effect (Furtado, 1967a) which indicates an altered affinity of the hormones for the receptor sites (Ariens & de Groot, 1954; van Rossum, 1963). The inhibition of stimulated water transfer is reversed on removal of bradykinin.

Theophylline, which inhibits cyclic AMP phosphodiesterase (Butcher & Sutherland, 1962) and induces water movement across the toad bladder (Orloff & Handler, 1962), was not affected by bradykinin (Furtado, 1967a; 1971) and this would appear to suggest that bradykinin inhibited the interaction of the hormones with their receptor sites rather than the cyclic AMP which is formed from such an interaction (Orloff & Handler, 1962).

Since both bradykinin (Furtado & Machado, 1966) and thiols (Bentley, 1964; Handler & Orloff, 1964) have been shown to inhibit vasopressin-induced water movement in toad bladder Furtado (1967b) looked for an interaction between cysteine and bradykinin. There was neither summation nor potentiation of the bradykinin inhibition of vasopressin, on the contrary, at doses up to 4mM cysteine inhibited the effects of bradykinin. It appeared to compete for a common binding site with bradykinin and so blocked the bradykinin effect without itself having much effect. At higher concentrations of cysteine it was possible to exceed even the 50% degree of inhibition seen with bradykinin alone, although this inhibition only reflected the effect seen with cysteine alone at this dose.

Furtado (1971) showed that kallidin, eledoisin and physalaemin will also, to varying degrees, inhibit vasopressin-and oxytocin-induced water transfer in toad bladder but angiotensin II was found to be ineffective.

Bradykinin has been shown to inhibit gastric acid secretion in dogs with a Pavlov pouch (Groza, Corneanu, Buzoianu, Ionescu & Busneag, 1966)

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being dose-dependent (Groza, Buzoianu & Ionescu, 1967; Groza, Corneanu, Ionescu, Rusovici & Ionescu, 1968). The gastro-inhibitory effect of bradykinin disappears after adrenalectomy and is replaced by an augmentation of histamine-induced secretion (Groza, Buzoianu, Ionescu & Rosovici, 1967). Hydrocortisone treatment restored the gastroinhibitory effect of bradykinin (Groza, Buzoianu & Ionescu, 1967) and it was proposed that bradykinin liberates an intermediary, under the influence of the adrenocortical hormones, which was considered to have a direct action on the oxyntic cells. More recently Groza, Corneanu-Dina & Ionescu (1972) showed that insulin-induced gastric secretion (Simici, Popescu & Diculescu, 1927) is inhibited in a dose-related manner by bradykinin.

Dennhardt & Haberich (1973) showed that kallikrein could affect the transfer of salt, water and hexoses across rat jejunum and colon in vivo. In normal rats kallikrein was shown to inhibit net transport of sodium and water in the colon irrespective of dose but in rats with ligated pancreatic ducts kallikrein produced a stimulation of sodium, chloride and water transport. A dose-related response was observed with doses of 5×10^{-4} mg/ml to 10^{-2} mg/ml. It was found that there was a marked increase in lumen to blood flux while blood to lumen flux tended to fall. Similar results were reported for the jejunum but the concentrations of kallikrein found to be effective were about one tenth of those used in the colon. It was noticeable that in this report the absorption rate of electrolytes and water in normal animals was markedly higher than in those with pancreatic ligations. Thus the effect seen with kallikrein is dependent in some way on the basal level of transfer of water and electrolytes. Dennhardt & Haberich considered that kallikrein was unable to stimulate further sodium transport in normal animals but since it is responsible for a change in the permeability properties of the mucosal membrane a net reduction in absorption would occur.

Kallikrein has been shown to stimulate glucose transport in intestinal preparations (Dennhardt & Haberich, 1973; Meng & Haberland, 1973) but contradictory results have been found with 3-OMe-glucose since Dennhardt

& Haberich (1973) observed no effect while Meng & Haberland (1973) reported stimulation. However Caspary & Creutzfeldt (1973) reported that kallikrein did not affect hexose transport, neither did it affect amino acid transport in rat small intestine.

Kallikrein has been reported to increase the intestinal lymph flow when infused into the duodenum of rats (Rohen, Moschler, Goebel & Dennhardt, 1973). It was also shown that the amount of sodium and potassium in the lymph increased after kallikrein treatment. Electron micrographs revealed a tubulo-vesicular system in the intestinal epithelium which enlarged after kallikrein treatment. These tubules open into the intercellular spaces and from there the fluid enters the stromal layers of the intestinal mucosa and the lumen of the lymph capillaries.

(d) Action of the Kallikrein-Kinin System on Smooth Muscle Contraction

(i) Response of Isolated Smooth Muscle

Kallikrein has been reported to contract dog, rat and pig intestine (Werle, 1936b), human appendix and cat intestine (Werle, Gotze & Keppler, 1937) but not rabbit intestine (Laborit, Letterier, Massart & Baron, 1964) The contractile action of kallikrein which took place after a delay and reached a maximum after 7 minutes (Werle, Gotze & Keppler, 1937) was abolished by boiling the enzyme (Werle, 1936b) suggesting that kinin activation was necessary.

Bradykinin and kallidin contract smooth muscle of rat uterus and guinea pig ileum (Konzett & Sturmer, 1960; Rocha e Silva, Beraldo & Rosenfeld, 1949; Werle, 1955) human myometrium and cat uterus (Berde & Saameli, 1961), longitudinal human ileum (Fishlock, 1966), hen rectal caecum (Bisset & Lewis, 1962), rat stomach strip (Vane, 1964), cat terminal ileum (Ferreira, Ng & Vane, 1973) and cat jejunum (Erspamer & Erspamer, 1962). Ferreira & Vane (1967a) concluded that cat jejunum was the most sensitive tissue to contract in response to bradykinin although others have found that rat uterus is sufficiently sensitive to bradykinin to be used as an assay

organ (Elliott, Horton & Lewis, 1960)

Bradykinin has been reported to relax smooth muscle of rat duodenum and colon (Horton, 1959a; Gaddum & Horton, 1959), circular muscle of human ileum (Fishlock, 1966), rabbit uterus (Erspamer & Erspamer, 1962), rabbit intestine (Rocha e Silva, 1962) and either contraction (Erspamer & Erspamer, 1962) or relaxation followed by contraction of rabbit intestine (Elliott, Horton & Lewis, 1960; Konzett & Sturmer, 1960; Walaszek & Huggins, 1959). Ferreira & Vane (1967b) reported that rat duodenum was the most sensitive of the tissues which relax in response to bradykinin.

(ii) Mode of Action of Kinins on Isolated Smooth Muscle

The mode of action of bradykinin on smooth muscle has not been established conclusively. It is known that bradykinin-induced contractions of guinea pig ileum, rat uterus, rat stomach strip, cat jejunum and cat terminal ileum are not affected by muscarinic receptor blockers, anti-cholinesterases, antihistamines, BOL 148, ganglion blockers, anoxia, cooling, tetrodotoxin, denervation or phenoxybenzamine (Ambache & Rocha e Silva, 1951; Day & Vane, 1963; Gershon, 1967; Khairallah & Page, 1961; 1963; Ohashi, Nonamura & Ohga 1967; Walaszek, Huggins & Smith, 1963; Werle, 1955) although it has been reported that bradykinin was potentiated by TEA and eserine on guinea pig ileum (Khairallah & Page, 1961; Wiegershausen, Stopp & Eichstadt, 1964) andrat uterus (Capek & Knesslova, 1959) and inhibited by atropine and morphine on guinea pig ileum (Wiegershausen, Stopp & Eichstadt, 1964). Potter & Walaszek (1972) showed that the potentiation of bradykinin by cysteine on guinea pig ileum (Picarelli, Henriques & Oliviera, 1962) was inhibited by atropine, morphine and tetrodotoxin but not by chlorpheniramine, mecamylamine, hexamethonium, BOL 148 or phentolamine and so postulated a cholinergic mechanism in this potentiation.

Thus it would appear that the concensus of opinion is that the contractile effect of bradykinin is not nervously mediated and does not involve the release of acetylcholine, histamine or 5-hydroxytryptamine.

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Thus it would appear that the concensus of opinion is that the contractile effect of bradykinin is not nervously mediated and does not involve the release of acetylcholine, histamine or 5-hydroxytryptamine.

Malone & Trottier (1973), using a series of prostaglandin synthetase inhibitors, reported that bradykinin-induced contractions of rat uterus were

not affected by calcium carbaspirin or phenylbutazone but were inhibited both competitively and non competitively by flufenamic acid and non competitively by indomethacin, a finding supported by Barabe, Park & Regoli (1975) for indomethacin but not by Collier & Shorley (1960) for aspirin and phenylbutazone on guinea pig ileum.

The prostaglandin receptor antagonists PPP, SC 19220 and 7 oxa-13-prostynoic acid have not been reported generally to affect bradykinin-induced contractions of various smooth muscle preparations (Sanner, 1974; Bennett, 1974) although Barabe, Park & Regoli (1975) did observe depression of the contractile action of bradykinin on rat uterus with PPP. However the action of these prostaglandin receptor antagonists will be considered more fully later.

It has been shown that the relaxatory action of bradykinin on rabbit ileum and rat duodenum was not affected by reserpine, (Turker, Kiran & Kaymakcalan, 1964; Ufkes & van der Meer, 1975), phentolamine or propralolol (Antonio, 1968; Hall & Bonta, 1973), guanethidine (Bauer, Ziegler & Konzett, 1966) or by tyramine-induced tachyphylaxis (Ufkes & van der Meer, 1975) although blockade of bradykinin-induced relaxations of rat duodenum and circular muscle of human ileum was seen with α and β receptor blockers (Fishlock, 1966; Montgomery, 1968; Turker, Kiran & Kaymakcalan, 1964).

Hall and Bonta (1972, 1973) showed that bradykinin could relax longitudinal muscle of guinea pig ileum which had been contracted with acetylcholine and that this was not affected by reserpinisation, phentolamine or propranolol.

In summary it would appear that most workers have concluded that the contractile and relaxatory actions of bradykinin are not mediated via the parasympathetic and sympathetic nerves respectively. However, the mechanism of its contractile and relaxatory actions have not been very closely established, although limited investigations have been made into their ion dependency (Aarsen & van Caspel de Bruyn, 1970; Antonio, 1968; Huidobro, 1963; Ohashi, Nonamura & Ohga, 1967) and into their susceptibility to the

effect of various drugs (Rocha e Silva & Garcia Leme, 1963, 1964; Garcia Leme & Rocha e Silva, 1965; Greene, Camargo, Krieger, Stewart & Ferreira, 1972; Stewart, 1968; Walaszek, 1970).

2. PROSTAGLANDINS

(a) Biochemistry

Semen and prostatic extracts were known to contain smooth muscle contracting ability (Battez & Boulet, 1913; Goldblatt, 1933; 1935) which was shown to be a lipid-soluble acid (von Euler, 1935; 1937). The active constituent which was called prostaglandin by von Euler (1934;1935;1937) lowered rabbit blood pressure (von Euler, 1935), had no action on the rabbit heart but increased blood flow through the perfused hind limb and kidney and decreased it through the pulmonary vascular bed (von Euler, 1939).

von Euler & Hammarstrom (1937) although finding prostaglandin activity in other tissues were unable to find concentrations comparable to those found in seminal plasma. More recent studies showed that prostaglandins are found in a large variety of tissues (Horton, 1972a,b).

The prostaglandins have been the subject of a considerable amount of research and there are many reviews in the literature (Bergstrom, 1966; Horton, 1968,1969,1972a,b; Karim, 1972; Ramwell,1973).

Bergstrom & Sjovall (1960a,b) isolated two pharmacologically active lipid-soluble acids from sheep prostate glands, one of which was more soluble in ether and lowered rabbit blood pressure(called prostaglandin E) and one which was more soluble in phosphate buffer (spelt with an "F" in Swedish) and did not affect rabbit blood pressure (called prostaglandin F). The prostaglandins were shown to be a group of oxygenated C_{20} fatty acids and subsequently the chemical identification of several naturally occurring prostaglandins was reported (Bergstrom, Krabisch, Samuelsson & Sjovall,1962; Bergstrom, Krabisch & Sjovall, 1960; Bergstrom, Ryhage, Samuelsson & Sjovall, 1963; Bergstrom & Samuelsson, 1962).

The enzymic formation of prostaglandins from certain fatty acids was reported in 1964 (van Dorp, Beerthuis, Nugteren & Vonkeman, 1964; Bergstrom, Danielsson & Samuelsson, 1964) and several reviews are available on this topic (Samuelsson, 1970,1972; Sih & Takeguchi, 1973; van Dorp, 1971). The enzyme responsible for this process is prostaglandin synthetase which is

thought to be a multienzyme complex associated with the endoplasmic reticulum (Sih & Takeguchi, 1973). Prostaglandin synthetase is widely distributed in animal tissues (Christ & van Dorp, 1972, 1973) and is thought to vary somewhat in its biochemical and pharmacological properties from tissue to tissue (Flower & Vane, 1974).

Only non-esterified fatty acids are substrates for the enzyme (Lands & Samuelsson, 1968) and prostaglandins formed from dihomo-δ-linolenic acid are denoted by the suffix '1', whereas from arachidonic acid the suffix '2' and from 5,8,11,14,17 - eicosapentaenoic acid the suffix is '3'.

In the formation of PGE_2 from arachidonic acid the first step involves the production of a cyclic endoperoxide which has been called PGG_2 (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974; Nugteren & Hazelhof, 1973). This is then followed by reduction of PGG_2 to PGH_2, another cyclic endoperoxide which was isolated by Hamberg & Samuelsson, (1973), followed by isomerisation into PGE_2 or by isomerisation of PGG_2 to 15-hydroperoxy PGE_2 followed by reduction to PGE_2 (Samuelsson & Hamberg, 1974). Formation of $PGF_{2\alpha}$ occurs from the same pool of endoperoxide as PGE_2 (Wlodawer & Samuelsson, 1973).

Dehydration of PGE produces PGA although the existence of a PGE dehydratase enzyme has not been unequivocally demonstrated despite interesting preliminary reports (Cammock, 1973; Russell, Alam & Clary, 1973) and it is possible that much of the PGA detected in tissues could have been formed non-enzymically during extraction and isolation procedures (Flower, 1974). However interconversion of PGA, PGB and PGC by a prostaglandin isomerase has been reported (Jones, 1970; Jones, Cammock & Horton, 1972).

The catabolism of prostaglandins (reviewed by Andersen & Ramwell, 1974; Piper, 1972; Samuelsson, 1970) occurs by four enzymic processes including formation of the 15 keto derivative (PG dehydrogenase), saturation of the 13-14 double bond (PG reductase), and oxidation of the hydrocarbon side chains at either the β or ω terminal. Prostaglandin dehydrogenase (PGOH) is the most important of these enzymes and is widely distributed in animal tissues (Anggard, Larsson & Samuelsson, 1971).

(b) Drugs which affect Prostaglandin Synthesis

Drugs capable of inhibiting prostaglandin synthetase have proved extremely useful and have been the subject of a considerable amount of investigation (see reviews by Flower, 1974; Robinson & Vane, 1974).

Aspirin has been widely used as an analgesic, antipyretic and antiinflammatory agent since the demonstration of the curative properties of an extract of willow bark, which contains salicylic acid, (Stone, 1754), although its mode of action was unclear until recently. Aspirin was said to work by uncoupling oxidative phosphorylation (Whitehouse, 1962, 1964, 1965) but more recently it was shown to be a potent inhibitor of prostaglandin synthetase (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Vane 1971). The evidence that this is the principal action of the aspirin-like drugs is now very strong (see reviews by Flower, 1974, and Vane 1971, 1972a, b, 1973a, b, 1974) and it is widely accepted that the actions of these substances reflect a decrease in PG synthesis although it has been argued that this is only a secondary effect of the aspirin-like drugs (Famaey & Whitehouse, 1973).

Many non steroidal antiinflammatory agents have been shown to be potent inhibitors of PG synthetase in tissue homogenates, isolated organs and whole animals although in the latter two situations the observations are complicated by the presence of PG metabolising enzymes, ability of drugs to reach the synthetase enzyme and the fact that one has to rely on PG release as an indirect measure of cellular synthesis. Since cells do not store PGs and release is in most cases equivalent to de novo synthesis (Piper & Vane, 1971) this latter assumption is usually justifiable. Most of the commonly used aspirin-like drugs are more potent than aspirin itself but there are variations in the activity of some of these drugs on enzymes from different tissues, supporting the idea that PG synthetase varies somewhat from tissue to tissue (Flower & Vane, 1974).

The mechanism of enzyme inhibition by the aspirin-like drugs has not been clearly established and their relatively wide chemical diversity

might suggest more than one mode of action (Flower, 1974). It has been suggested that these drugs inhibit the initial attack of the enzyme on the substrate (Downing, 1972) since there is an absence of intermediates in inhibited preparations of PG synthetase (Gryglewski & Vane, 1972; Piper & Vane, 1969a; Tomlinson, Ringold, Qureshi & Forchielli, 1972). The findings of Ku & Wasvary (1973), Lands, Letellier, Rome & Vanderhoek (1973) and Smith & Lands (1971) suggested that rather than a simple competition between drug and substrate for the catalytic site of the enzyme the aspirin-like drugs were 'competitive irreversible' inhibitors. It would appear that there are two possible kinetic situations. In the first case the inhibitor combines in an irreversible time-dependent fashion with the catalytic enzyme site and presence of substrate at this site reduces the velocity of the combination, but provided that the inhibitor is in excess of the enzyme it cannot prevent ultimate inhibition of the enzyme. Since the initial degree of inhibition depends on the substrate combination, a competitive effect obtains (Flower, 1974). In the second case the inhibitor does not bind to the catalytic site but to another site which is sufficiently close to the catalytic site to reduce its affinity for the substrate, perhaps by some allosteric effect. The latter possibility is favoured by Lands, Letellier, Rome & Vanderhoek (1973). Phenylbutazone is different from the other aspirin-like drugs in that it is thought to interfere with the breakdown of the endoperoxide (Flower, 1974). The action of most of the aspirin-like drugs is irreversible or only slowly reversible (Ku & Wasvary, 1973; Smith & Lands, 1971).

Aspirin-like drugs can affect other enzymes and cellular systems (see Flower 1974) but the concentration of drugs required to inhibit PG synthetase are usually much lower than those which inhibit other enzymes, although indomethacin will inhibit phosphodiesterase at concentrations close to those which inhibit PG synthetase (Flores & Sharp, 1972).

(c) Physiological and Pharmacological Actions of Prostaglandins

The apparently ubiquitous nature of the prostaglandins has led to innumerable suggestions about their physiological significance, which are adequately covered elsewhere (Andersen & Ramwell, 1974; Horton, 1969, 1972a,b; Karim, 1972; Ramwell, 1973; Weeks, 1972). It is considered pertinent to restrict the present work to a review of the actions of prostaglandins on intestinal smooth muscle contractility and to the action of prostaglandins on sodium and water movement in the mammalian intestine.

(d) Actions of Prostaglandins on Contractility of the Gastrointestinal Tract

Mechanical, chemical and electrical stimulation has been shown to release prostaglandins of the E and F type from the gastrointestinal tracts of rat (Bennett, Friedmann & Vane, 1967; Coceani, Pace-Asciak, Volta & Wolfe, 1967; Collier, 1974; Pace-Asciak, Morawska, Coceani & Wolfe, 1968; Ramwell & Shaw, 1968), man (Bennett, Murray & Wyllie, 1968), frog (Vogt, Suzuki & Babilli, 1966), pig (Miyazaki, 1968) and both guinea pig and rabbit (Ambache, Brummer, Rose & Whiting, 1966). Rat stomach and frog intestine have also been shown to be capable of synthesising PGE and PGF from arachidonic acid (Kunze, 1970; Pace-Asciak, 1973; Pace-Asciak & Wolfe, 1971). Most of the prostaglandins found in human and rat stomach are thought to occur in the mucosa rather than the muscle (Bennett, Friedman & Vane, 1967; Bennett, Murray & Wyllie, 1968) with the greatest amounts near to the inner surface in human gastric mucosa (Bennett, 1972). The gut is also capable of inactivating prostaglandins in vitro and in vivo (Kunze, 1970; Pace-Asciak, Morawska & Wolfe, 1970; Parkinson & Schneider, 1969)

Of the primary prostaglandins only types E and F affect the contractility of gastrointestinal motility and there are several good reviews available (Bass & Bennett, 1968; Bennett, 1972; Bennett & Flesher, 1970). Prostaglandins E and F usually contract longitudinal gastrointestinal muscle of man (Bennett, Eley & Scholes, 1968; Bennett & Flesher, 1970; Bennett, Murray

& Wyllie, 1968; Bennett & Posner, 1971; Vanasin, Greenough & Schuster, 1970) and animals including guinea pig, rat, rabbit, cat, bat, hamster, bird, dog and chicken (Anggard, & Bergstrom 1963; Bennett, Eley & Scholes, 1968; Bennett, Eley & Stockley, 1975; Bergstrom, Carlsson & Weeks, 1968; Ferreira & Vane, 1967[b]; Karim & Hillier, 1968; Vanasin, Greenough & Schuster, 1970). However, PGE has been reported to relax longitudinal muscle of human gastric antrum (Bennett, Murray & Wyllie, 1968) and rat duodenum (Turker & Ozer 1970) while PGF has been observed to occasionally relax longitudinal muscle of human distal colon (Bennett & Flesher, 1970). In circular muscle of the gastrointestinal tract of animals including man, guinea pig, rat and dog PGE usually produces a contraction (Bennett, Eley & Stockley, 1975; Bennett & Flesher, 1970; Bennett & Posner, 1971; Flesher & Bennett, 1969; Vanasin Greenough & Schuster, 1970) although Bennett & Posner (1971) observed either contraction or relaxation of human proximal colon. On the other hand PGE inhibits circular muscle in the gastrointestinal tract (Bennett, Eley & Scholes, 1968; Bennett, Murray & Wylie, 1968; Flesher & Bennett, 1969; Vanasin, Greenough & Schuster, 1970) although contraction of the circular muscle of rat stomach (Bennett & Flesher, 1970) and human ileum (Bennett, Eley & Scholes, 1968) has been reported.

Prostaglandin endoperoxides, PGG_2 and PGH_2 (Hamberg & Samuelsson, 1973; Hamberg, Svensson, Wakabayashi & Samuelsson, 1974) are reported to contract gerbil colon and rat stomach to the same extent as PGE_2 and PGF_2 but were more active on rabbit aorta and guinea pig trachea (Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975) and it is the belief of these workers that prostaglandins may exert their biological action through the endoperoxides.

(e) Effect of Prostaglandins and Cyclic AMP on Sodium and Water Transfer in Small Intestine

(i) Salt and Water Transfer

There are many excellent reviews concerning ion and water transfer

by the mammalian small intestine (Fordtran & Dietschy, 1966; Fordtran & Ingelfinger, 1968; Schultz & Curran, 1968, 1970; Schultz & Frizzell, 1972; Schultz, Frizzel & Nellans, 1974) where a full description of the mechanisms concerned are described.

(ii) Shunt Pathway

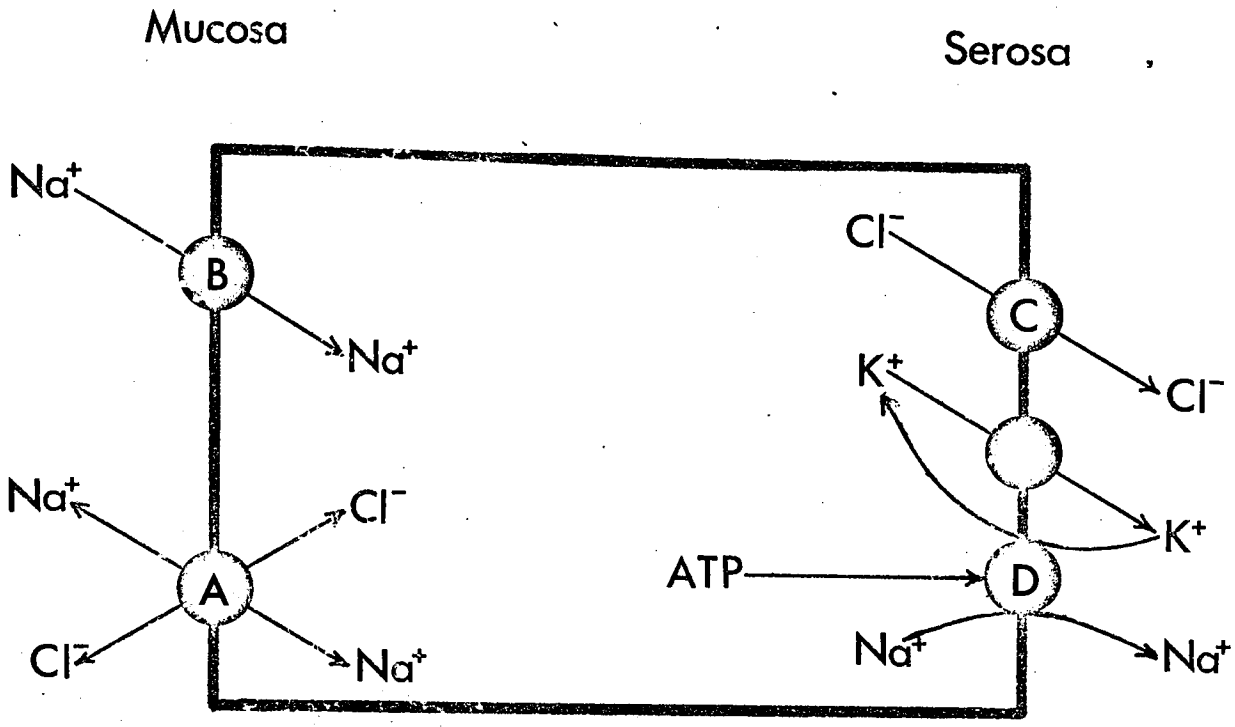
Solute transfer across epithelia can occur via a transcellular route which involves movement across at least two membranes in series or via an extracellular route which circumvents the membranes surrounding the epithelial cells. The latter route is called the shunt pathway and comprises the tight junctions and underlying intercellular spaces (Fromter & Diamond, 1972). The shunt is thought to play a major role in the movement of ions, small non electrolytes and water across the small intestine(Schultz, 1973; Schultz, Frizzell & Nellans, 1974), accounting for 85-90% of the total tissue conductance (Frizzell & Schultz, 1972). A more extensive discussion of the shunt pathway is found in recent reviews (Fromter & Diamond, 1972; Schultz, 1973; Schultz, Frizzell & Nellans, 1974).

(iii) Transcellular Ion and Water Transfer

Most of the published work has been performed on ileal tissue and there are relatively few studies on mammalian jejunum. Thus a general model for ion transfer in ileum will be discussed and, where relevant, information from studies on jejunum will be considered.

In human ileum sodium and chloride are absorbed and bicarbonate secreted, all three movements taking place against steep electrochemical potential difference (Turnberg, Bieberdorf, Morawski & Fordtran, 1970) whereas bicarbonate is absorbed in the jejunum (Fordtran, Rector & Carter, 1968). The results of studies on intracellular ion concentrations (Koopman & Schultz, 1969; Schultz, Fuisz & Curran, 1966), the electrical potential profile (Rose & Schultz, 1971), ion influxes across the brush border (Frizzell, Nellans, Rose, Markscheid-Kampi & Schultz, 1973) and transepithelial movements of sodium and chloride across short-circuited rabbit ileum (Nellans, Frizzell

& Schultz, 1974) are consistent with the model below:-



In the absence of actively transported sugars or amino acids, sodium may cross the brush border via two routes. One (A) is tightly coupled to chloride influx and the second (B) is chloride-independent. In both cases sodium entry is down a steep electrochemical potential difference (Koopman & Schultz, 1969; Rose & Schultz, 1971) and is not inhibited by ouabain or metabolic inhibitors (Chez, Palmer, Schultz & Curran, 1967). Process A appears to be a neutral sodium-chloride influx which is not linked to metabolic energy and which conforms to a classical carrier model (Nellans, Frizzell & Schultz, 1973). Process A is thought to be reversible, mediating the efflux of sodium chloride, sodium bicarbonate or both out of the cell into the mucosal solution. Process B is unclear and may be diffusional or carrier-mediated (Schultz, Frizzell & Nellans, 1974)

Sodium extrusion from the cell into the serosal solution (Process D) is active and is inhibited by ouabain (Schultz & Zalusky, 1964) suggesting that this process is Na-K-ATPase mediated. Process D involves the extrusion of sodium at a higher rate than the coupled uptake of potassium (Frizzell & Schultz, 1972; Rose & Schultz, 1971). Process C is unclear but

simple diffusion might suffice (Schultz, Frizzell & Nellans, 1974).

Actively transported sugars and amino acids stimulate sodium absorption and this phenomenon has been reviewed by Schultz & Curran(1970) and Kimmich (1973). This stimulation of sodium absorption is not via the coupled sodium-chloride influx across the brush border (Schultz, Frizzell & Nellans, 1974).

Water absorption is secondary to sodium absorption (Curran & Solomon, 1957) and Diamond & Tormey (1966) described how the intercellular spaces of intestinal mucosa could act as concentration chambers for the sodium expelled across the basolateral membranes and it has been shown that these spaces become open and distended when transporting fluid rapidly due to the entry of water through the tight junctions.

(iv) Prostaglandins and Cyclic AMP

The actions of prostaglandins and cyclic nucleotides on intestinal salt and water transfer is the subject of several excellent reviews(Field, 1974; Kimberg, 1974; Scratcherd & Case, 1973).

Prostaglandins, which have been implicated in diarrhoea associated with medullary carcinoma of the thyroid (Sandler, Karim & Williams, 1968), produced net electrolyte and water secretion when infused into the superior mesenteric artery of dogs (Pierce, Carpenter, Elliott & Greenough, 1971) or given intraluminally (Matuchansky & Bernier, 1973) or intravenously in man (Cummings, Newman, Misiewicz, Milton-Thompson & Billings, 1973). Secretion of salt and water has also been seen in isolated rabbit ileal mucosa after addition of PGE_1, PGE_2 or $PGF_{2\alpha}$ (Al-Awqati & Greenough, 1972; Kimberg, Field, Johnson, Henderson & Gershon, 1971).

Prostaglandins have been shown to enhance intestinal adenyl cyclase activity (Kimberg, Field, Johnson, Henderson & Gershon, 1971; Sharp & Hynie, 1971) and to increase cyclic AMP level in the intestinal mucosa at the same time as affecting short circuit current (SCC). Adenyl cyclase is present in the small intestinal mucosa of rodents (Ishikawa, Ishikawa, Davis & Sutherland, 1969; Kimberg, Field, Johnson, Henderson & Gershon, 1971;

Parkinson, Ebel, di Pona & Sharp, 1972; Sharp & Hynie, 1971), dogs (Evans, Chen, Carlin & Evans, 1972; Schafer, Lust, Sircar & Goldberg, 1970) and man (Chen, Rohde & Sharp, 1972). It is probably therefore that prostaglandinselicit intestinal secretion through cyclic AMP (Field, 1974; Kimberg, 1974).

It is also known that the enterotoxin of vibrio cholerae and Eschericia coli produce intestinal secretion which is believed to involve cyclic AMP (see reviews by Banwell & Sherr, 1973; Field, 1974 and Kimberg, 1974). It has been suggested that cholera toxin might affect also prostaglandins (Bennett, 1971; Finck & Katz, 1972; Gots, Formal & Gianella, 1974; Jacoby & Marshall, 1972; Vaisrub, 1972; Valiulus & Long 1973) although there are a number of contrary reports (Bourne, 1973; Cuatrecasas, 1973; Kimberg, Field, Gershon & Henderson, 1974; Kimberg, Field, Johnson, Henderson & Gershon, 1971; Sharp & Hynie, 1971).

Theophylline, in rabbit ileal mucosa, caused a rapid increase in SCC which mimicked that produced by cyclic AMP (Field, 1971), and during the altered SCC absorption of sodium and chloride was replaced by net secretion (Field, Fromm & Silen, 1969). Since small intestinal fluid transport is isosmotic with its fluid of origin (Lee, 1968) predictable amounts of water accompanied the secreted solutes. Secretory stimuli such as theophylline do not affect glucose-stimulated sodium transport (Al-Awqati, Cameron, & Greenough, 1973; Field, 1971; Field, Fromm, Al-Awqati & Greenough, 1972). Nellans, Frizzell & Schultz, (1973) showed that theophylline inhibited the coupled sodium chloride influx process. This does not explain the secretion seen with theophylline and it would appear that either an underlying active secretion of salt and water is unmasked by the action of cyclic AMP or that cyclic AMP has two effects, one inhibiting sodium chloride influx and the other enhancing sodium chloride efflux (Field, 1974). Field (1974) considered that there is strong circumstantial evidence for the presence of an active sodium chloride extrusion mechanism and that this shares the same carrier mechanism as the sodium chloride influx process.

3. BRADYKININ-PROSTAGLANDIN INTERACTIONS

Collier (1969) considered that the antagonism by aspirin of the pharmacological actions of bradykinin might be due to inhibition of a carrier mechanism by which bradykinin reached its receptors or inhibition of the release of an unidentified active substance. The observation that the aspirin-like drugs are potent inhibitors of prostaglandin synthetase (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Vane, 1971) showed that the latter suggestion was the more likely.

Aspirin-like drugs have been reported to antagonise the nociceptive action of bradykinin in mouse (Collier, Dineen, Johnson & Schneider, 1968), rat (Deffenu, Pegrassi & Lumach, 1966; Gilfoil, Kravins & Dunn, 1964), dog (Dickerson, Engle, Guzman, Rodgers & Lim, 1965; Guzman, Braun, Lim, Potters & Rodgers, 1964), guinea pig (Gjuris, Heicke & Westerman, 1964)[a,b] and man (Coffman, 1974; Lim, Miller, Guzman, Rodgers, Rogers, Wang, Chao & Shih, 1967) although contrary results have been obtained in guinea pig (Collier & Lee, 1963) and man (Lewis, 1963).

The inflammatory action of bradykinin was antagonised by aspirin-like drugs in the rat (Lisin & Leclerq, 1963; Martelli, 1967; Starr & West 1967) and rabbit (Lecomte & Troquet, 1960; Lish & McKinney, 1963) but Lewis (1963) did not observe selective antagonism in the rat.

Bradykinin-induced bronchoconstriction was antagonised by aspirin like drugs in the guinea pig in vivo (Collier, Holgate, Schacter & Shorley, 1959, 1960) but not in the rat or rabbit (Bhoola, Collier, Schacter & Shorley, 1962). Bradykinin-induced tachypnoea (Gjuris, Heicke & Westerman, 1964a), contraction of isolated trachea (Bhoola, Collier, Schacter & Shorley, 1962) and bronchoconstriction of isolated perfused lungs (Greef & Moog, 1964) were all antagonised by aspirin-like drugs although Aarsen (1966) found contrary results in the latter system.

Aspirin-like drugs in rat, dog, guinea pig and rabbit reduced bradykinin induced hypotension in duration but not degree (Collier, Dineen, Perkins & Piper, 1968; Collier & Shorley, 1960; Deby, Barac & Bacq, 1974;

Vargaftig, 1966) and antagonised its effect on pulmonary blood vessels (Greef & Moog , 1964; Klupp & Konzett, 1965; Konzett & Bauer, 1966; Lecomte & Troquet, 1960) although non selective blockade was seen in guinea pig and rabbit (Hauge, Lunde & Waaler, 1966; Starr & West, 1966).

Piper & Vane (1969,a,b) first showed release from guinea pig lungs of PGE_2 and $PGF_{2\alpha}$ and a previously unknown substance which contracted the rabbit aorta. The generation of 'rabbit aorta contracting substance' (RCS) was prevented by aspirin (Piper & Vane, 1969a,b) and augmented by arachidonic acid (Palmer, Piper & Vane, 1973; Vargaftig & Dao, 1972). RCS is now thought to be thromboxane A_2 which is formed from PGG_2 or PGH_2 (Bunting, Moncada, Needleman & Vane, 1976; Samuelsson, 1975). Bradykinin has also increased prostaglandin release from dog kidney (McGiff, Terragno, Malik & Lonigro, 1972), in vivo and in vitro dog spleen (Ferreira, Moncada & Vane, 1973; Moncada, Ferreira & Vane, 1972), dog knee joint (Moncada, Ferreira & Vane, 1975), isolated rabbit heart (Needleman, Key, Denny, Isakson & Marshall, 1975; Needleman, Marshall & Sobel, 1975), rat lungs (Damas & Bourdon, 1974), gravid dog uterus (Terragno, Terragno, Pacholczyk & McGiff, 1974) and bovine mesenteric arteries and veins (Terragno, Crowshaw, Terragno & McGiff, 1975) but Sirois & Gagnon (1974) did not observe an effect of bradykinin on PG release from rabbit renal medulla.

Vargaftig & Dao (1972) showed that mepacrine, which inhibits phospholipase A (Markus & Ball, 1969), suppressed release of RCS by bradykinin from guinea pig lungs and suggested that bradykinin activated an acylhydrolase to release prostaglandin precursors. Stoner, Manganiello & Vaughan (1973) observed that bradykinin increased cyclic GMP and cyclic AMP levels in guinea pig lung and that aspirin or indomethacin prevented the rise in cyclic AMP but not cyclic GMP. It was considered that bradykinin enhanced the synthesis and release of prostaglandins as a consequence of its effect on cyclic GMP and that the release of prostaglandins mediated the rise in cyclic AMP.

Vane & Ferreira (1975) have argued that the prostaglandins released by bradykinin can act as a mediator or potentiator of the response observed. The renal vasodilator action of bradykinin is associated with increased PGE levels in renal venous blood (McGiff, Terragno, Malik & Lonigro, 1972) and is reduced by indomethacin (McGiff, Itskovitz & Terragno, 1975). The effect of bradykinin on solute-free-water excretion was abolished by indomethacin. Thus it is likely that all of the effects on solute free water excretion and part of the vasodilation induced by bradykinin are prostaglandin-mediated. It is also thought that prostaglandin release mediates the action of bradykinin on blood pressure (Vane & Ferreira, 1975), the coronary vasculature of rabbit (Needleman, Key, Denny, Isakson & Marshall, 1975), uterine blood vessels of the dog (Terragno, Terragno, Pacholczyk & McGiff, 1974) , rat cremaster muscle vasculature (Messina, Weiner & Kaley (1975) and rabbit coeliac artery (Aiken, 1974). The bronchoconstrictor action of bradykinin may be mediated by the prostaglandins which are released, since RCS and $PGF_{2\alpha}$ are potent contractile agents on isolated human bronchial muscle (Piper & Walker, 1973). It was observed that on repeated exposure of guinea pig tracheal muscle to bradykinin the responses gradually changed from contraction to relaxation (Iorio & Constantine, 1969) and the relaxations were prevented by discontinuing aeration or phenylbutazone. Thus it is possible that the appearance of a relaxation can be explained by the induction of a PGE.

In all these situations the prostaglandin is thought in some way to mediate the response to bradykinin. However, the nociceptive action of bradykinin is thought to work through a different mechanism since prostaglandins were shown to produce long lasting overt pain only when given in high concentrations (Ferreira, 1972; Karim, 1971). Also, bradykinin, which is algesic in the dog spleen (Guzman, Braun, Lim, Potter & Rodgers, 1964) caused a similar release of prostaglandins from this preparation as did adrenaline which is not algesic in the dog spleen. Ferreira (1972) suggested a sensitisation of the sensory nerve endings

by prostaglandins to mechanical or chemical stimulation. Intrasplenic injection of bradykinin in the dog produced a pressor response which was an autonomic response to stimulation of different nerve endings and which was reduced by aspirin-like drugs (Guzman, Braun, Lim, Potter & Rodgers, 1964). Bradykinin increased the basal output of prostaglandins from the spleen (Ferreira, Moncada & Vane, 1973). Indomethacin blocked the release of PGs and reduced the pressor response to bradykinin, and infusion of low concentrations of PGE_1 restored this response. The analgesic action of indomethacin was explained as removal of the generation of prostaglandins which sensitise pain receptors to the action of bradykinin. A similar situation has been found in the dog knee joint to that in the dog spleen (Moncada, Ferreira & Vane, 1974, 1975). Moncada, Ferreira & Vane (1973) showed that the vascular-permeability-increasing effect of bradykinin was substantially increased when low concentrations of PGE_1 were present and sensitisation of bradykinin-induced permeability by prostaglandins has been confirmed by Thomas & West (1973) and Williams & Morley (1973).

In conclusion, it has been shown that many pharmacological actions of bradykinin are inhibited by the aspirin-like drugs which are potent prostaglandin synthetase inhibitors and that these pharmacological actions are often accompanied by increased prostaglandin release. Thus it would appear that there is considerable interaction between bradykinin and the prostaglandins and that prostaglandins may mediate the action of bradykinin or sensitise the target tissue to the action of bradykinin.

METHODS

SECTION 1 SODIUM AND WATER TRANSFER EXPERIMENTS

(a) Everted Gut Sac Technique

The rat jejunum was used for these experiments since it is the site of principal sodium and water absorption in the gastrointestinal tract and because of its previous use (Lee 1968; Turnberg 1973).

Male Wistar rats (100-200 g body weight unless otherwise stated) were starved for 18 hours before experimentation. Rats were killed by a blow on the head and the whole of the small intestine was rinsed through with sodium chloride solution (0.154M). The intestine was everted according to the technique of Wilson & Wiseman (1954) and then divided into five equal segments (Barry, Matthews & Smyth, 1961). The jejunal segment (sac 11) was subdivided into two equal sacs so that each experimental animal provided two adjacent sacs. One of these sacs served as a control and was filled with Krebs bicarbonate solution containing 27.75mM glucose while the other sac was filled with the same solution to which had been added the substance under investigation. The proximal sac made from the jejunal segment of each animal was used alternatively as a control or an experimental sac so that any effect that might have been due to either the delay in using the sacs, or to differences in transport characteristics along the intestine, were minimised.

Each sac was weighed, then filled with the appropriate solution and tied off. The sac was reweighed and incubated in an Ehrlenmeyer flask containing 15ml Krebs bicarbonate solution which had been gassed with 95% O_2 + 5% CO_2. The composition of the Krebs bicarbonate solution was the same in mucosal and serosal solutions and contained (mM): NaCI 118, KCI 4.7, $MgSo_4\ 7H_2O$ 1.18, KH_2PO_4 1.17, $NaHCO_3$ 25, $CaCI_2 2H_2O$ 2.5, glucose 27.75, pH 7.4. The sacs were incubated at 37°C for an hour in a shaking water bath at 40 oscillations per minute. At the end of the incubation period the sacs were blotted and reweighed. Samples of the mucosal solutions were estimated

for sodium using a Pye Unicam SP90 series 2 Atomic Absorption spectrophotometer.

Results were expressed as mucosal transfer, that is the amount of sodium or water leaving the mucosal solution during the incubation period as described by Parsons, Smyth & Taylor (1958) and discussed by Smyth(1963). The mucosal water transfer was calculated from the weight changes in the sacs after incubation and the mucosal sodium transfer was calculated from a knowledge of the quantities of sodium present initially and finally in the mucosal solution. Water transfer was expressed as ml/g initial wet weight of jejunal sac.hr and sodium transfer as μ equiv/g initial wet weight of jejunal sac.hr.

In the results section a negative (-) sign is used to denote an inhibition of control transfer while a positive (+) sign is used to denote a stimulation of control transfer. The values quoted in the text refer to the percentage change from the control transfer.

Unless stated to the contrary drugs, wherever used, were placed in both mucosal and serosal solutions.

(b) Operative Procedures

In this study bilaterally adrenalectomised, nephrectomised and adrenalectomised-nephrectomised rats were used. The rats used for this purpose were heavier (300-350g body weight) than those used in the rest of the study since it was found that lighter rats did not survive the operative procedures sufficiently well. All operations were carried out on rats under anaesthesia induced by halothane in nitrous oxide/oxygen. After removal of adrenals, kidneys or both, rats were caged individually and placed in a warm room. Adrenalectomised rats were maintained on 1% saline and all animals were fed the regular diet 41B. Experiments were performed on these rats 48 hours after the operations.

(c) Modification of Diet

In most of the experiments reported here rats were fed on normal rat diet with tap water for drinking. However in some experiments the dietary intake of sodium was modified. In order to produce a high sodium intake rats were maintained on normal rat diet but their drinking water was replaced by 1% saline for 14 days prior to experimentation. Other rats were fed on a low sodium diet (Nutritional Biochemicals Co., Ohio) for 3 weeks and given tap water to drink.

SECTION 11 SMOOTH MUSCLE CONTRACTION EXPERIMENTS

(a) Organ Bath Technique

(i) Rat Terminal Ileum

Male Wistar rats (150–300g) were starved for 18 hours prior to experimentation. Rats were killed by a blow on the head and sections of terminal ileum, 2.5cm long, were immediately removed and flushed through with Tyrode solution. Ileal preparations were suspended under a load of 1g in 20ml organ baths containing aerated Tyrode solution at 30°C. The composition of Tyrode solution used was (mM): NaCI 137, KCI 2.7, $NaH_2PO_4 2H_2O$ 0.42, $NaHCO_3$ 11.9, $MgSO_4 7H_2O$ 1.1, $CaCI_2 2H_2O$ 1.8, glucose 5.6. In experiments involving anoxia the Tyrode solution was gassed with nitrogen instead of air.

Preparations of terminal ileum were equilibrated for 90 minutes and then contractile responses to the various agonists used were recorded. A tissue contact time of 90 seconds was employed for bradykinin, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ while for acetylcholine 45 seconds was sufficient to produce a complete contraction. In all experiments reported here concentrations of acetylcholine, bradykinin, PGE_2 and $PGF_{2\alpha}$ were chosen which produced contractile responses approximately equal to the 50% maximal acetylcholine response. Dose cycles of 10 minutes were used to avoid tachyphylaxis and because of the relatively slow recovery time of the tissue after bradykinin or the prostaglandins.

The isotonic contractile responses of rat terminal ileum were recorded using Devices 2LD01 and 3194 isotonic transducers which were coupled to Devices M2 recorders.

In the experiments reported below the contractile responses of the various agonists in the presence of drugs are expressed as percentages of the control contractile responses to each agonist.

(ii) Rat Stomach Strip

This preparation, described by Vane (1957), was used in the bioassay of prostaglandins which is described subsequently.

Male Wistar rats (200-300g) were starved for 18 hours prior to experimentation. Rats were killed by a blow on the head and the fundal region of the stomach was removed. The fundus was cut along its lesser margin and opened to produce a flap of muscular tissue, which was washed in Tyrode solution to remove any loose filaments and stomach contents. Cuts were made on alternate sides of the flap of fundal tissue and the tissue was pulled from its upper and lower edges in order to produce a strip of muscular tissue. The rat fundic strip was cleaned with a pair of scissors and suspended in a 10ml organ bath containing aerated Tyrode solution at 30°C. The stomach strip was subjected to a load of 2g and a dose cycle of 15 minutes was used because of the longer relaxation time of this tissue. PGE_2 was the only agonist used on this tissue and a contact time of 2 minutes was employed.

The Tyrode solution used was the same as that described previously except that a series of blocking drugs were used in order to increase the sensitivity and specificity of this tissue to prostaglandins. The blocking drugs used were: indomethacin (2.8μM); methysergide (0.47μM); hyoscine (0.23μM); mepyramine (0.21μM); propranolol (6.76μM) and phentolamine (5.29μM).

Contractile responses of this tissue were measured in the same way as those of rat terminal ileum.

(b) Prostaglandin Bioassay

(i) Extraction of Prostaglandins

Sections of rat terminal ileum were set up in the usual manner in 10ml organ baths. After equilibration the tissues were washed and then left for an hour without washing. The organ bath fluid was removed at the end of an hour and acidified to approximately pH3 with IN hydrochloric acid. The bath fluid was extracted twice with an equal volume of diethyl ether (Analar). The ether phases were pooled and evaporated to dryness under reduced pressure at 40°C. Residues were taken up in 1ml of Tyrode solution and assayed against standard PGE_2 on the rat stomach strip using the modified Tyrode solution described above.

The effect of bradykinin or other agents on the release of prostaglandin-like material was tested by incubating the terminal ileum with the appropriate agents for the first hour after equilibration. Recovery of prostaglandin-like material was evaluated by extraction of standard solutions of PGE_2 and was found to be 52.96 ± 4.55% (n= 16). Bradykinin solutions of ten times the concentration found in the organ bath fluid were also subjected to the extraction procedure but it was not possible to detect any extracted bradykinin on the rat stomach strip. The section of terminal ileum was weighed at the end of the experiment since the prostaglandin-like material found in the organ bath fluid was expressed as ng of PGE_2 equivalent/g of tissue.hr.

(ii) Thin Layer Chromatography

The thin layer chromatographic technique used was that described by Green & Samuelsson (1964) and their A1 solvent system (Benzene-Dioxan-Acetic acid 20:20:1) was employed.

Ready made glass chromatoplates (0.40 x 20 x 20 cm) precoated with silica gel to a thickness of 0.25mm were activated by heating for 35 minutes at 110°C (chromatoplates supplied by Merck). The extracts of organ bath fluid prepared as described above were taken up in 0.4ml of absolute ethanol and applied to the plates as single spots alongside standard prostaglandins

(50–100µg). The spots were dried in a stream of warm air. The chromatoplates were run in the A1 solvent system until the solvent front had reached about 2–3cm from the top of the chromatoplates which took about 2 hours.

After development the plates were removed from the solvent system, the solvent front was marked and the plates were dried at 100°C. The chromatoplates were sprayed with 10% phosphomolybdic acid in ethanol and heated at 110°C for 15 minutes which resulted in the appearance of blue spots on a yellow-green background.

SECTION 111 DRUGS AND CHEMICALS

Bradykinin (Sigma) was prepared in a sterile solution (7.86×10^{-5} M and 7.86×10^{-8}M) in bidistilled water and stored frozen in 1ml ampoules until required.

Prostaglandins E_1, E_2, A_1 and $F_{2\alpha}$ which were gifts from Dr. J.E. Pike, Upjohn Company Ltd., were stored in an absolute alcohol solution in sterile ampoules at 0°C and diluted with Tyrode or Krebs solution as required.

Cyclic AMP (Sigma), dibutyryl cyclic AMP (Sigma) and PPP & DPP (gifts from Drs Hogberg & Fex, AB Leo, Sweden) were stored at 0–4°C until use. All other chemicals were made up into solution as required. Indomethacin, aspirin, flufenamic acid, meclofenamic acid and SC 19220 were made up in absolute alcohol and further diluted with either Tyrode or Krebs solution. Care was taken to ensure that the final concentration of alcohol did not affect the response being measured.

Other drugs used, with their source, are as follows:

Flufenamic acid Meclofenamic acid	Gifts from Parke Davis Ltd.
Indomethacin	Gift from Merck, Sharp & Dohme Ltd.
SC 19220	Gift from Dr.J. Sanner, G.D. Searle & Co., Chicago, U.S.A.
7 oxa-13-prostynoic acid	Gift from Prof. J. Fried, University of Chicago, U.S.A.
Methysergide	Gift from Sandoz Ltd.

SQ 20881	Gift from Dr. M. Ondetti, Squibb, U.S.A.
Aspirin	B.D.H.
Mepyramine	B.D.H.
hyoscine	B.D.H.
propranolol	I.C.I.
phentolamine	CIBA
phosphomolybdic acid	Williams & Hopkins
Theophylline	Sigma
Ethanol	B.D.H.

SECTION IV CALCULATION OF RESULTS

The results of experiments performed on both water transfer and on contractile action of rat terminal ileum are expressed as mean and standard error of the mean. The effect of experimental treatments has been analysed using the Students 't' test.

RESULTS

SECTION 1 WATER TRANSFER

(A) Experiments to Investigate the Effect of Bradykinin on Sodium and Water Transfer

It was the purpose of this part of the study to investigate the action of bradykinin on sodium and water transfer across an epithelial membrane. The isolated gut sac technique of Wilson & Wiseman (1954) was chosen for this purpose and the rat jejunum was the tissue used.

(i) Effect on Mucosal Sodium and Water Transfer of Varying the Concentration of Bradykinin in Mucosal and Serosal Solutions

Initially it appeared that the effect of bradykinin on sodium and water transfer was variable but upon closer scrutiny of the results it became apparent that the effect produced by bradykinin was dependent upon the level of sodium and water transfer in the paired control sac which had been incubated in Krebs bicarbonate solution alone. The results were divided into three groups on the basis of the effect observed. Thus when the level of water transfer was less than 0.6ml/g wet wt hr. in the control sacs a stimulation of transfer was observed in all the experimental sacs. When the level of water transfer in the control sacs was between 0.6 and 1.0 ml/g wet wt hr. bradykinin produced either a stimulation, an inhibition or no effect at all in the experimental sacs. When the level of water transfer in the control sacs was greater than 1.0ml/g wet wt hr. bradykinin inhibited water transfer in all sacs. The effects of bradykinin, 7.86×10^{-12}M, in both mucosal and serosal solutions on water transfer are shown in Fig.1 and were associated with corresponding changes in sodium transfer as reported by Curran & Solomon (1957).

The results in Table 1 show that a similar pattern of effects on water transfer were obtained when bradykinin at 7.86×10^{-11} or 7.86×10^{-13}M was present in both mucosal and serosal solutions. At low levels of control water transfer (i.e. less than 0.6ml/g wet wt hr.) bradykinin 7.86×10^{-11}, 7.86×10^{-12} and 7.86×10^{-13}M significantly stimulated water transfer by

Mean Mucosal Water Transfer

Control	BK	Control	BK	Control	BK
0.519	0.715	0.780	0.758	1.139	0.883
± 0.017	± 0.047	± 0.035	± 0.041	± 0.026	± 0.053
n = 17****		n = 31 n.s.		n = 17****	

Mean Mucosal Sodium Transfer

46.3	68.9	87.1	85.9	159.4	127.7
± 5.4	± 7.7	± 5.8	± 5.7	± 5.3	± 5.9
n = 12**		n = 22 n.s.		n = 15***	

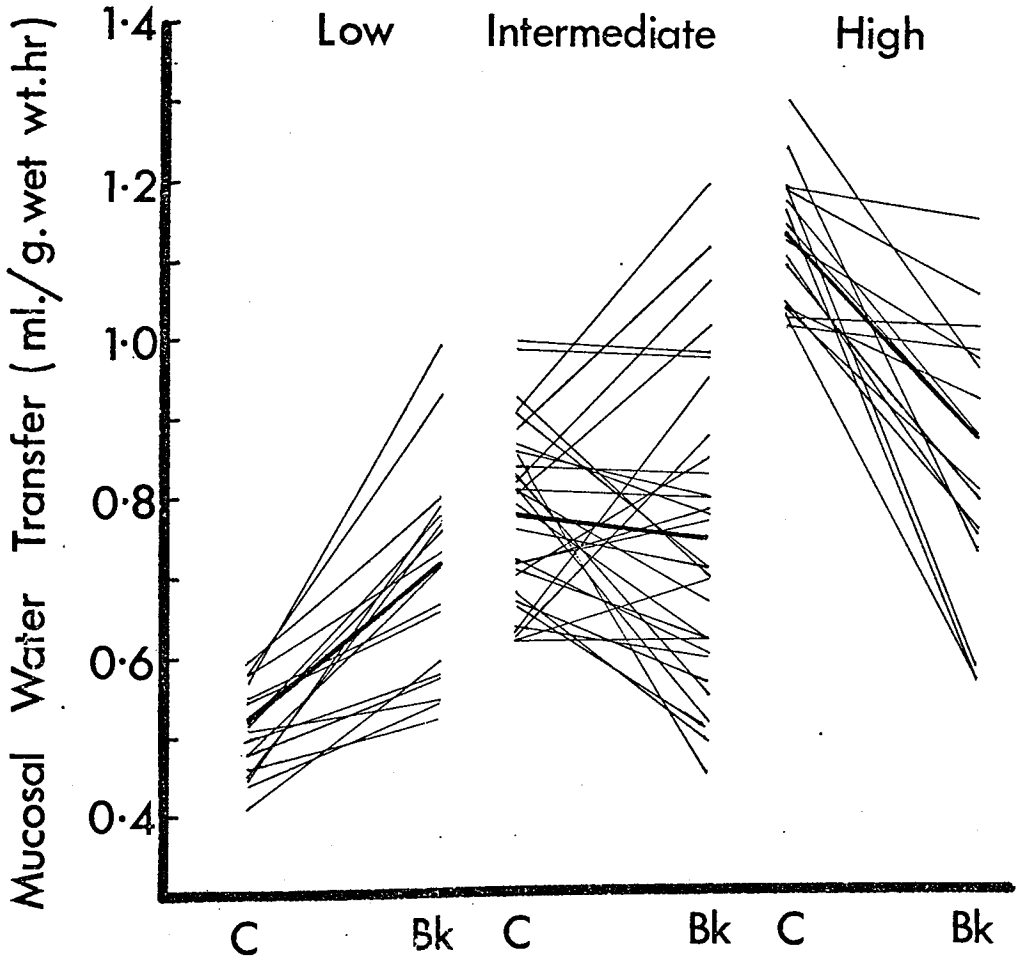


Fig. 1. Effect of bradykinin (BK), 7.86 x 10^{-12}M, in mucosal and serosal solutions on sodium and water transfer expressed as ml. or μequiv/g wet wt. of jejunal sac.hr. The degrees of significance are expressed as follows: * = 0.05; ** = 0.02; *** = 0.01; **** = 0.001; n.s. = not significant. The number of observations are given in parentheses. Means are represented by heavy line.

TABLE 1 The effect of bradykinin 7.86×10^{-11} and 7.86×10^{-13}M on water transfer in rat jejunal sacs. The degree of significance for each set of results is expressed as follows: * = < 0.05; ** = < 0.02; *** = < 0.01; **** = < 0.001; n.s. = not significant. The number of observations in each case is given in parentheses.

Level of Mucosal Water Transfer (ml/g wet wt.hr)

Group	Low	Intermediate	High
Control	0.528 ± 0.033	0.728 ± 0.037	1.178 ± 0.045
Bradykinin (7.86×10^{-11}M)	0.884 ± 0.064*** (4)	0.794 ± 0.051 n.s. (18)	0.789 ± 0.046**** (9)
Control	0.468 ± 0.019	0.761 ± 0.027	1.081 ± 0.054
Bradykinin (7.86×10^{-13}M)	0.596 ± 0.053** (14)	0.738 ± 0.043 n.s. (28)	0.865 ± 0.083* (6)

+67.2%, +37.8% and +27.9% respectively. Water transfer was unaffected by bradykinin at any concentration when control water transfer was intermediate (i.e. between 0.6 and 1.0 ml/g wet wt hr.) but at high control water transfer (i.e. greater than 1.0 ml/g wet wt hr.) bradykinin at concentrations of 7.86×10^{-11}, 7.86×10^{-12} and 7.86×10^{-13}M significantly inhibited water transfer by -33.4 %, -22.4% and -19.9% respectively. Bradykinin, 7.86×10^{-12}M, produced a consistent qualitative and quantitative effect in subsequent experiments reported here.

(ii) The Effect of Bradykinin in Mucosal and Serosal Solutions, or in Mucosal or Serosal Solutions alone on Mucosal Water Transfer

Sacs were incubated with bradykinin, 7.86×10^{-12}M, either in the mucosal and serosal solutions or in the mucosal or serosal solutions alone. It can be seen from Table 2 that water transfer was inhibited by bradykinin at high control water transfer when it was present in either the mucosal or serosal solutions or both, but at intermediate levels of control water transfer bradykinin had no significant effect. A significant stimulation of water transfer at low levels of control water transfer was obtained when bradykinin was in both solutions, or in the serosal solution alone, but no effect was observed when bradykinin was present only in the mucosal solution.

The control level of water transfer in the rat jejunum is capable of great variation, between about 0.4 and 1.4 ml/g wet wt hr., but most sacs transfer water at a rate of 0.7 - 0.9 ml/g wet wt hr. In the present study bradykinin-induced effects are obtained at transfer rates which are the least commonly observed, that is in sacs with low or high rates of transfer. Because it was sometimes difficult to obtain a sufficient number of these values to do a statistical analysis between various treatments a series of experiments was carried out to find what factors might affect basal levels of water transfer in the hope of increasing the efficiency of data collection. In this series of experiments involving adrenalectomy,

TABLE 2 The effect of bradykinin, 7.86×10^{-12} M, in either mucosal or serosal solution in rat jejunal sacs. The degrees of significance are expressed as in Fig. 1. The number of observations are given in parentheses.

Group	Level of Mucosal Water Transfer (ml/g wet wt.hr) Low	Intermediate	High
Control	0.451 ± 0.021	0.722 ± 0.062	1.145 ± 0.043
Bradykinin (Mucosal+Serosal)	0.612 ± 0.033**** (14)	0.668 ± 0.062n.s. (6)	0.927 ± 0.062*** (11)
Control	0.557 ± 0.027	0.742 ± 0.026	1.179 ± 0.043
Bradykinin (Mucosal)	0.579 ± 0.042 n.s. (6)	0.725 ± 0.043n.s. (18)	0.814 ± 0.081**** (9)
Control	0.527 ± 0.036	0.786 ± 0.034	1.083 ± 0.044
Bradykinin (Serosal)	0.750 ± 0.072*** (7)	0.761 ± 0.043n.s. (9)	0.841 ± 0.062*** (4)

nephrectomy and variation of sodium intake some attempt was made to manipulate the levels of control transfer and to see the effects of these measures on the responses to bradykinin.

(iii) Effect of Adrenalectomy, Nephrectomy and Combined Adrenalectomy-Nephrectomy on Bradykinin Effects on Water Transfer

It was the purpose of this experiment to investigate the effect of removal of the animals kidneys and/or adrenals on the action of bradykinin on water transfer. Removal of the kidneys reduces renin-angiotensin levels while adrenalectomy reduces mineralocorticoid levels in the rat.

This experiment shows the effect of adrenalectomy, nephrectomy and combined adrenalectomy-nephrectomy on the stimulation of water transfer seen with bradykinin, 7.86×10^{-12}M, at low levels of control water transfer since it was not possible to obtain animals with high control water transfer. It can be seen from Table 3 that adrenalectomy, nephrectomy or combined operations did not affect the stimulation of water transfer in rat jejunum seen with bradykinin at low control transfer. It can also be seen that each of these operative procedures caused a significant reduction in the level of control water transfer.

(iv) Effect of 1% Saline Pretreatment on Bradykinin Action on Water Transfer

It is known that in rats given saline as drinking fluid the renin-angiotensin-aldosterone system is depressed (Gross, Brunner & Ziegler, 1965). It was the purpose of this experiment to see if saline loading had an effect on bradykinin-induced changes in water transfer.

Rats pretreated with 1% saline as drinking fluid for 14 days exhibited significantly lower mean values of mucosal water transfer (Table 4). Bradykinin, 7.86×10^{-12}M, produced its usual spectrum of action in the jejunum of normal rats given water as drinking fluid. Thus a stimulation was observed at low control water transfer and an inhibition was observed at high control water transfer. In the saline-pretreated animals bradykinin was seen to cause a significant stimulation of water transfer at low control

TABLE 3 The effect of adrenalectomy, nephrectomy and combined adrenalectomy-nephrectomy on the effect of bradykinin, 7.86×10^{-12}M, on water transfer in rat jejunal sacs. The degree of significance for each set of results is expressed as in Fig. 1. The number of observations in each case is given in parentheses.

			ml/g wet wt.hr.
Normal	(12)	Control	0.491 ± 0.032
		Bradykinin	$0.628 \pm 0.047^{**}$
Adrenalectomised	(14)	Control	0.346 ± 0.023
		Bradykinin	$0.484 \pm 0.024^{****}$
Normal	(7)	Control	0.545 ± 0.015
		Bradykinin	$0.665 \pm 0.042^{**}$
Nephrectomised	(13)	Control	0.425 ± 0.043
		Bradykinin	$0.548 \pm 0.027^{**}$
Normal	(9)	Control	0.402 ± 0.036
		Bradykinin	$0.591 \pm 0.033^{****}$
Adrenalectomised - Nephrectomised	(12)	Control	0.370 ± 0.032
		Bradykinin	$0.484 \pm 0.034^{**}$

TABLE 4 The effect of (a) 1% saline pretreatment for 14 days and (b) low sodium diet for 21 days on the action of bradykinin, 7.86 x 10^{-12}M, on water transfer in rat jejunal sacs. The degree of significance for each set of results is expressed as in Fig. 1. The number of observations in each case is given in parentheses.

		Level of Mucosal Water Transfer (ml/g wet wt.hr)		
		Low	Intermediate	High
(a)				
Normal	Control	0.467 ± 0.056	0.758 ± 0.037	1.070 ± 0.036
	Bradykinin	0.677 ± 0.063* (5)	0.758 ± 0.061 n.s. (10)	0.813 ± 0.061** (3)
Saline	Control	0.446 ± 0.044	0.742 ± 0.063	
	Bradykinin	0.606 ± 0.053* (13)	0.763 ± 0.037 n.s. (6)	
(b)				
Normal	Control	0.500 ± 0.043	0.752 ± 0.026	1.117 ± 0.044
	Bradykinin	0.636 ± 0.055* (8)	0.738 ± 0.041 n.s. (21)	0.815 ± 0.053*** (5)
Low Sodium	Control	0.478 ± 0.022	0.744 ± 0.033	1.352 ± 0.025
	Bradykinin	0.602 ± 0.040** (9)	0.747 ± 0.056 n.s. (23)	0.959 ± 0.191 (3)

water transfer. It was not possible to investigate the inhibitory action of bradykinin at high control water transfer in saline-pretreated rats because none of the rats showed high levels of control transfer. There was no effect of bradykinin at intermediate levels of control transfer in either group of rats.

(v) Effect of Low Sodium Diet on the Effects of Bradykinin on Water Transfer

Rats were fed on a low sodium diet and tap water for three weeks prior to experimentation to increase circulating levels of mineralocorticoids (Singer & Stacke-Dunne, 1955).

It can be seen from Table 4 that in sacs of jejunum from normal rats bradykinin, 7.86×10^{-12}M, produced its usual spectrum of activity, stimulating water transfer at low control transfer and inhibiting water transfer at high control transfer. Jejuna from rats fed on a low sodium diet exhibited the same spectrum of activity as those from normal rats, when incubated with bradykinin, 7.86×10^{-12}M in both mucosal and serosal solutions.

The above experiments which were aimed at manipulating levels of the circulating renin-angiotensin-aldosterone system proved to be somewhat disappointing and in subsequent experiments random sampling of animals was used.

(B) Role of Prostaglandins in the Bradykinin Action on Water Transfer

Prostaglandins inhibit intestinal absorption of sodium and water (Al-Awqati & Greenough, 1972; Kimberg, Field, Johnson, Henderson & Gershon, 1971; Pierce, Carpenter, Elliott & Greenough, 1971) and they have been implicated in the action of bradykinin in several situations (Vane & Ferreira, 1975). It was decided therefore to investigate the possibility that prostaglandins were involved in the inhibitory actions of bradykinin on water transfer. In these experiments the non-steroidal antiinflammatory drug, meclofenamic acid, was used since it has been reported to be a potent inhibitor of prostaglandin synthetase (Flower, 1974). The interaction between bradykinin and several prostaglandins on water transfer was also investigated.

(i) Effect of Meclofenamic Acid on Bradykinin Action on Water Transfer

Since it was not possible to obtain sufficient animals showing low levels of control water transfer the effect of meclofenamic acid, 6.8μM, on the action of bradykinin, 7.86×10^{-12}M, was investigated only on intermediate and high levels of control water transfer. In these experiments both bradykinin and meclofenamic acid were present in mucosal and serosal solutions.

Meclofenamic acid alone had no effect on water transfer at intermediate or high control transfer (Table 5). At high control water transfer the inhibitory action of bradykinin (-26.22± 5.69%) was abolished in the presence of meclofenamic acid (-2.18 ± 6.57%), which alone had no significant effect (-6.86 ± 5.97%). Neither bradykinin (+1.22 ± 7.70%) nor meclofenamic acid (-5.93 ± 3.20%) had a significant effect on water transfer at intermediate control transfer but the combination of meclofenamic acid and bradykinin caused a significant stimulation of water transfer (+33.2 ± 9.30%; $p < 0.02$) at this level. This degree of stimulation was significantly different ($p < 0.05$) from the effect of bradykinin alone.

TABLE 5 The effect of meclofenamic acid (6.8 μM) and bradykinin 7.86×10^{-12} M, on water transfer in rat jejunal sacs. Degrees of significance for each set of results are expressed as in Fig. 1. The numbers of observations are given in parentheses.

	Level of Mucosal Water Transfer (ml/g wet wt.hr)		
	Low	Intermediate	High
Control		0.751 ± 0.031	1.279 ± 0.077
Bradykinin		0.772 ± 0.061 n.s. (4)	0.934 ± 0.069* (6)
Control		0.867 ± 0.034	1.354 ± 0.075
Meclofenamic Acid		0.816 ± 0.046 n.s. (5)	1.259 ± 0.100n.s. (12)
Control		0.783 ± 0.029	1.277 ± 0.063
Bradykinin + Meclofenamic Acid		1.038 ± 0.041** (5)	1.214 ± 0.070n.s. (15)

(ii) Effect of PGA_1 on Water Transfer and on the Action of Bradykinin on Water Transfer

PGA_1, 5.95×10^{-5}M, caused a significant inhibition of water transfer at both intermediate ($p < 0.01$) and high ($p < 0.001$) control transfer but had no significant effect at low control transfer (Table 6). At high control transfer the combination of PGA_1 and bradykinin, 7.86×10^{-12}M, produced an inhibition of water transfer ($-38.56 \pm 3.22\%$) which was significantly greater than that produced by either PGA_1 ($-28.06 \pm 2.34\%$; $p < 0.05$) or bradykinin ($-15.80 \pm 3.23\%$; $p < 0.01$) alone. Bradykinin and PGA_1 at intermediate control transfer inhibited water transfer to a degree ($-28.53 \pm 3.75\%$) which was significantly greater than the effects seen with either PGA_1 ($-11.68 \pm 3.75\%$; p 0.01) or bradykinin ($-1.70 \pm 8.98\%$; $p < 0.01$) alone and it is clear that the increased inhibition could not be an additive effect. Bradykinin significantly stimulated water transfer at low control transfer ($+25.88 \pm 10.53\%$; $p < 0.05$) but in the presence of PGA_1 which itself was without significant effect ($-9.04 \pm 4.46\%$) produced a non significant inhibition of water transfer ($-12.75 \pm 7.86\%$) and the combined effect of PGA_1 and bradykinin was significantly different from that of bradykinin alone ($p < 0.02$)

(iii) Effect of PGE_1 on Water Transfer and on the Action of Bradykinin on Water Transfer

It can be seen from Table 7 that PGE_1, 5.65×10^{-5}M, significantly inhibited water transfer at high ($p < 0.001$) and intermediate ($p < 0.02$) levels of control transfer but had no significant effect at low control transfer. The combination of PGE_1 and bradykinin, 7.86×10^{-12}M, produced a significant inhibition of water transfer at high control transfer ($-41.11 \pm 3.71\%$; $p < 0.001$) which was significantly greater than that seen with either PGE_1 ($-27.46 \pm 3.28\%$; $p < 0.05$) or bradykinin ($-18.18 \pm 3.68\%$; $p < 0.001$) alone. At intermediate control transfer the combination of bradykinin and PGE_1 significantly inhibited water transfer to a degree ($-35.45 \pm 4.37\%$; $p < 0.001$) which was significantly greater than the effects seen with PGE_1

TABLE 6 The effect of prostaglandin A_1, 5.95×10^{-5}M and bradykinin, 7.86×10^{-12}M, on water transfer in rat jejunal sacs. Degrees of significance for each set of results are expressed as in Fig. 1. The numbers of observations are given in parentheses.

	Level of Mucosal Water Transfer (ml/g wet wt.hr.)		
	Low	Intermediate	High
Control	0.412 ± 0.033	0.750 ± 0.039	1.108 ± 0.034
Bradykinin	0.499 ± 0.031* (10)	0.743 ± 0.052n.s. (9)	0.931 ± 0.033*** (5)
Control	0.528 ± 0.013	0.682 ± 0.011	1.110 ± 0.034
PGA_1	0.479 ± 0.027n.s. (15)	0.601 ± 0.024*** (9)	0.797 ± 0.029**** (5)
Control	0.522 ± 0.023	0.777 ± 0.028	1.132 ± 0.049
Bradykinin + PGA_1	0.452 ± 0.023n.s.	0.556 ± 0.036****	0.699 ± 0.063****

TABLE 7 The effect of prostaglandin E_1, 5.65×10^{-5}M, and bradykinin, 7.86×10^{-12}M, on water transfer in rat jejunal sacs. Degrees of significance for each set of results are expressed as in Fig. 1. The numbers of observations are given in parentheses.

	Level of Mucosal Water Transfer (ml/g wet wt.hr.)		
	Low	Intermediate	High
Control	0.493 ± 0.054	0.848 ± 0.043	1.156 ± 0.035
Bradykinin	0.698 ± 0.064* (6)	0.840 ± 0.076 n.s. (7)	0.941 ± 0.030**** (6)
Control	0.467 ± 0.047	0.761 ± 0.032	1.136 ± 0.038
PGE_1	0.395 ± 0.044 n.s. (5)	0.631 ± 0.034** (7)	0.825 ± 0.051**** (6)
Control	0.517 ± 0.023	0.770 ± 0.046	1.247 ± 0.100
Bradykinin + PGE_1	0.488 ± 0.046 n.s. (6)	0.500 ± 0.054**** (9)	0.726 ± 0.057**** (6)

(−16.54 ± 4.45%; $p < 0.01$) or bradykinin (−1.04 ± 11.70%; $p < 0.001$) alone. Bradykinin significantly stimulated water transfer at low control transfer (+45.57 ± 15.54%; $p < 0.05$) but in combination with PGE_1, which alone had no significant effect (−13.68 ± 9.43%), caused a non significant inhibition of transfer (−5.54 ± 1.55%) which was significantly different ($p < 0.01$) from the effect of bradykinin alone.

(iv) Effect of $PGF_{2\alpha}$ on Water Transfer and on the Action of Bradykinin on Water Transfer

$PGF_{2\alpha}$, 5.65×10^{-5}M, significantly inhibited water transfer at high ($p < 0.001$) and intermediate ($p < 0.01$) control transfer but had no effect on low control transfer (Table 8). At high control transfer the combination of $PGF_{2\alpha}$ and bradykinin, 7.86×10^{-12}M, significantly inhibited ($p < 0.01$) water transfer by 35.51 ± 6.62% which was significantly greater than the inhibition seen with bradykinin alone (−18.90 ± 2.73%; $p < 0.05$) but not significantly different from that produced by $PGF_{2\alpha}$ alone (−32.94 ± 3.06%). Table 8 shows that the significant inhibition ($p < 0.01$) seen with bradykinin and $PGF_{2\alpha}$ (−22.74 ± 4.93%) at intermediate control transfer was not significantly different from the inhibition seen with $PGF_{2\alpha}$ alone (−15.70 ± 4.35%) but was significantly different from bradykinin (−3.94 ± 5.37%; $p < 0.01$). At low control transfer the significant stimulation of water seen with bradykinin (+30.99 ± 3.46%; $p < 0.05$) was converted to a significant inhibition of water transfer (−27.33 ± 5.93%; $p < 0.05$) by $PGF_{2\alpha}$ which alone produced a non significant stimulation of water transfer (+ 13.96 ± 6.00%) at this level of control transfer. The inhibition seen with bradykinin and $PGF_{2\alpha}$ was significantly different from the effects seen with bradykinin ($p < 0.001$) and $PGF_{2\alpha}$ ($p < 0.001$) alone.

TABLE 8 The effect of prostaglandin $F_{2\alpha}$, 5.65×10^{-5}M, and bradykinin, 7.86×10^{-12}M, on water transfer in rat jejunal sacs. Degrees of significance for each set of results are expressed as in Fig. 1. The numbers of observations are given in parentheses.

	Level of Mucosal Water Transfer (ml/g wet wet.hr.)		
	Low	Intermediate	High
Control	0.407 ± 0.038	0.761 ± 0.033	1.163 ± 0.023
Bradykinin	0.534 ± 0.054* (7)	0.731 ± 0.056n.s. (13)	0.943 ± 0.036**** (5)
Control	0.469 ± 0.052	0.748 ± 0.022	1.223 ± 0.063
$PGF_{2\alpha}$	0.535 ± 0.066n.s. (5)	0.622 ± 0.028*** (19)	0.813 ± 0.015**** (5)
Control	0.443 ± 0.039	0.806 ± 0.033	1.146 ± 0.032
Bradykinin + $PGF_{2\alpha}$	0.296 ± 0.032* (5)	0.625 ± 0.044*** (14)	0.804 ± 0.082*** (5)

(C) Role of Cyclic AMP in the Bradykinin Action on Water Transfer

Cyclic AMP is known to affect intestinal transfer of sodium and water (Field, 1974; Kimberg, 1974). Bearing in mind the above results suggesting an interaction between bradykinin and prostaglandins, and the knowledge that prostaglandins increase adenyl cyclase activity in intestinal mucosa (Kimberg, Field, Johnson, Henderson & Gershon, 1974; Sharp & Hynie, 1971) it was considered pertinent to investigate the role of cyclic AMP in the inhibitory effect of bradykinin on water transfer. In order to do this use was made of Theophylline, a methyl xanthine derivative which is known to inhibit the enzyme which breaks down cyclic AMP, phosphodiesterase (Butcher & Sutherland, 1962). Both cyclic AMP and its dibutyryl derivative were also used. The dibutyryl derivative was used because of its greater resistance to degradation by phosphodiesterase and its greater ability to penetrate membranes than cyclic AMP itself (Posternak, Sutherland & Merion, 1962).

(i) Effect of Theophylline on Water Transfer

The effect of theophylline at concentrations of 0.1, 1.0 and 10.0 mM on water transfer is shown in Table 9. Theophylline, 10mM, inhibited water transfer at high (-73.4%), intermediate (-61.1%) and low (-30.8%) control transfer, while theophylline 1mM and 0.1mM inhibited transfer at high control transfer (-39.9% and -32.5% respectively) but had no significant effects at low and intermediate control transfer. At high control transfer there was a dose-related effect.

(ii) Effect of Theophylline on the Bradykinin Action on Water Transfer

At low levels of control transfer the stimulation produced by bradykinin, 7.86×10^{-12}M, (+33.68 ± 12.79%) was converted to an inhibition (-14.38 ± 1.66%) by a concentration of theophylline, 1mM, which alone was

TABLE 9 The effect of theophylline on water transfer in rat jejunal sacs. Degrees of significance for each set of results are expressed as in Fig. 1. The numbers of observations are given in parentheses.

	Level of Mucosal Water Transfer (ml/g wet wet.hr.)		
	Low	Intermediate	High
Control	0.487 ± 0.064	0.825 ± 0.073	1.240 ± 0.091
Theophylline (0.1mM)	0.603 ± 0.131n.s. (6)	0.704 ± 0.128n.s. (4)	0.837 ± 0.122* (5)
Control	0.471 ± 0.034	0.797 ± 0.032	1.210 ± 0.081
Theophylline (1mM)	0.468 ± 0.029n.s. (6)	0.648 ± 0.036n.s. (10)	0.728 ± 0.045**** (6)
Control	0.448 ± 0.086	0.748 ± 0.055	1.033
Theophylline (10mM)	0.300 ± 0.043n.s. (3)	0.291 ± 0.043**** (5)	0.275 (1)

TABLE 10 The effect of theophylline, 1mM, and bradykinin, 7.86×10^{-12}M, on water transfer in rat jejunal sacs. Degrees of significance for each set of results are expressed as in Fig. 1. The numbers of observations are given in parentheses.

	Level of Mucosal Water Transfer (ml/g wet wt.hr.)		
	Low	Intermediate	High
Control	0.491 ± 0.033	0.725 ± 0.025	1.152 ± 0.046
Bradykinin	0.653 ± 0.063* (6)	0.768 ± 0.051n.s. (10)	0.978 ± 0.019*** (5)
Control	0.471 ± 0.034	0.797 ± 0.032	1.210 ± 0.081
Theophylline	0.468 ± 0.029n.s. (6)	0.648 ± 0.036n.s. (10)	0.728 ± 0.045**** (6)
Control	0.517 ± 0.033	0.823 ± 0.023	1.148 ± 0.061
Bradykinin + Theophylline	0.442 ± 0.029* (5)	0.545 ± 0.032**** (10)	0.612 ± 0.037**** (5)

without effect (-2.65 ± 2.42%; Table 10). Bradykinin and theophylline produced a significant inhibition of water transfer (-33.45 ± 3.17%) at intermediate levels of control transfer which was significantly greater ($p < 0.01$) than that produced by theophylline alone (-17.81 ± 4.30%). At high control transfer bradykinin and theophylline produced a significant inhibition of water transfer (-45.95 ± 5.32%) which was significantly greater than that produced by bradykinin (-14.48 ± 3.63%) but not significantly different from that produced by theophylline (-38.64 ± 4.43%).

(iii) Effect of Cyclic AMP on Water Transfer

Cyclic AMP (1mM) which was present only in the serosal solution inhibited water transfer at high control water transfer (-23.4%) but no significant effect was seen at intermediate transfer levels. Insufficient observations were made at low control transfer but there did not appear to be any effect of cyclic AMP (Table 11a)

(iv) Effect of Dibutyryl Cyclic AMP on Water Transfer and on Bradykinin Action on Water Transfer

In this experiment the effect of dibutyryl cyclic AMP, alone or in combination with bradykinin, on water transfer was investigated (Table 11b). Bradykinin, 7.86×10^{-12}M, was present in both mucosal and serosal solutions while dibutyryl cyclic AMP was only present in the serosal solution.

Dibutyryl cyclic AMP inhibited water transfer significantly when the control transfer was low (-19.65 ± 2.69%), intermediate (-13.49 ± 5.19%) or high (-13.62 ± 2.73%). At low levels of control transfer bradykinin and dibutyryl cyclic AMP together stimulated transfer by a smaller amount (+15.31 ± 2.47%) than that produced by bradykinin alone (+28.37 ± 8.45%) and at intermediate levels of control transfer produced an inhibition of water transfer (-28.59 ± 3.05%) which was significantly greater ($p < 0.01$) than that produced by dibutyryl cyclic AMP alone (-13.49 ± 5.19%). When

TABLE 11 The effect of (a) cyclic AMP, 1mM, and (b) dibutyryl cyclic AMP, 1mM, and bradykinin, 7.86×10^{-12}M, on water transfer in rat jejunal sacs. Degrees of significance are expressed as in Fig. 1. The numbers of observations are given in parentheses.

	Level of Mucosal Water Transfer (ml/g wet wt.hr.)		
	Low	Intermediate	High
(a)			
Control	0.583 ± 0.026	0.819 ± 0.041	1.134 ± 0.052
Cyclic AMP	0.564 ± 0.103 (2)	0.826 ± 0.046n.s. (7)	0.868 ± 0.043*** (6)
(b)			
Control	0.500 ± 0.033	0.704 ± 0.023	1.123 ± 0.034
Bradykinin	0.636 ± 0.051* (8)	0.699 ± 0.056n.s. (12)	0.929 ± 0.035*** (6)
Control	0.548 ± 0.021	0.801 ± 0.033	1.094 ± 0.046
Dibutyryl cyclic AMP	0.438 ± 0.026*** (5)	0.675 ± 0.023*** (16)	0.942 ± 0.022*** (6)
Control	0.525 ± 0.026	0.811 ± 0.023	1.147 ± 0.043
Bradykinin + dibutyryl cyclic AMP	0.606 ± 0.023 (4)	0.579 ± 0.034**** (14)	0.758 ± 0.036*** (7)

control transfer was high bradykinin and dibutyryl cyclic AMP produced an inhibition of water transfer (-33.56 ± 2.99%) which was greater than that produced by bradykinin (-17.02 ± 3.00%) or dibutyryl cyclic AMP (-15.62 ± 2.73%) separately.

SECTION 11 SMOOTH MUSCLE CONTRACTION

The experiments described above suggested an involvement of prostaglandins and cyclic AMP in the inhibitory actions of bradykinin upon sodium and water transfer in the rat jejunum. It was decided to extend the investigation and to consider whether it was possible to observe such an involvement in the action of bradykinin on smooth muscle contractility. The longitudinal smooth muscle of the rat terminal ileum is sensitive to bradykinin and responds with a slow contraction.

In all these experiments acetylcholine was used as a control agonist. The contractile response of smooth muscle to acetylcholine is thought to be the result of an increase in passive sodium permeability (Bolton, 1972). Thus it was hoped that any non specific effects of the drugs used (apart from hyoscine) on the ability of the smooth muscle to contract would show up as a reduction of the acetylcholine-induced contractions.

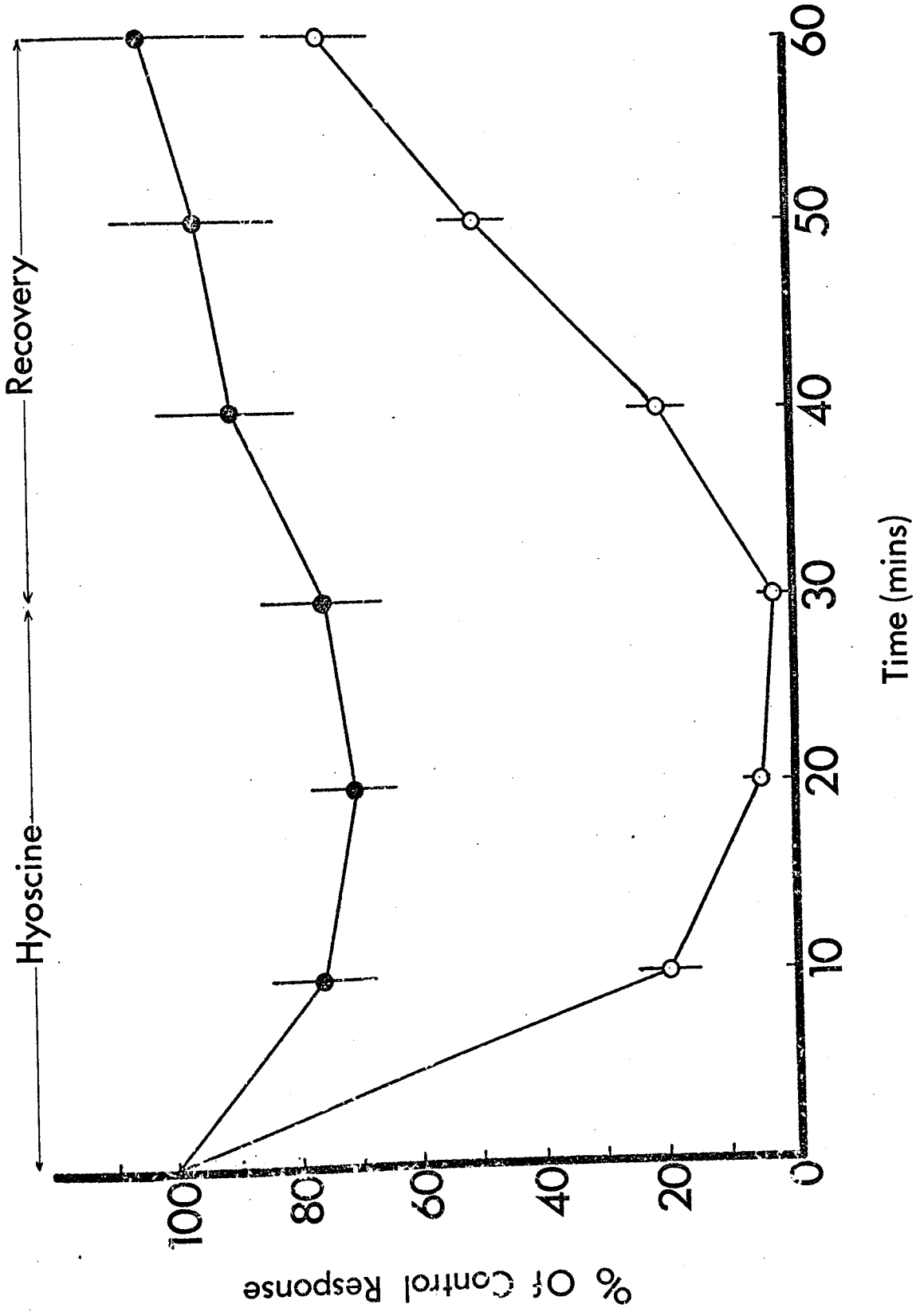
(A) Experiments to Investigate Possible Cholinergic Nervous Mediation of Bradykinin-Induced Contraction of Rat Terminal Ileum

The contractile action of bradykinin has been reported to be due to a direct action on the smooth muscle rather than via an action on nerves within the tissue (Day & Vane, 1963; Khairallah & Page, 1961;1963). It was the purpose of this part of the investigation to consider whether such a conclusion could be drawn concerning the contractile action of bradykinin on the longitudinal muscle of the rat terminal ileum.

(i) Effect of Hyoscine on the Responses of Rat Terminal Ileum to Acetylcholine and Bradykinin

The effect of hyoscine, 2.28μM, on the contractile responses of rat terminal ileum to acetylcholine and bradykinin can be seen in Fig 2. Incubation of the preparation with hyoscine caused a reduction of the acetylcholine-induced contractions to 20.3 ± 4.5% (n = 10) of the control response after 10 minutes, and virtual abolition of the responses after

Fig. 2 The effect of hyoscine, 2.28μM, upon contractile responses of rat terminal ileum to acetylcholine (O) and bradykinin (●). Graph shows mean % of control response with the standard error of the mean.



20 minutes (4.9 ± 2.1%; n=10) and 30 minutes (1.4 ± 0.1%; n=10). The responses of rat terminal ileum to bradykinin were only reduced to 76.4 ± 8.5% (n=10) of the control after 10 minutes incubation with hyoscine, 71.0 ± 7.1% (n=10) after 20 minutes incubation and 75.7 ± 10.7% (n=10) after 30 minutes. There was a highly significant difference ($p < 0.001$) between the responses of the two agonists at 10, 20 and 30 minutes incubation. On removal of hyoscine the responses to bradykinin quickly recovered to 90.6 ± 11.0% (n=10) after 10 minutes, 95.3 ± 12.8% (n=10) after 20 minutes and 104.6 ± 17.2% (n=10) after 30 minutes while acetylcholine was slower in recovering (21.4 ± 4.1%; n=10, after 10 minutes; 50.1 ± 4.8%; n=10, after 20 minutes and 75.2 ± 8.7%; n=10, after 30 minutes.

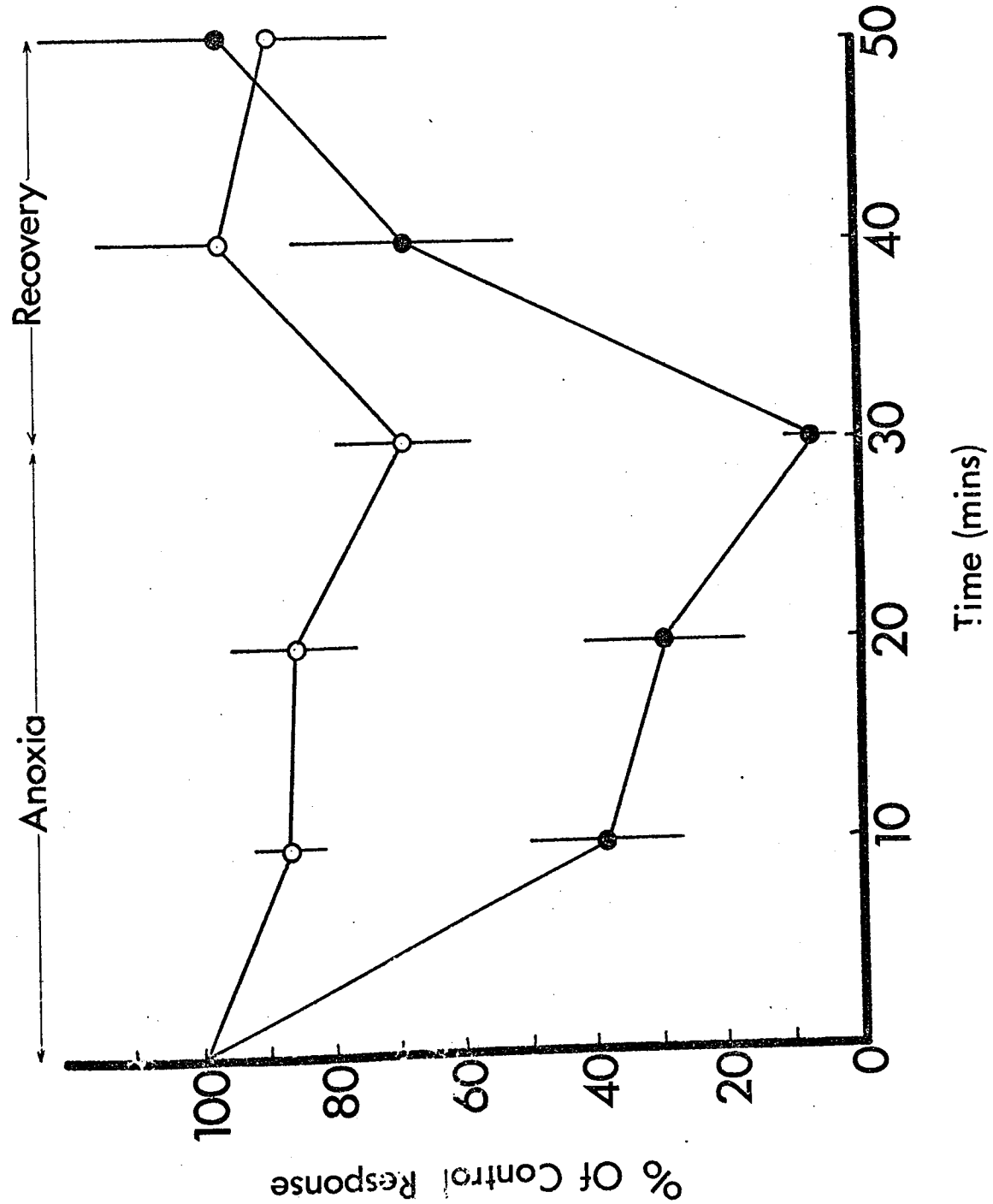
(B) Experiments Involving the use of Metabolic Inhibition

The metabolic requirments for the contraction of smooth muscle have been extensively studied (Coe, Detar & Bohr, 1968; Furchgott & Shorr, 1948; Prasad, 1935a,b;) However, the metabolic requirements of individual spasmogens has not been well investigated. The involvement of secondary messengers such as prostaglandins or cyclic AMP in the contractile action of bradykinin would probably have a metabolic requirement in addition to that of the contractile process itself.

(i) Effect of Anoxia on Responses of Rat Terminal Ileum to Acetylcholine and Bradykinin

Anoxia, which was induced by replacement of air by nitrogen as the gassing agent, caused a dramatic reduction of the bradykinin-induced responses to 38.2 ± 11.6% (n=8) at 10 minutes, 29.0 ± 12.4% (n=8) at 20 minutes and 7.4 ± 2.6% (n=8) at 30 minutes while the responses to acetylcholine were significantly less affected at each time (86.4 ± 4.8%, n=8, $p < 0.02$, at 10 minutes; 85.6 ± 8.9, n=8, $p < 0.01$ at 20 minutes; 68.7 ± 10.7%, n=8, $p < 0.001$ at 30 minutes, Fig 3). Preliminary observations using 2,4 dinitrophenol, an uncoupler of oxidative phosphorylation, suggest that bradykinin-induced contractions of rat terminal ileum are considerably more dependent on oxidative metabolism than are those of acetylcholine.

Fig. 3 The effect of nitrogen-induced anoxia upon contractile responses of rat terminal ileum to acetylcholine (0) and bradykinin (●). Graph shows mean % of control response with the standard error of the mean.



(c) Role of Cyclic AMP in the Contractile Action of Bradykinin

(i) Effect of Theophylline on Contractile Responses of Rat Terminal Ileum to Acetylcholine and Bradykinin

Theophylline was used to investigate if there was an involvement of cyclic AMP in the action of bradykinin on rat terminal ileum. It can be seen from Fig 4 that exposure of the rat terminal ileum to theophylline, 1mM, caused a partial reduction of the contractile responses to acetylcholine to 75.86 ± 7.45% (n=7) after 10 minutes, 58.27 ± 4.34% (n=7) after 30 minutes and 69.07 ± 7.96% (n=7) after 50 minutes. However, bradykinin-induced responses were reduced to 27.66 ± 10.91% (n=7) after 10 minutes, 27.92 ± 8.20% (n=8) after 30 minutes and 27.25 ± 7.79% (n=8) after 50 minutes. Responses to acetylcholine and bradykinin were significantly different ($p < 0.01$ in each case) at 10, 30 and 50 minutes. Recovery of responses to both acetylcholine and bradykinin was rapid after removal of theophylline, 109.94 ± 5.88% (n=7) and 94.76 ± 5.85% (n=8) respectively after 10 minutes.

Theophylline, 10mM, caused complete abolition of both acetylcholine and bradykinin contractile responses within 5 minutes of exposure of the tissue to the drug (Fig 5). However, recovery on removal of theophylline was rapid, the responses to acetylcholine and bradykinin after 10 minutes being 114.33 ± 2.83% (n=6) and 95.09 ± 9.24% (n=4) respectively.

Fig. 4 The effect of theophylline, 1mM, upon contractile responses of rat terminal ileum to acetylcholine (O) and bradykinin (●). Graph shows mean % of control response with the standard error of the mean.

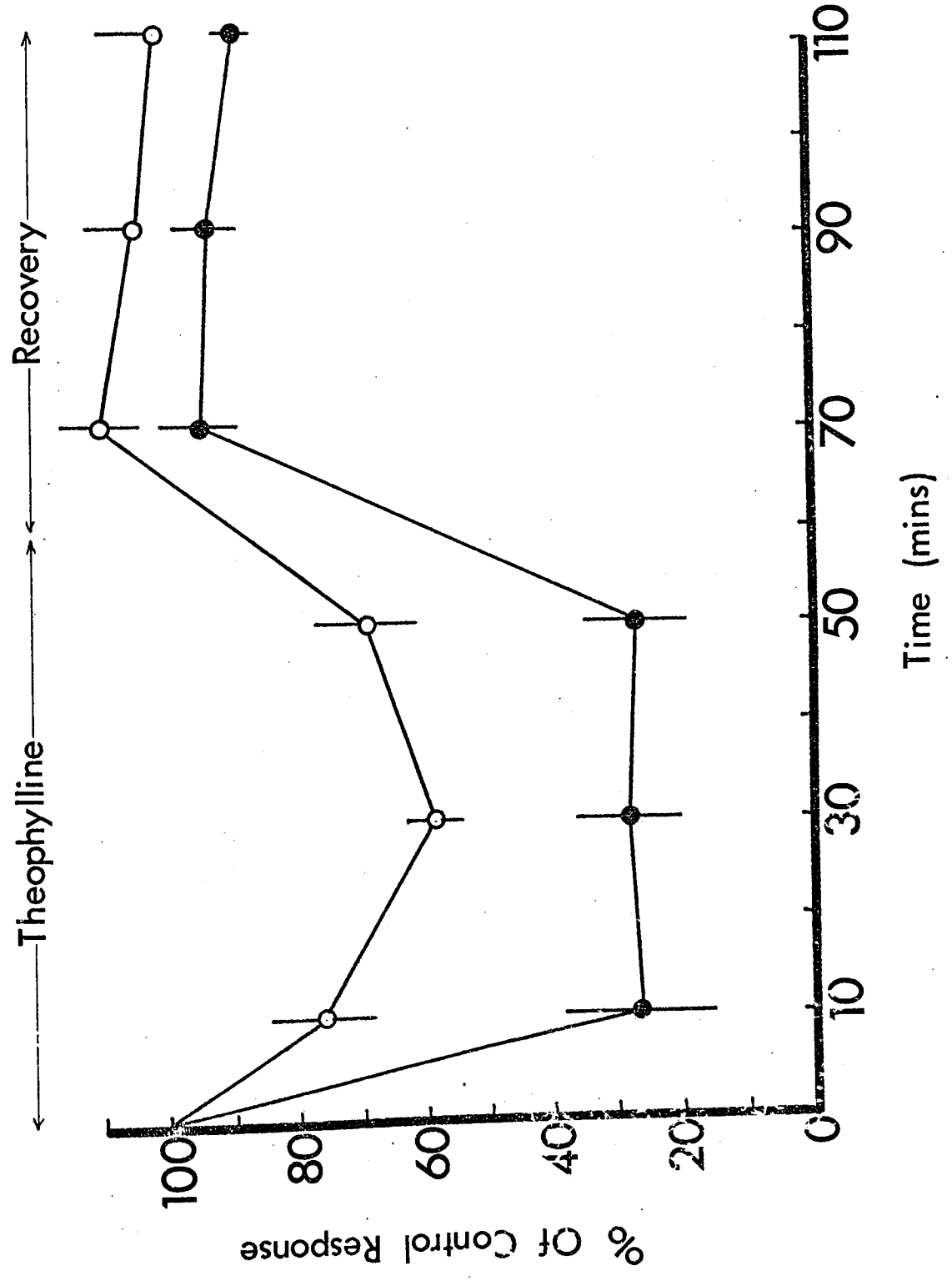
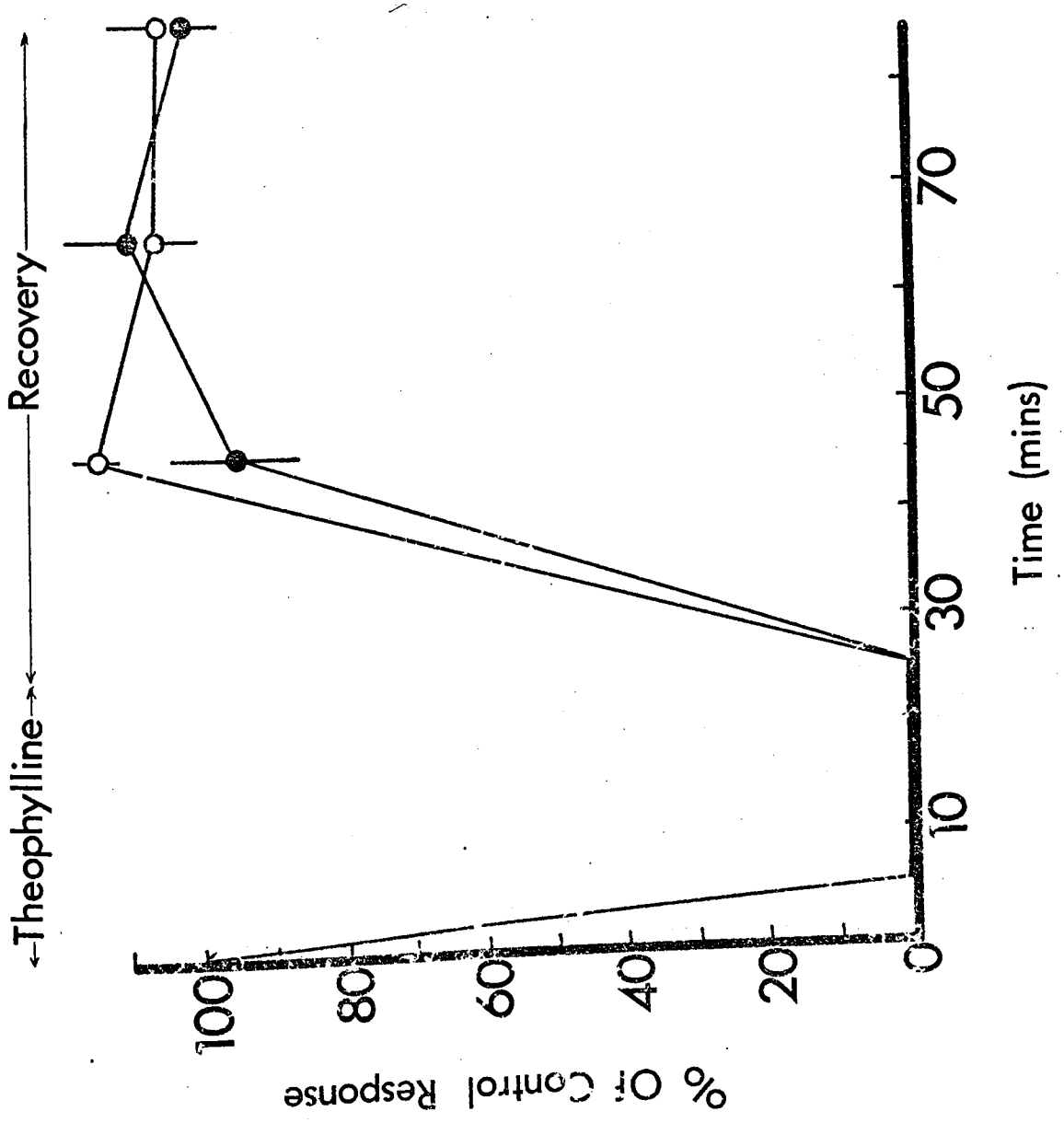


Fig. 5 The effect of theophylline, 10mM, upon contractile responses of rat terminal ileum to acetylcholine (0) and bradykinin (●). Graph shows mean % of control response with the standard error of the mean.



(D) Role of Prostaglandins in the Contractile Action of Bradykinin

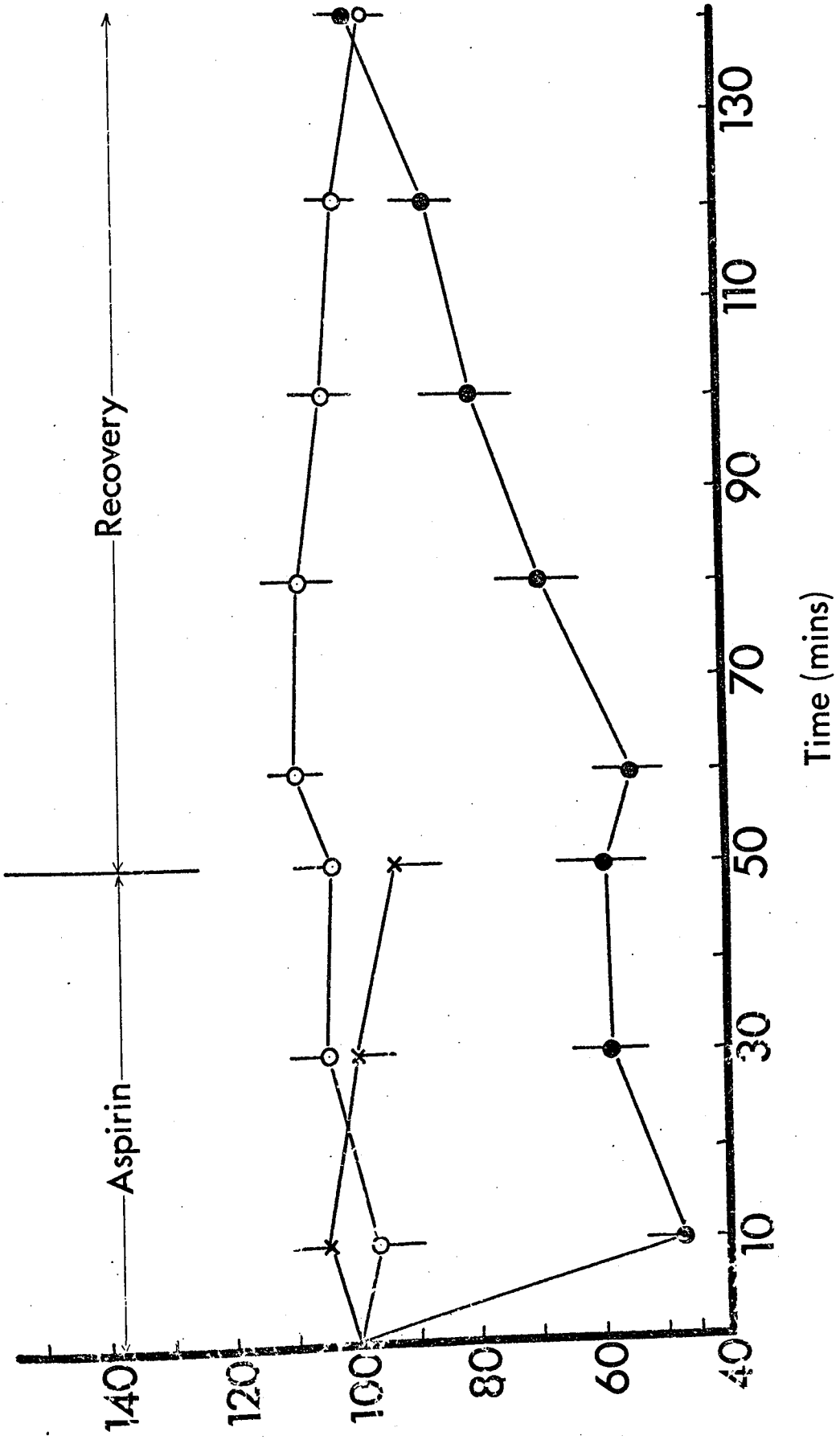
(i) Experiments using Prostaglandin Synthetase Inhibitors

The discovery that aspirin-like drugs specifically inhibit prostaglandin synthetase (Vane, 1971) was of major importance and has provided a simple yet reliable method of investigating the possible involvement of prostaglandins in a variety of situations. In the present experiments use was made of four such inhibitors of prostaglandin synthetase, namely aspirin, indomethacin, meclofenamic acid and flufenamic acid, in concentrations which have been reported to be effective in inhibiting the enzyme (see review by Flower, 1974 for references). In these experiments either PGE_2 or both PGE_2 and $PGF_{2\alpha}$ were also used as agonists in addition to acetylcholine and bradykinin in order to assess the specificity of the prostaglandin synthetase inhibitors used since it has been reported that they can affect other enzymes (see Flower, 1974) as well as directly affecting prostaglandin receptors (Collier & Sweatman, 1968).

(a) Effect of Aspirin on Responses of Rat Terminal Ileum to Acetylcholine, Bradykinin and PGE_2

Exposure of preparations of rat terminal ileum to Tyrode solution containing 610μM aspirin had no effect on the ability of the preparation to respond to acetylcholine (Fig 6), the contractions produced by this agonist being 95.79 ± 7.68% (n=10) of the control contraction after 10 minutes, 105.12 ± 6.23% (n=14) after 30 minutes and 101.94 ± 6.17% (n=13) after 50 minutes. In contrast bradykinin-induced contractions in the presence of aspirin were reduced to 47.64 ± 5.97% (n=12) after 10 minutes, 57.84 ± 6.13% (n=13) after 30 minutes and 57.99 ± 7.17% (n=14) after 50 minutes. Thus the inhibitory action of aspirin on bradykinin responses did not appear to be progressive. In the presence of aspirin the PGE_2-induced contractions were 104.2 ± 5.8% (n=10) after 10 minutes, 98.8 ± 6.1% (n=10) after 30 minutes and 92.8 ± 8.3% (n=10) after 50 minutes. There was a highly significant difference ($p < 0.001$) between the contractile

Fig. 6 The effect of aspirin, 610μM, upon contractile responses of rat terminal ileum to acetylcholine (O) and bradykinin (●) and PGE_2 (X). Graph shows mean % of control response with the standard error of the mean.



responses to both acetylcholine and PGE_2 and those to bradykinin at 10, 30 and 50 minutes.

Removal of aspirin did not affect significantly acetylcholine-induced responses of rat ileum but those of bradykinin showed a gradual recovery. However there was still a significant difference ($p < 0.02$) between the responses of rat terminal ileum to acetylcholine and bradykinin 70 minutes after removal of aspirin. After 90 minutes the bradykinin responses had recovered to 97.7 ± 4.2% (n=7) of control contractions, a value which was not significantly different from the responses to acetylcholine.

Aspirin occasionally caused a reduction of tone in 12 out of 22 tissues.

(b) Effect of Indomethacin on Contractile Responses of Rat Terminal Ileum to Acetylcholine, Bradykinin PGE_2

The presence of 2.8μM indomethacin in the Tyrode solution bathing rat terminal ileum caused a slight depression of both acetylcholine (88.66 ± 4.48% of control contraction, n=10) and bradykinin (78.85 ± 4.73%, n=10) responses after 10 minutes incubation (Fig 7). There was no significant difference between the responses to both agonists. The responses of terminal ileum to bradykinin were reduced to 84.31 ± 5.44%, (n=10) at 30 minutes and 74.35 ± 7.26% (n=9) at 50 minutes, therefore the depression of these responses was not progressive. Acetylcholine-induced contractions recovered to normal values after 30 minutes incubation (104.47 ± 7.17%; n=9) and were slightly bigger at 50 minutes (119.85 ± 7.16%; n=10). There were significant differences between acetylcholine and bradykinin induced responses at 30 minutes ($p < 0.05$) and 50 minutes ($p < 0.001$). It was observed that responses of terminal ileum to bradykinin gradually recovered on removal of indomethacin, reaching 99.40 ± 8.63% (n=9) after 50 minutes. Responses to acetylcholine remained slightly bigger during the recovery phase so that they were 117.15 ± 6.59% (n=9) after 50 minutes.

Fig. 7 The effect of indomethacin, 2.8μM, upon contractile responses of rat terminal ileum to acetylcholine (O) and bradykinin (●). Graph shows mean % of control response with the standard error of the mean.

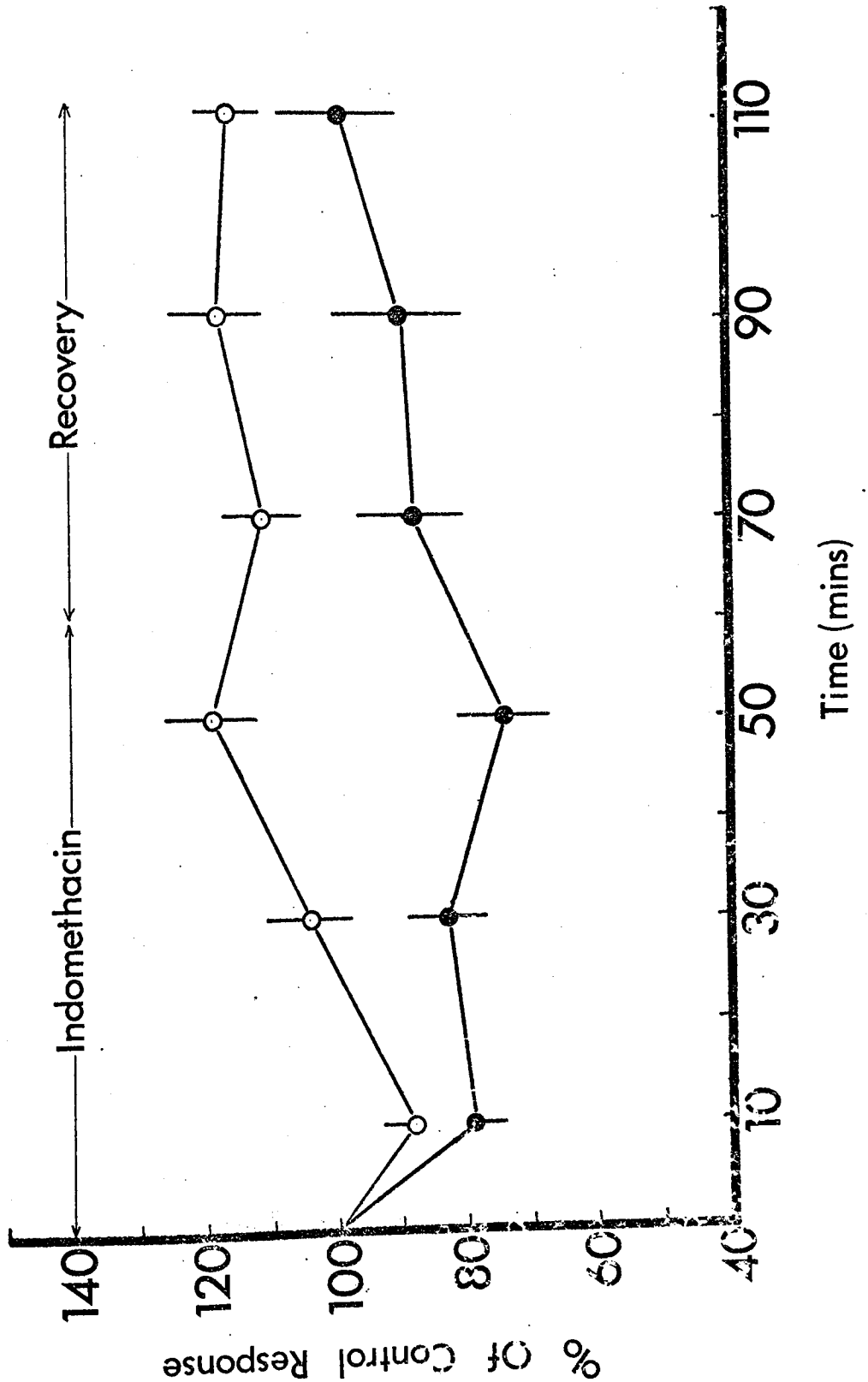
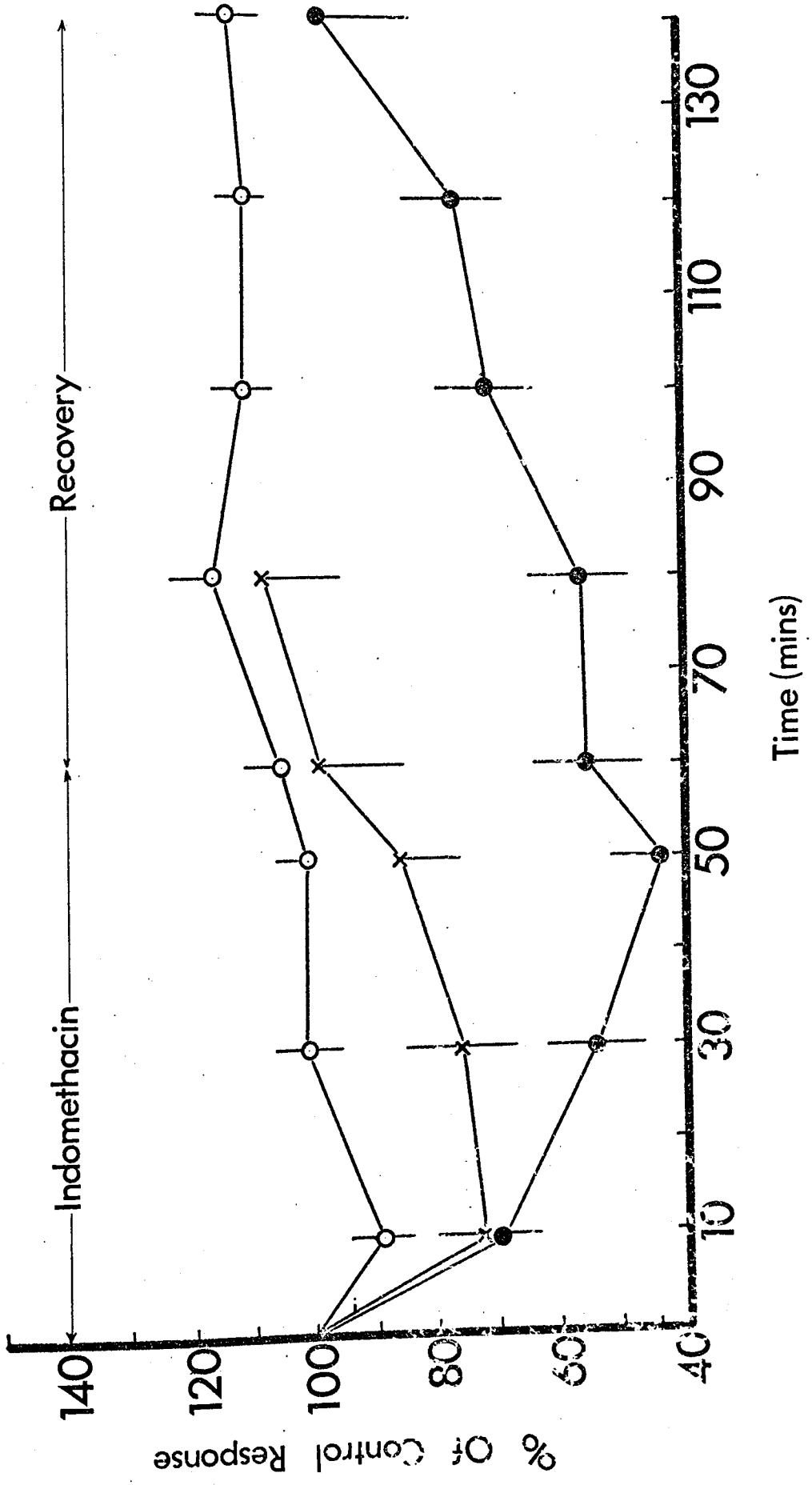


Fig. 8 The effect of indomethacin, 28.0μM, upon contractile responses of rat terminal ileum to acetylcholine (○), bradykinin (●), and PGE_2 (X). Graph shows mean % of control response with the standard error of the mean.



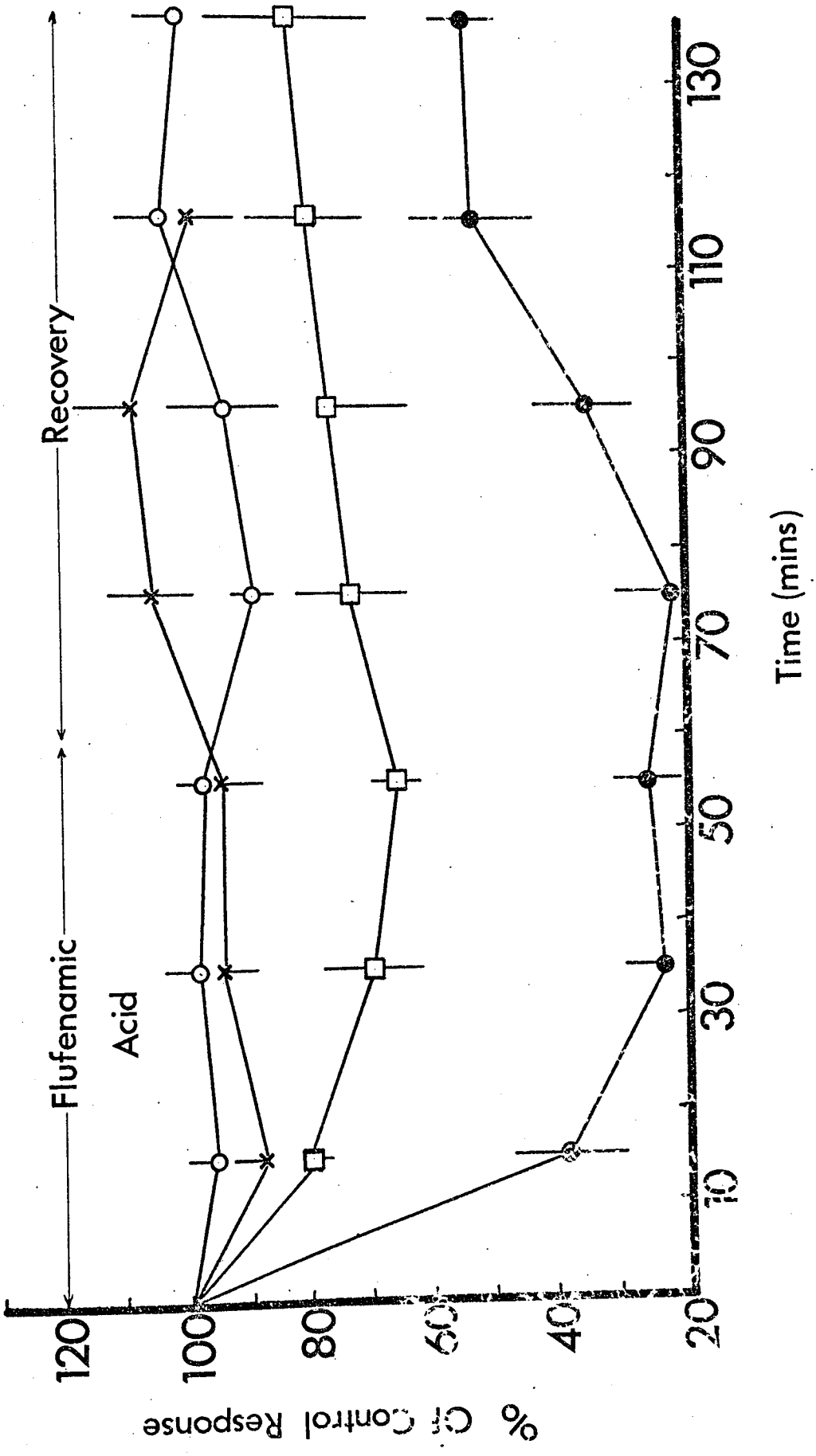
Exposure of rat terminal ileum to 28.0μM indomethacin had no significant effect on the contractile responses of the tissue to acetylcholine (Fig 8) which were 88.47 ± 4.54% (n=14) after 10 minutes, 106.09 ± 5.42% (n=14) after 30 minutes and 105.93 ± 5.08% (n=13) after 50 minutes. In contrast the responses to bradykinin were progressively reduced to 71.02 ± 5.76% (n=13) after 10 minutes, 54.21 ± 7.75% (n=14) after 30 minutes and 42.79 ± 7.28% (n=13) after 50 minutes. In the presence of 28.0μM indomethacin PGE_2 responses were reduced to 69.9 ± 7.9% (n=10) at 10 minutes, 76.2 ± 9.7% (n=10) at 30 minutes and 86.2 ± 13.9% (n=10) at 50 minutes. There were significant differences between acetylcholine and bradykinin responses at 10 minutes ($p < 0.05$), 30 minutes ($p < 0.001$) and 50 minutes ($p < 0.001$) and also between the responses to bradykinin and PGE_2 at 50 minutes ($p < 0.02$). After removal of 28.0μM indomethacin acetylcholine-induced responses were slightly elevated to 113.63 ± 4.49% (n=8) after 90 minutes. The slight depression of $PGE_{2\alpha}$ induced contractions recovered rapidly on removal of indomethacin (98.8 ± 13.9%, n=10, after 10 minutes) whereas the depression of bradykinin-induced responses recovered only gradually until the responses were 98.15 ± 15.12% (n=5) after 90 minutes. The difference in the rates of recovery of bradykinin and PGE_2 would suggest that there may be a different inhibitory mechanism operating in each case.

A reduction of tone of the tissue was frequently seen in the presence of indomethacin, particularly when a concentration of 28.0uM was used. Tone was reduced in 20 out of 24 preparations at 28.0μm and 6 out of 11 preparations at 2.8μm.

(c) Effect of Flufenamic Acid on Contractile Responses of Rat Terminal Ileum to Acetylcholine, Bradykinin, PGE_2 and $PGF_{2\alpha}$

Contractile responses of the rat terminal ileum to acetylcholine and PGE_2 were not significantly affected by the incubation of the tissue with Tyrode solution containing 3.6μM flufenamic acid (Fig 9). Acetylcholine-and PGE_2-induced contractions were 96.37 ± 4.02% (n=7) and

Fig. 9 The effect of flufenamic acid, 3.6μM, upon contractile responses of rat terminal ileum to acetylcholine (○), bradykinin (●), PGE_2 (X) and $PGF_{2\alpha}$ (□). Graph shows mean % of control response with the standard error of the mean.



89.14 ± 5.00% (n=10) after 15 minutes, 99.35 ± 4.99% (n=8) and 95.80 ± 5.1% (n=10) after 35 minutes and 96.82 ± 3.64% (n=11) and 96.6 ± 7.8% (n=10) respectively after 55 minutes. Flufenamic acid dramatically reduced contractile responses to bradykinin to 39.79 ± 8.78% (n=6), 23.14 ± 5.60% (n=9) and 26.55 ± 5.33% (n=5) after 15, 35 and 55 minutes respectively. Thus there was a progressive increase in the degree of inhibition of bradykinin responses. $PGF_{2\alpha}$-induced responses were reduced but less so than those of bradykinin. The responses to $PGF_{2\alpha}$ were 81.66 ± 2.96% (n=7), 70.41 ± 8.14% (n=4) and 66.97 ± 3.75% (n=5) after 15, 35 and 55 minutes respectively, and were significantly different from the responses to acetylcholine at 15 ($p < 0.02$), 35 ($p < 0.01$) and 55 ($p < 0.001$) minutes. The contractile responses to bradykinin were highly significantly different ($p < 0.001$) from those of acetylcholine at each of the times tested and they were significantly different from $PGF_{2\alpha}$ and PGE_2 at 15 minutes ($p < 0.001$ and $p < 0.001$), 35 minutes ($p < 0.01$ and $p < 0.001$) and 55 minutes ($p < 0.001$ and $p < 0.001$).

After removal of flufenamic acid responses to bradykinin showed a gradual and partial recovery to 55.24 ± 4.48% (n=5) after 75 minutes while $PGF_{2\alpha}$ contractile responses had recovered to 84.58 ± 13.60% (n=7). Acetylcholine-and PGE_2-induced responses were not affected by the removal of flufenamic acid.

Incubation of the tissue with flufenamic acid was sometimes associated with a reduction of tone, falling in 11 out of 21 preparations.

(d) Effect of Meclofenamic Acid on Contractile Responses of Rat Terminal Ileum to Acetylcholine, Bradykinin, PGE_2 and $PGF_{2\alpha}$

Exposure of the rat terminal ileum to 3.4μM meclofenamic acid had no effect on acetylcholine after 15 minutes (101.94 ± 6.36%, n=14) and caused a slight potentiation of acetylcholine after 35 minutes (113.98 ± 5.96%, n=14) and 55 minutes (113.32 ± 4.52%, n=15). Both $PGF_{2\alpha}$ and PGE_2 were slightly reduced in the presence of meclofenamic acid (Fig 10) to

Fig. 10 The effect of meclofenamic acid, 3.4μM, upon contractile responses of rat terminal ileum to acetylcholine (O), bradykinin (●), PGE_2 (X) and $PGF_{2\alpha}$ (□). Graph shows mean % of control response with the standard error of the mean.

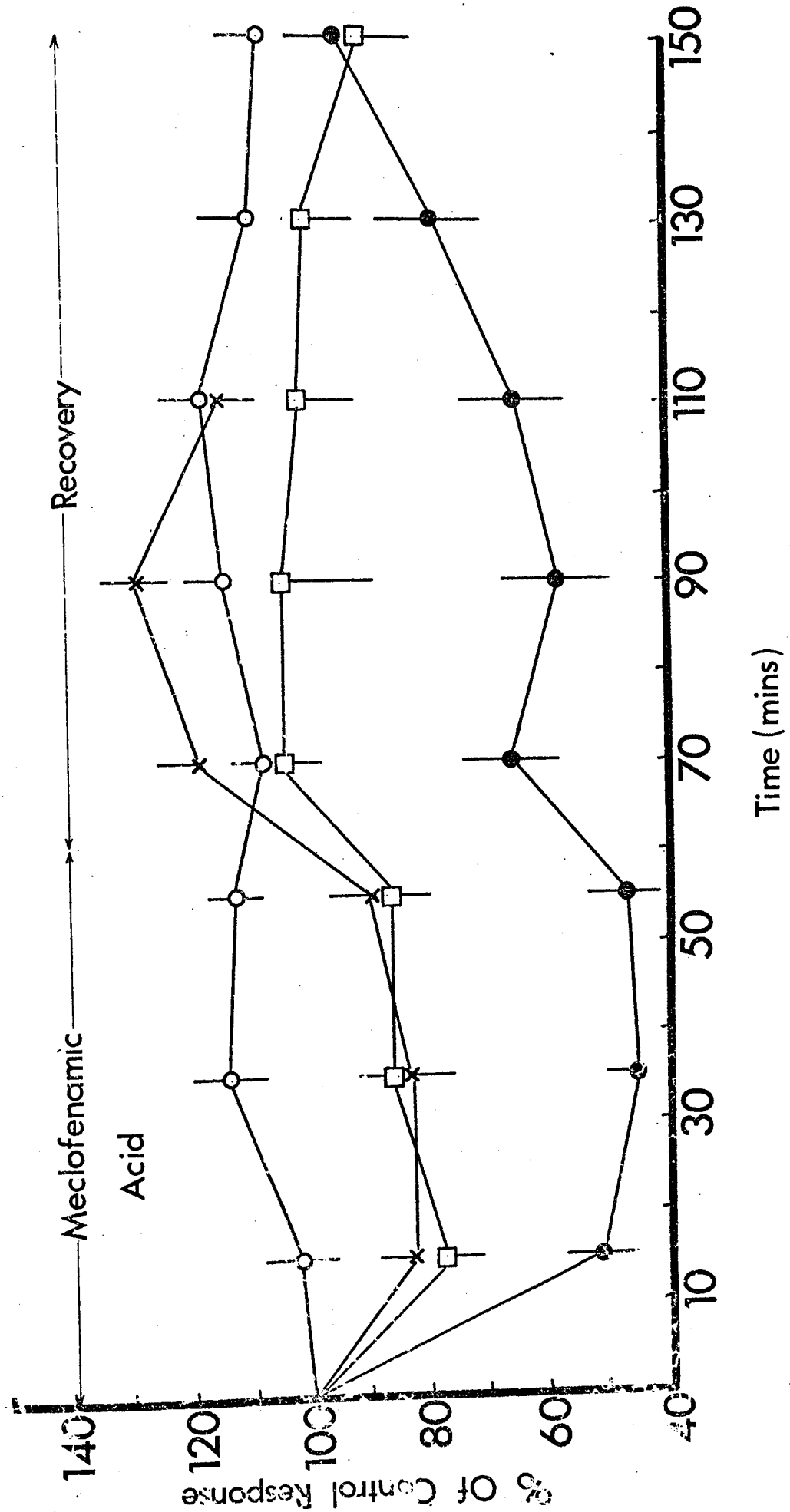
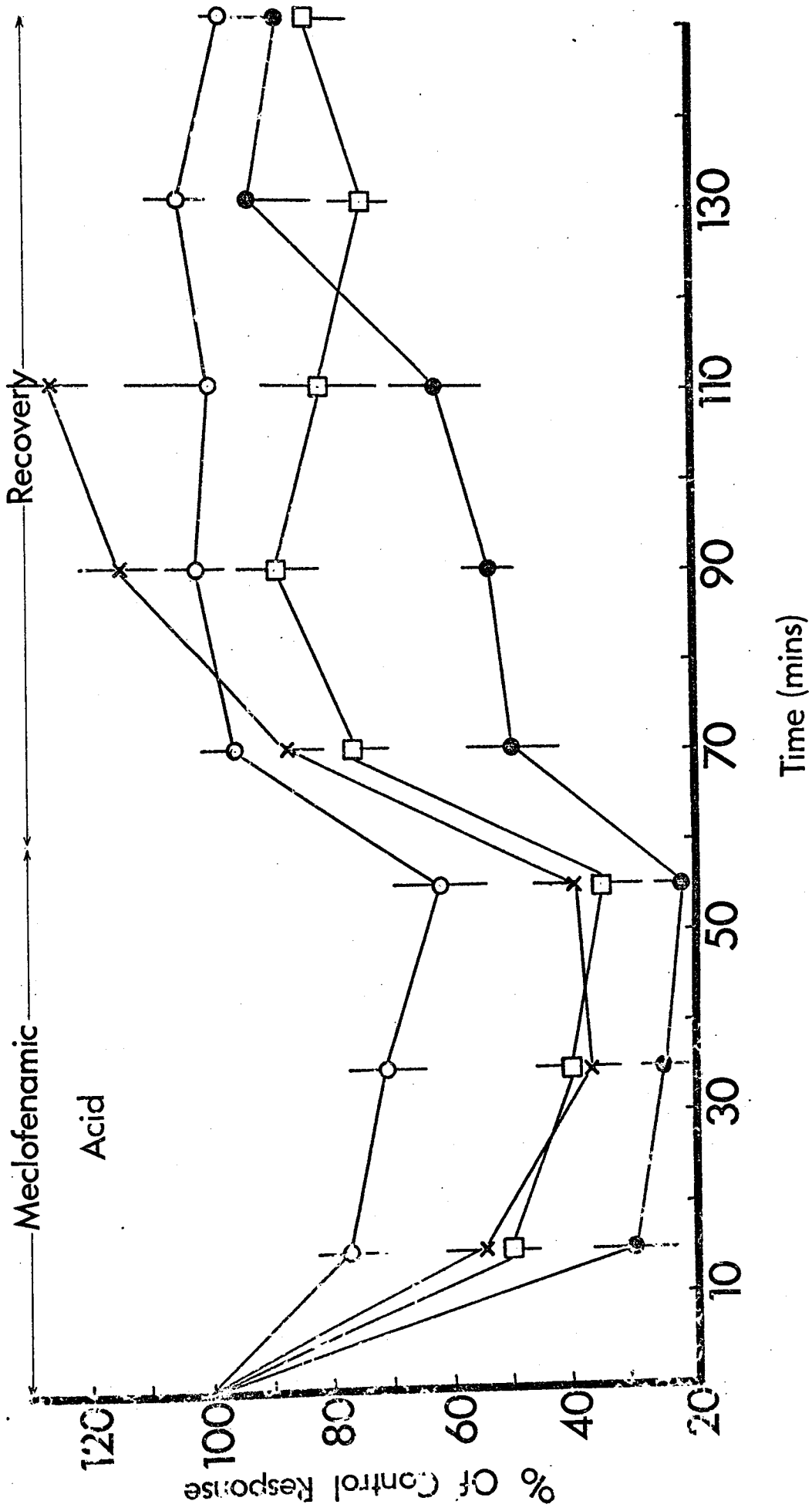


Fig. 11. The effect of meclofenamic acid, 17.0μM, upon contractile responses of rat terminal ileum to acetylcholine (0), bradykinin (●), PGE_2 (X) and $PGF_{2\alpha}$ (□). Graph shows mean % of control response with the standard error of the mean.



77.96 ± 6.35% (n=8) and 83.2 ± 6.1% (n=10) after 15 minutes, 84.75 ± 5.67% (n=7) and 83.6 ± 7.4% (n=10) after 35 minutes and 87.62 ± 6.52% (n=8) and 88.3 ± 7.4% (n=10) after 55 minutes respectively. Thus there was no difference in the responses to PGE_2 and $PGF_{2\alpha}$. It can be seen from Fig IC that bradykinin-induced contractions were markedly inhibited in the presence of 3.4μM meclofenamic acid to 51.47 ± 5.93% (n=15) after 15 minutes, 44.91 ± 4.48% (n=13) after 35 minutes and 46.91 ± 5.76% (n=13) after 55 minutes. The responses to bradykinin were highly significantly different ($p < 0.001$) from those to acetylcholine at each time tested and were significantly different from those to PGE_2 and $PGF_{2\alpha}$ at 15 minutes ($p < 0.02$), 35 minutes ($p < 0.001$) and 55 minutes ($p < 0.001$). On removal of 3.4μM meclofenamic acid there was an immediate recovery of contractile responses to $PGF_{2\alpha}$ (105.82 ± 6.68%, n=6, at 10 minutes) and PGE_2, which was slightly bigger (119.3 ± 7.3%, n=10, after 10 minutes). In contrast the recovery of bradykinin in meclofenamic acid-free-Tyrode solution was gradual, the responses reaching 95.02 ± 8.28% (n=6) after 95 minutes. Acetylcholine-induced contractions were unaffected by removal of meclofenamic acid.

In the presence of 17.0μM meclofenamic acid bradykinin induced contractions of rat terminal ileum were reduced to 30.10 ± 6.93% (n=9) after 15 minutes, 24.86 ± 4.41% (n=10) after 35 minutes and 20.83 ± 3.93% (n=11) after 55 minutes (Fig II) but the responses to acetylcholine were also reduced to 78.01 ± 6.25% (n=13) after 15 minutes, 76.33 ± 6.78% (n=12) after 35 minutes and 62.29 ± 7.91% (n=9) after 55 minutes. Contractile responses to $PGF_{2\alpha}$ and PGE_2 were reduced by 17.0μM meclofenamic acid to 54.79 ± 4.05% (n=11) and 50.2 ± 7.3% (n=10) after 15 minutes, 39.13 ± 5.03% (n=11) and 38.7 ± 6.1% (n=10) after 35 minutes and 35.87 ± 7.57% (n=8) and 40.3 ± 6.7% (n=10) after 55 minutes. There was no significant differences in the effect on PGE_2 and $PGF_{2\alpha}$. The reduction of responses was progressive for all four agonists. The contractile responses to bradykinin were highly significantly different ($p < 0.001$) from those of acetylcholine at 15, 35 and 55 minutes and were significantly

different ($p < 0.05$) from those of $PGF_{2\alpha}$ after 15, 35 and 55 minutes and those of PGE_2 after 55 minutes ($p < 0.05$). On removal of 17.0μM meclofenamic acid from the bathing solution there was a rapid recovery of the responses to acetylcholine (97.10 ± 5.99%, n=15, after 15 minutes), PGE_2 (88.4 ± 6.6%, n=10, after 15 minutes and $PGF_{2\alpha}$ (76.77 ± 6.30%, n=6, after 15 minutes). However the contractile responses to bradykinin recovered much more gradually reaching a value of 87.63 ± 9.21% (n=7) after 95 minutes.

Reduction of tone was often seen in tissues incubated in Tyrode containing meclofenamic acid, especially at a concentration of 17.0μM, when tone was reduced in 21 out of 25 preparations. At 3.4μM tone fell in 14 out of 24 preparations.

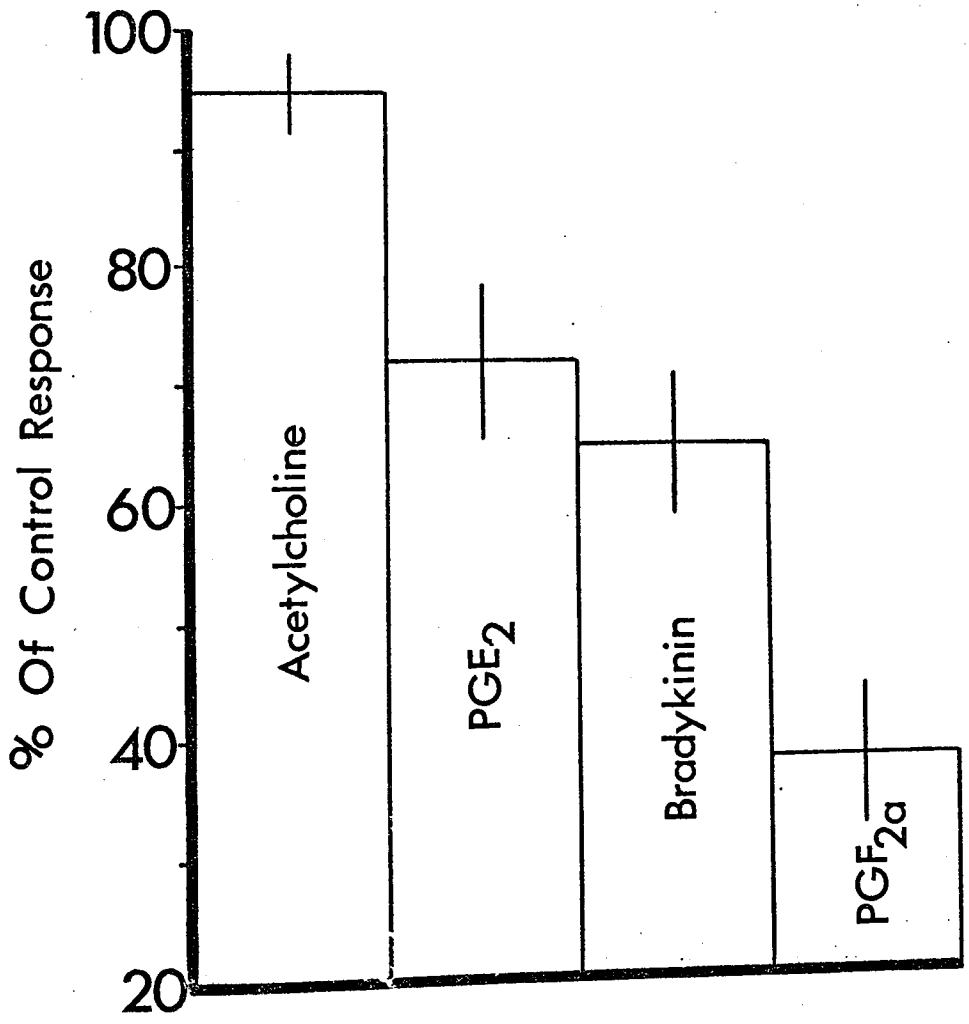
(ii) Experiments using Prostaglandin Receptor Blocking Agents

Several substances have been discovered which prevent the normal interactions of prostaglandins with their receptor sites. There are three main types: namely, polyphloretin phosphate and its derivaties (Eakins, Karim & Miller, 1970), SC 19220 (Sanner, 1969) and 7 oxa-13-prostynoic acid (Fried, Santhana Krishnan, Himizu, Lin, Ford, Rubin & Grigas, 1969). The properties of these prostaglandin receptor blocking agents have been extensively reviewed recently by Bennett (1974). The disocvery of such agents could be of great value for investigating the involvement of prostaglandins in physiological, pharmacological and pathological phenomena. It was hoped that the use of several prostaglandin receptor blocking drugs in the subsequently reported experiments would clarify the results described above.

(a) Effect of Polyphloretin Phosphate on Contractile Responses of Rat Terminal Ileum to Acetylcholine, Bradykinin, PGE_2 and $PGF_{2\alpha}$

Polyphloretin phosphate (PPP), incubated for 2 minutes at a concentration of 10μg/ml, did not affect the contractile response of rat terminal ileum to acetylcholine (94.53 ± 3.63%, n=10) but the response to bradykinin was reduced to 64.82 ± 5.79% (n=8), a value which was

Fig. 12 The effect of polyphloretin phosphate, 10μg/ml, upon contractile responses of rat terminal ileum to acetylcholine, bradykinin, PGE_2 and $PGF_{2\alpha}$. The histogram shows mean % of control response with the standard error of the mean.



significantly different ($p < 0.001$) from that of acetylcholine (Fig 12). $PGF_{2\alpha}$-induced contractions were inhibited (37.06 ± 6.03%, n=11) significantly greater ($p < 0.01$) than those of bradykinin while the response to PGE_2 was inhibited to a value which was not significantly different from that of bradykinin (71.6 ± 6.6%, n=10) but which was significantly different from those of acetylcholine ($p < 0.01$) and $PGF_{2\alpha}$ ($p < 0.001$).

The blocking action of PPP on bradykinin and the prostaglandins was reversible, the agonist responses returning to normal within 15 minutes after its removal. PPP did not consistently affect the tone of the rat terminal ileum.

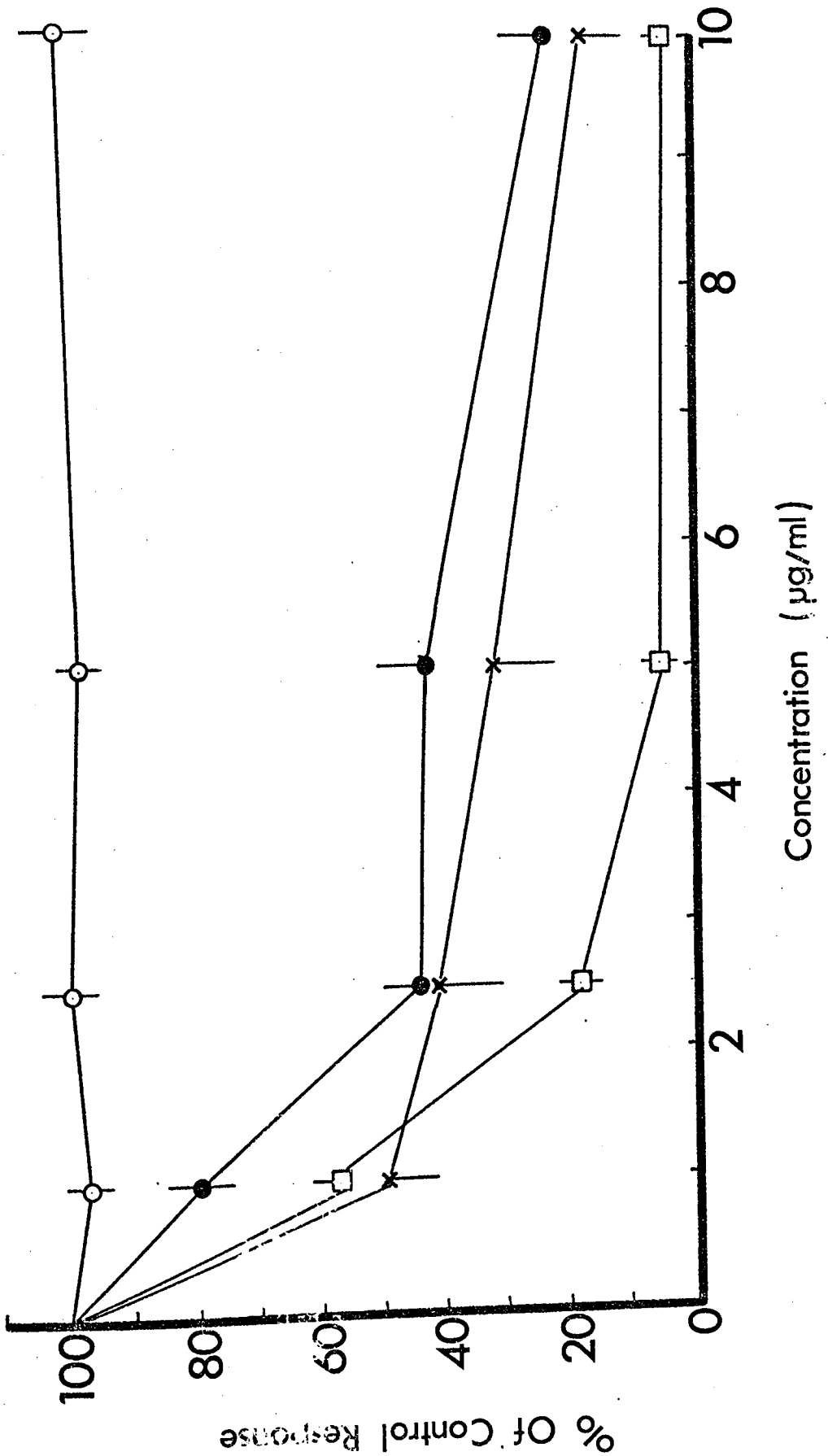
(b) Effect of Diphloretin Phosphate on Contractile Responses of Rat Terminal Ileum to Acetylcholine, Bradykinin, PGE_2 and $PGF_{2\alpha}$

Acetylcholine-induced responses of rat terminal ileum were not affected by a 2 minute exposure of the tissue to diphloretin phosphate (DPP) at 1µg/ml (97.38 ± 3.18%, n=13), 2.5µg/ml (99.87 ± 3.20%, n=16), 5µg/ml (98.49 ± 3.24%, n=14) or 10µg/ml (101.01 ± 4.85%, n=9) (Fig 13).

Bradykinin, $PGF_{2\alpha}$ and PGE_2 induced contractions were significantly reduced, when compared to acetylcholine, in the presence of 1µg/ml DPP to 70.02 ± 4.73 % (n=8), 57.60 ± 4.67% (n=6) and 49.85 ± 7.94% (n=5) respectively. Higher concentrations of DPP progressively reduced the ability of bradykinin and PGE_2 equally to contract the rat terminal ileum; the responses were reduced to 43.89 ± 5.74% (n=7) and 41.47 ± 10.23% (n=5) at 2.5µg/ml; 42.91 ± 7.90% (n=10) and 32.07 ± 9.75% (n=5) at 5.0µg/ml and 23.22 ± 7.14% (n=5) and 16.76 ± 7.46% (n=6) at 10µg/ml respectively. The responses to bradykinin and PGE_2 at 2.5, 5.0 and 10ug/ml were not significantly different from each other but were highly significantly different ($p < 0.001$) from those of acetylcholine at these concentrations.

$PGF_{2\alpha}$-induced contractile responses were reduced to 18.53 ± 3.60% (n=8) at 2.5 µg/ml, 4.95 ± 1.98% (n=6) at 5µg/ml and 3.97 ± 2.36% (n=4) at 10µg/ml. These inhibitions were significantly different from those seen for bradykinin and PGE_2 at 2.5µg/ml ($p < 0.01$ and $p < 0.02$), 5.0µg/ml

Fig. 13 The effect of diphloretin phosphate, at various concentrations, upon contractile responses of rat terminal ileum to acetylcholine (○), bradykinin (●), PGE_2 (X) and $PGF_{2\alpha}$ (□). Graph shows mean % of control response with the standard error of the mean.



($p < 0.001$ and $p < 0.01$) and 10µg/ml ($p < 0.05$ and $p < 0.05$) respectively, and highly significantly different ($p < 0.001$) from those of acetylcholine at each concentration.

Thus it can be seen that $PGF_{2\alpha}$ was more susceptible to the prostaglandin receptor blocking action of DPP than PGE_2. It is also of interest that the inhibition patterns for bradykinin and PGE_2 are very similar, apart from the effect observed with the lowest dose of DPP. DPP did not appear to have a non specific depressant action on the contractile ability of the smooth muscle since acetylcholine responses were not affected. DPP was also seen to be a more potent inhibitor than PPP.

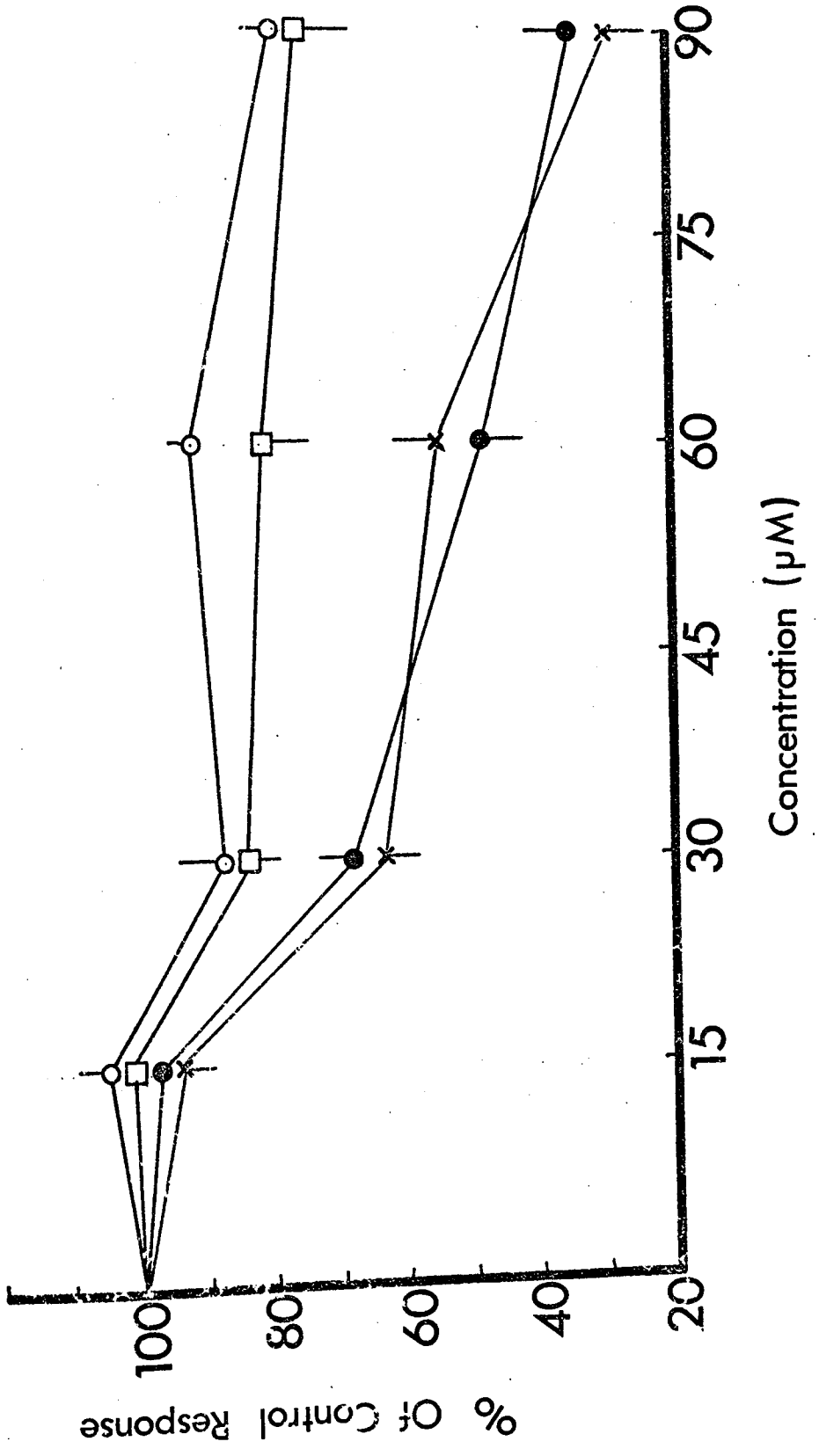
(c) Effect of SC 19220 on Contractions of Rat Terminal Ileum to Acetylcholine, Bradykinin, PGE_2 and $PGF_{2\alpha}$

Exposure of the rat terminal ileum for 10 minutes to 1.51×10^{-5}M SC 19220 did not affect contractile responses to acetylcholine (103.57 ± 3.67%, n=5), bradykinin (100.69 ± 5.04%, n=5), $PGF_{2\alpha}$ (101.09 ± 12.78%, n=6) or PGE_2 (97.49 ± 4.84%, n=5) (Fig 14).

Both acetylcholine-and $PGF_{2\alpha}$-induced contractions of rat terminal ileum were slightly and equally reduced to 87.10 ± 6.62% (n=7) and 84.91 ± 4.47% (n=10) in the presence of 3.02×10^{-5}M SC 19220. At this concentration of receptor blocking agent responses to both bradykinin (68.34 ± 4.95%, n=7) and PGE_2 (63.21 ± 4.75%, n=5) were reduced significantly more than either acetylcholine or $PGF_{2\alpha}$ ($p < 0.05$ in each case). The inhibition of contractile responses to acetylcholine and $PGF_{2\alpha}$ was not increased by exposure of the tissue to 6.03×10^{-5}M (91.87 ± 3.28%, n=15, and 81.36 ± 6.81%, n=9, respectively) or 9.06×10^{-5}M (78.96 ± 3.38%, n=17 and 78.51 ± 8.89%, n=7, respectively. There was no significant difference in the effects of SC 19220 on acetylcholine or $PGF_{2\alpha}$-induced responses at any concentration.

In marked contrast the contractile responses of bradykinin and PGE_2 were progressively more inhibited in the presence of increased

Fig. 14 The effect of SC 19220, at various concentrations, upon contractile responses of rat terminal ileum to acetylcholine (O), bradykinin (●), PGE_2 (X), and $PGF_{2\alpha}$ (□). Graph shows mean % of control response with the standard error of the mean.



SC 19220 concentrations. At 6.03×10^{-5}M SC 19220 the responses to bradykinin and PGE_2 were reduced to 48.49 ± 6.22% (n=10) and 55.54 ± 6.42% (n=7), respectively, both of which were significantly different from the responses to acetylcholine ($p < 0.001$ in each case) and $PGF_{2\alpha}$ ($p < 0.01$ in each case). At 9.06×10^{-5}M SC 19220 the responses to bradykinin and PGE_2 were reduced to 34.54 ± 6.68% (n=10) and 29.34 ± 7.24% (n=8) respectively, both of which were significantly different from the responses to acetylcholine ($p < 0.001$ in each case) and $PGF_{2\alpha}$ ($p < 0.001$ in each case).

Thus it is clear that in this tissue PGE_2 is more susceptible than $PGF_{2\alpha}$ to blockade by SC 19220. It is of interest that the inhibition pattern seen for bradykinin is very similar to that seen for PGE_2. It is clear that there is little non specific depression of contractile ability of the muscle since acetylcholine responses are only reduced partially even at high concentrations of SC 19220. The presence of SC 19220 was not associated with loss of tone in the muscular preparation.

(d) Effect of 7 Oxa-13-Prostynoic Acid on Contractile Responses to Acetylcholine, Bradykinin and PGE_2

The rat terminal ileum was exposed to Tyrode solution containing 6.49×10^{-5}M 7 oxa-13-prostynoic acid and contractile responses to acetylcholine, bradykinin and PGE_2 were reduced to 62.1%, 64.1% and 73.8 % (n=2 in each case) respectively. Unfortunately there was not enough 7 oxa-13-prostynoic acid available for it to be possible to provide adequate quantitative data for this experiment but from the results obtained it would appear that 7 oxa-13-prostynoic acid was having a non specific depressant action on the muscle at the concentration used, since contractile responses to acetylcholine were markedly reduced.

(E) Experiments to Measure and Identify Possible Prostaglandin Release by Rat Terminal Ileum

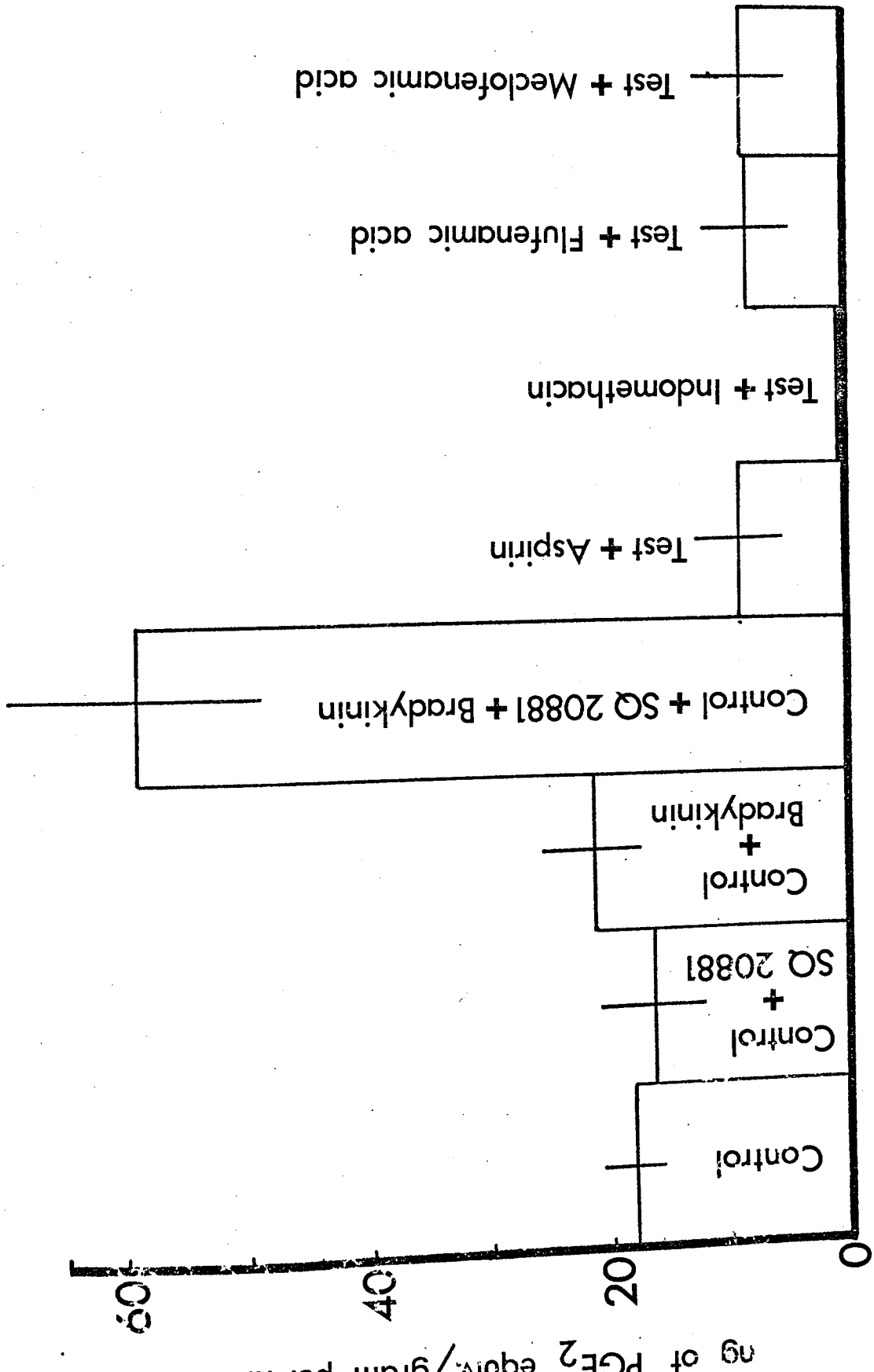
(i) Prostaglandin Bioassay

In view of the findings reported above concerning the experiments performed with several prostaglandin synthetase inhibitors and prostaglandin receptor blocking drugs, it was decided to investigate if prostaglandins were released by rat terminal ileum into the surrounding Tyrode solution before and during the administration of a concentration of bradykinin which would elicit a contractile response. It was also decided to investigate the effect of the previously used prostaglandin synthetase inhibitors upon such a release of prostaglandins.

PGE_2 was used as standard prostaglandin in this bioassay because it has been reported that PGE_2 is the principal prostaglandin in the rat gastrointestinal tract (Collier, 1974) and because of the similarity between bradykinin and PGE_2 action in some of the previous experiments.

The basal output of prostaglandin-like material in the effluent from the organ bath was 17.7 ± 2.7 ng of PGE_2 equivalent gram of tissue hr. (n=17). This was not increased by incubation with 60ng/ml bradykinin (21.2 ± 4.0, n=8) or 1µg/ml SQ 20881 (16.0 ± 4.6, n=10), which is an inhibitor of the kininase enzyme which causes breakdown of bradykinin. However, the presence of both bradykinin and SQ 20881 in the organ bath increased the production of prostaglandin-like material in the effluent to 55.4 ± 10.9 ng equivalents of PGE_2/g of tissue hr (n=17) which was significantly greater ($p < 0.01$) than that seen with the control (i.e. only SQ 20881). It can be seen from Fig 15 that this stimulation of prostaglandin-like material was significantly reduced in the presence of 3.4µM meclofenamic acid (7.56 ± 3.7, n=6, $p < 0.02$), 610µM aspirin (8.6 ± 3.7, n=6, $p < 0.05$) and 3.55µM flufenamic acid (7.21 ± 3.39, n=6, $p < 0.02$). Indomethacin, 28.0µM reduced the release of PG-like material to undetectable levels ($p < 0.01$). These concentrations of prostaglandin synthetase inhibitors were the same as those used previously in the contractile

Fig. 15 The effect of bradykinin (60ng/ml), SQ 20881 (1µg/ml), aspirin (610µM), indomethacin (28.0µM), flufenamic acid (3.55µM) and meclofenamic acid (3.4µM) on the release of prostaglandin-like material from rat terminal ileum.



experiments.

(ii) Characterisation by T.L.C. of Prostaglandin-like Material Released by Rat Terminal Ileum

When chromatographed on silica gel plates the acidic ether extract prepared previously showed three main spots with Rf values 0.36, 0.66 - 0.72 and 0.86 - 0.89 (Fig 16). The most prominent spot was that with Rf value 0.66 - 0.72. A similar pattern of spots was seen in extracts of organ bath fluid taken from tissue incubated with and without bradykinin and SQ 20881 but the spots were more prominent when bradykinin and SQ 20881 were present. These spots did not coincide with those produced by standard prostaglandins which were PGE_1 (0.53), PGE_2 (0.55), $PGF_{2\alpha}$ (0.41) and PGA_1 (0.77).

The extract from tissues incubated with indomethacin 28.0μM, showed a different pattern of spots on TLC plates. There were no spots with Rf values 0.36 or 0.66 - 0.72 but the spot at 0.86 - 0.89 was unaffected. In addition a spot appeared at Rf 0.79 (Fig. 17).

Extraction of organ bath fluid from tissues incubated with indomethacin, 28.0μM, and standard PGE_2 for 1 hour showed three spots. One spot corresponded to PGE_2 (Rf 0.55), and two had similar Rf values to those seen with bradykinin (0.68 - 0.71 and 0.85 - 0.89).

From Fig. 18 it can be seen that 15 keto-prostaglandin E_2 produced a single spot which had the same mobility as the second of the spots seen with the bradykinin extract (Rf 0.69).

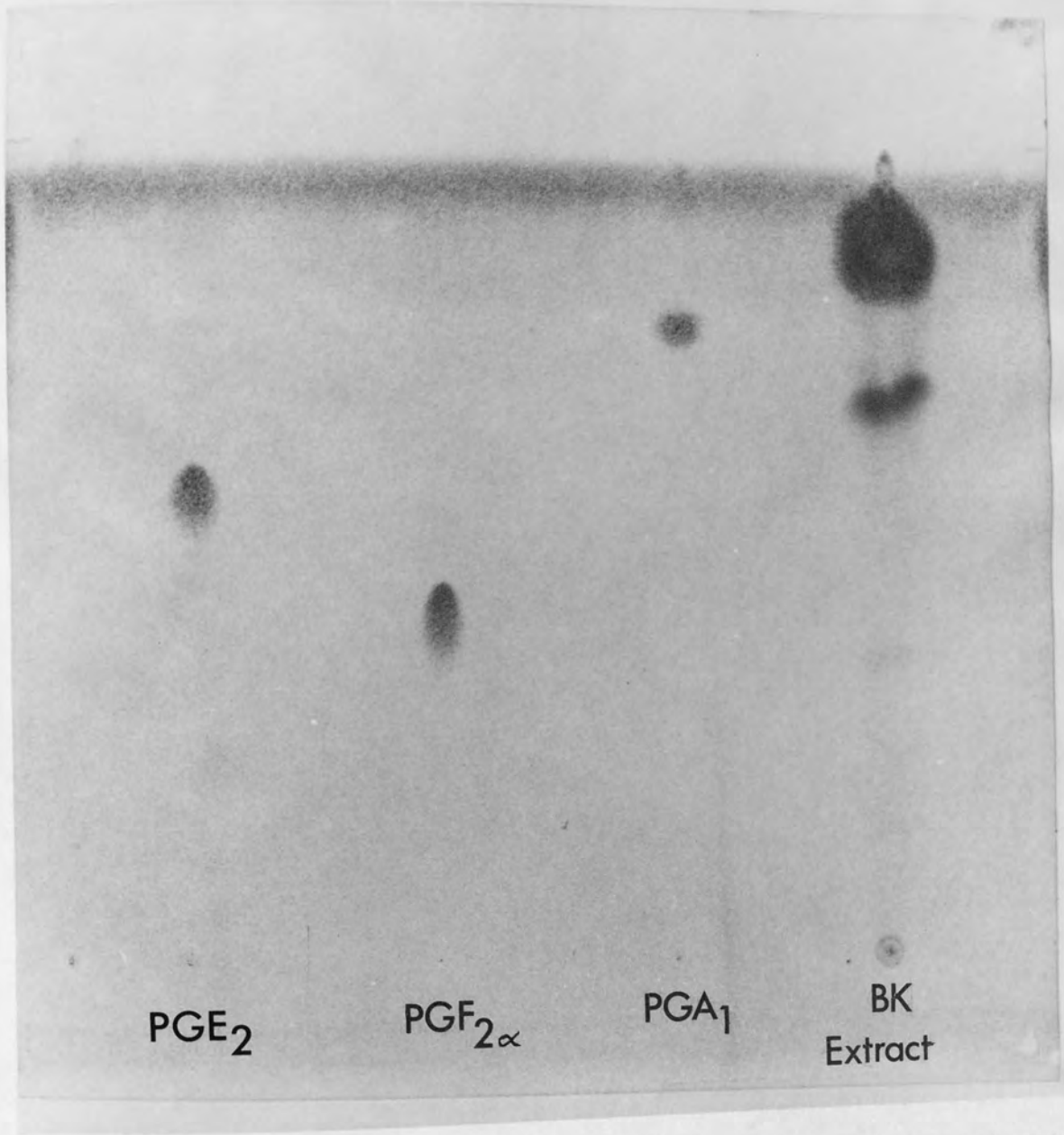


Fig 16. Thin layer chromatogram showing the mobility of the extract of prostaglandin-like material and the relative mobilities of standard PGE_2, $PGF_{2\alpha}$, and PGA_1 in Benzene:Dioxan:Acetic acid (20:20:1).

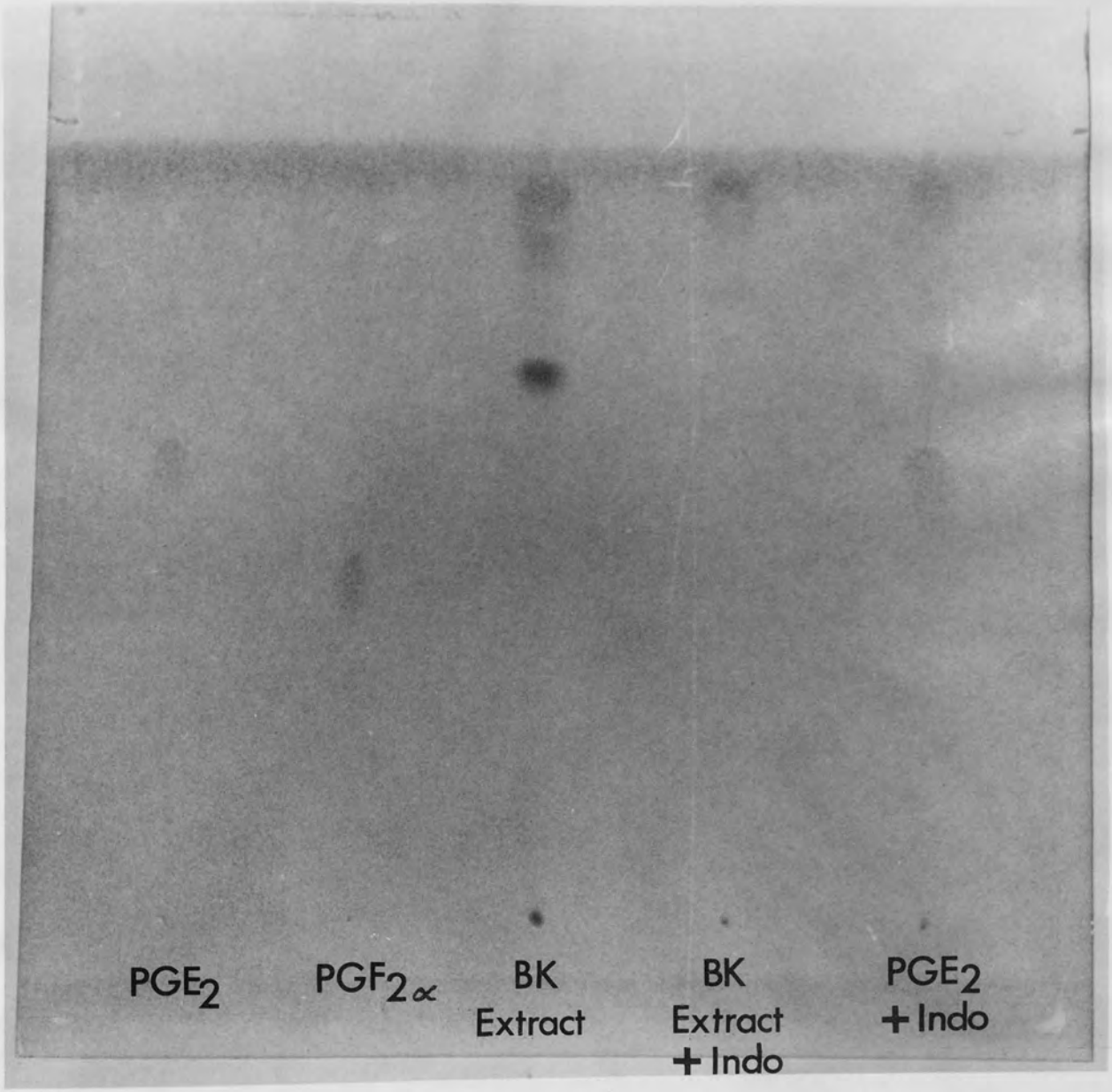


Fig 17. Thin layer chromatogram showing the effect of indomethacin, 28.0 μM, on bradykinin-induced release of prostaglandin-like material and the effect of incubation of rat terminal ileum with standard PGE_2.

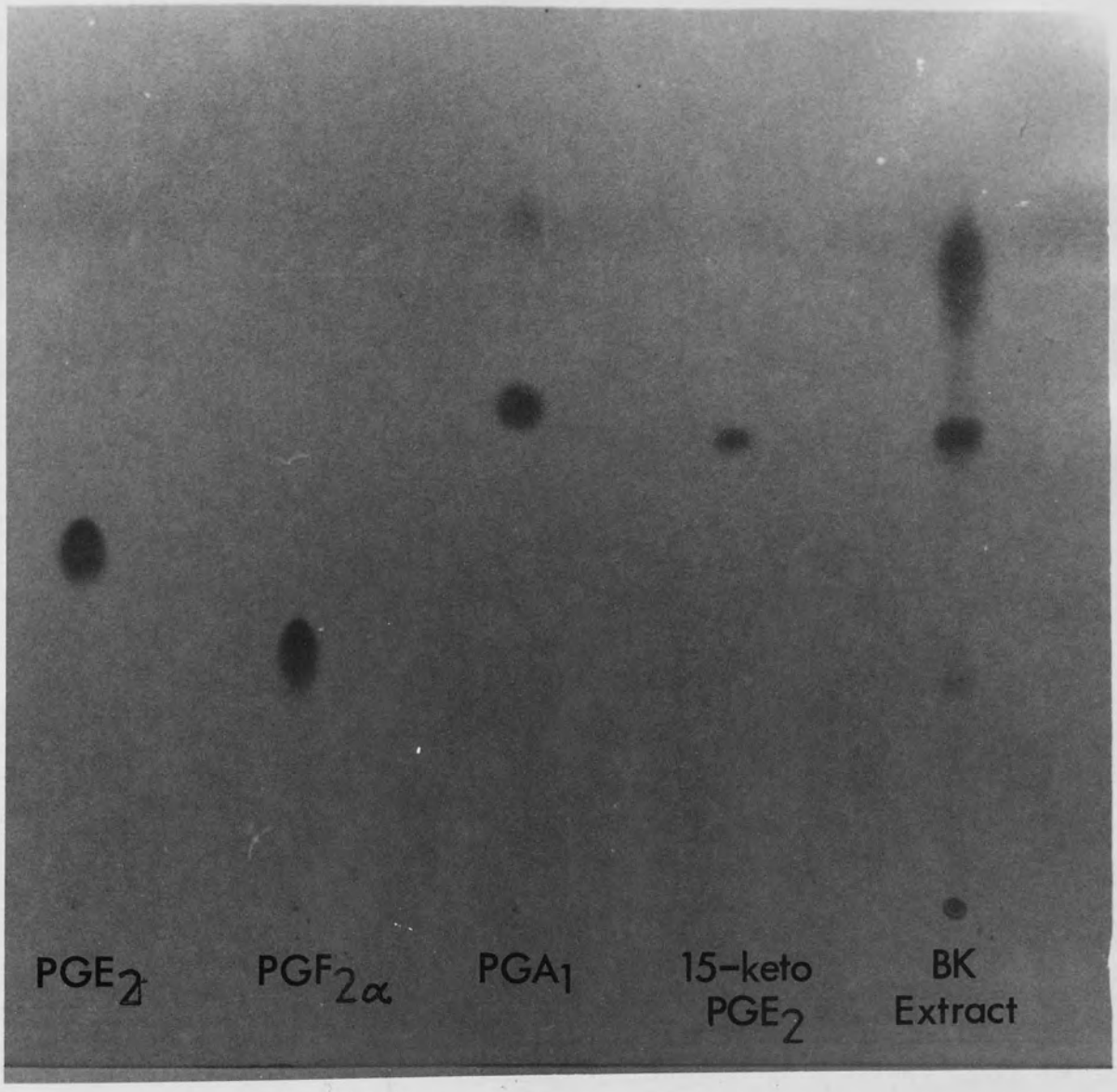


Fig 18. Thin layer chromatogram showing the relative chromatographic mobilities of the extract of prostaglandin-like material and standard samples of PGE_2, $PGF_{2\alpha}$, PGA_1 and 15 keto-PGE_2.

DISCUSSION

1. GENERAL INTRODUCTION

An attempt has been made in this study to investigate the action bradykinin on sodium and water transfer in the isolated rat jejunum and to elucidate the mechanisms by which bradykinin affects this system. In addition the mechanism by which bradykinin contracts the longitudinal smooth muscle of the rat terminal ileum has been investigated. The results appertaining to each system will be discussed separately but they will subsequently be compared and contrasted.

2. ACTION OF BRADYKININ ON SODIUM AND WATER TRANSFER IN RAT JEJUNUM

(a) Effect of Bradykinin on Rat Jejunum

The method of Wilson & Wiseman (1954) which has been used frequently for studying the action of substances on the intestinal transfer of sodium and water was used in this study to investigate the action of bradykinin on sodium and water transfer in rat jejunum.

Bradykinin, at low concentrations (7.86×10^{-11} to 7.86×10^{-13} M), was observed to have two different effects on the transfer of sodium and water and it appeared that the effect seen was dependent on the control level of sodium and water transfer. Thus when control transfer was high an inhibition was observed. These findings are of interest because Dennhardt & Haberich (1973) showed that kallikrein could have both stimulatory and inhibitory actions on the transport of sodium and water by rat jejunum and colon. In rats with ligated pancreatic ducts, in which control transfer was low, kallikrein stimulated sodium and water transfer in both rat jejunum and colon whereas in normal rats, which had high control transfer, kallikrein inhibited sodium and water transfer in jejunum and colon. Thus it seems possible that the observations of Dennhardt & Haberich (1973) may have been due to the generation of bradykinin by kallikrein within the intestine.

The idea that bradykinin may have two opposing effects and that either of these effects may be dominant depending on the prevailing conditions is not new. The usual effects of bradykinin on rat blood pressure and guinea pig ileum are to cause hypotension and contraction, respectively, but bradykinin-induced hypertension is seen in rats with low blood pressure after nephrectomy and ganglion blockade (Croxatto & Belmar, 1961; Croxatto, Belmar, Pereda & Labarca, 1960) and a relaxation in response to bradykinin occurs in guinea pig ileum whose tone has been elevated with acetylcholine or histamine (Hall & Bonta, 1972). It has also been shown that bradykinin can have successively both relaxatory and

stimulatory actions on intestinal preparations (Aarsen & van Caspel de Bruyn, 1970; Elliott, Horton & Lewis, 1960a; Ohashi, Nonamura & Ohga, 1967). Further, Ohashi, Nonamura & Ohga (1967) suggested that the action of bradykinin on taenia coli was dependent on the electrical activity of the membrane at the moment of addition of bradykinin. Groza, Buzoiamu & St. Ionescu (1967) observed that the usual inhibition of gastric acid secretion seen by them with bradykinin could be replaced by an augmentation of acid secretion by manipulation of the experimental conditions.

In the present study the stimulatory action of bradykinin exhibited a clear dose response effect so that a greater stimulatory effect was seen with higher concentrations. However, the relationship was less clear when the inhibitory action of bradykinin was considered. Concentrations of 7.86×10^{-12} and 7.86×10^{-13}M produced a similar inhibition of transfer but 7.86×10^{-11}M produced a greater degree of inhibition. It is possible that the concentrations chosen are towards the lower end of the dose response curve for the inhibitory effect of bradykinin since the dose response curves for the stimulatory and inhibitory effects of bradykinin may not be the same.

It was observed that bradykinin-induced inhibition of water transfer at high control transfer was present when the peptide was incubated in either mucosal or serosal solution and it is interesting that Furtado (1971) had reported that either mucosal or serosal bradykinin inhibited the vasopressin-or oxytocin-induced increase in water permeability of toad bladder. On the other hand the stimulatory action of bradykinin at low control transfer was only observed when bradykinin was present in the serosal solution.

Kallikrein is present in large amounts in the gastrointestinal tract (Werle & Vogel, 1961; Werle, Vogel & Kaliampetsos, 1963) and there are large amounts of kallikrein in the pancreas (Kraut, Frey & Werle, 1930) which can reach the gastrointestinal tract via the pancreatic duct. Thus there is large potential kinin-generating system which can act on the

gastrointestinal tract and it is tempting to speculate that bradykinin may have a physiological or pathological role to play in the movement of sodium and water across the gastrointestinal tract.

In patients with carcinoid tumours, dumping syndrome and ganglioneuroma tumours there is frequently nausea, vomiting and diarrhoea and there have been reports of elevated kallikrein and kinin levels in these conditions (Cameron, Warner & Szabo, 1967; Mason & Melmon, 1966; Oates, Melmon, Sjoerdsma, Gillespie & Mason, 1964; Stickler, Hallenbeck, Flock & Rosevear, 1962; Werle, Trautschold & Schievelbein, 1966; Zeitlin & Smith, 1966,1970). It is possible that bradykinin contributes to the diarrhoea observed in these conditions by contributing to the intestinal hypermotility observed (Bennett, 1971a; Oates, Melmon, Sjoerdsma, Gillespie & Mason, 1964; Zeitlin & Smith, 1966, 1970). The results reported in the present study raise the possibility that bradykinin may also contribute to the observed diarrhoea by an action on sodium and water transfer across the gastrointestinal tract. Singleton (1969) suggested that the kinins should be considered as condidates for the diarrhoea hormone in such cases. Thus bradykinin may have a role to play in the physiological and pathological control of gastrointestinal transfer of electrolytes and water.

(b) Effect of the Kidneys and Adrenal Glands on the Action of Bradykinin on Sodium and Water Transfer

These experiments were designed to show if modification of the renin-angiotensin-aldosterone system or sodium balance affected the action of bradykinin on intestinal sodium and water movement. Adrenalectomy and nephrectomy were performed in order to reduce circulating levels of aldosterone and renin-angiotensin, respectively. It was shown that the stimulation of water transfer by bradykinin at low control water transfer was not affected by previous nephrectomy, adrenalectomy or combined nephrectomy-adrenalectomy, which would suggest that the presence of a normally functioning renin-angiotensin-aldosterone system is probably not necessary for the stimulation of water transfer seen with bradykinin at low control transfer.

In addition it was shown in rats drinking 1% saline that bradykinin was still capable of stimulating water transfer at low control transfer and in rats on a low sodium diet that bradykinin showed its usual stimulatory and inhibitory actions at low and high control transfer. It has been reported that the renin-angiotensin-aldosterone system is suppressed in animals drinking saline (Gross, Brunner & Ziegler, 1965) and activated in animals on a low sodium diet (Singer & Stacke-Dunne, 1955).

The data from animals on varied sodium intake supports the previous data from nephrectomised and adrenalectomised rats and so it is probable that neither the stimulatory nor the inhibitory action of bradykinin on water transfer in rat jejunum is dependent on the presence of the adrenal glands or the kidneys, although experimental procedures were not assessed for their effectiveness by measurement of hormone levels. These findings are in contrast with those of Groza, Buzoianu, St. Ionescu & Rosovici (1967) who showed that the inhibition of gastric secretion produced by bradykinin in Shay rats is abolished by adrenalectomy.

(c) Role of Prostaglandins in the Action of Bradykinin on Water Transfer in Rat Jejunum

There is an increasing body of evidence suggesting a relation between prostaglandins and bradykinin in several systems (see Introduction). It was the purpose of this part of the study to investigate the possible role of prostaglandins in the inhibitory action of bradykinin at high control water transfer since it has been shown that prostaglandins inhibit sodium and water transfer in intestinal preparations (Al-Awqati & Greenough, 1972; Matuchansky & Bernier, 1973; Pierce, Carpenter, Elliott, & Greenough, 1971). It has been suggested that cholera-induced diarrhoea (Becquerel, 1849) may involve prostaglandins (Bennett, 1971b; Finck & Katz, 1972; Gots, Formal & Gianella, 1974; Jacoby & Marshall, 1972; Vaisrub, 1972) although there are contrary reports (Bourne, 1973; Cuatrecasas, 1973; Kimberg, Field, Gershon & Henderson, 1974; Kimberg, Field, Johnson, Henderson & Gershon, 1971; Sharp & Hynie, 1971).

The non steroidal antinflammatory agent meclofenamic acid which has been shown to be a potent inhibitor of prostaglandin synthesis (Flower, Gryglewski, Herbaczynska-Cedro & Vane, 1972) was used in this study rather than the more commonly used indomethacin since indomethacin has been reported to affect phosphodiesterase and hence cyclic AMP levels (Flores & Sharp, 1972) and so is less suitable for separating the involvement of prostaglandins and cyclic AMP in various actions (Flower, 1974).

It was shown that meclofenamic acid, 6.8μM, caused an abolition of the inhibitory action of bradykinin at high control transfer in rat jejunum and it is interesting that at intermediate control water transfer where neither bradykinin nor meclofenamic acid alone had any effect on water transfer, the combination of the two agents significantly stimulated transfer. Since Vane & Ferreira (1975) suggested that antagonism of a pharmacological action by low concentrations of agents such as meclofenamic acid indicates prostaglandin involvement in that action it was considered

that prostaglandins may have a role to play in the inhibitory action of bradykinin seen at high control transfer. The appearance of a stimulation of transfer at intermediate control transfer in the presence of bradykinin and meclofenamic acid suggests that at this level of transfer the stimulatory and inhibitory actions of bradykinin normally cancel each other and that when one of these opposing actions is removed the other is evident. It is also likely that the stimulatory action of bradykinin does not involve prostaglandins.

The possible involvement of prostaglandins in the inhibitory action of bradykinin was further tested by studying the actions of PGE_1, PGA_1 and $PGF_{2\alpha}$. At high and intermediate transfer PGE_1 inhibited water transfer as previously reported (Al-Awqati & Greenough, 1972) and in combination with bradykinin inhibited water transfer to a significantly greater degree than seen with either agent alone. The increased inhibition of water transfer was somewhat less than the combined degrees of inhibition of both agents separately suggesting that there had been an additive effect and that a maximum degree of inhibition had been reached. At intermediate control transfer bradykinin and PGE_1 inhibited transfer significantly more than either agent alone, an effect which was clearly not additive, whereas at low control transfer the stimulatory action of bradykinin was abolished in the presence of PGE_1 which itself had no effect. Thus it would appear that bradykinin and PGE_1 had synergistic effects on inhibition of water transfer by rat jejunum.

PGA_1 inhibited significantly water transfer at high and intermediate control water transfer. In the presence of bradykinin and PGA_1 there was a significantly greater inhibition of water transfer at both high and intermediate control transfer which at high control transfer was less than the combined degrees of inhibition suggesting a maximum degree of inhibition and at intermediate levels was much greater than the combined effects of bradykinin and PGA_1. At low control transfer bradykinin-induced stimulation of water transfer was abolished in the presence of

PGA_1. Thus again it would appear that the inhibitions of water transfer seen with bradykinin and PGA_1 were synergistic.

$PGF_{2\alpha}$ inhibited significantly water transfer at high and intermediate control levels of transfer. The combination of bradykinin and $PGF_{2\alpha}$ inhibited water transfer at high control transfer to the same degree as $PGF_{2\alpha}$ alone, perhaps suggesting a common mechanism, while at intermediate control transfer the two agents inhibited transfer to a significantly greater degree than either agent alone. Bradykinin-induced stimulation at low control transfer was converted in the presence of $PGF_{2\alpha}$ into a significant inhibition of water transfer. It can be seen that there was a synergistic effect of bradykinin and $PGF_{2\alpha}$ on inhibition of water transfer.

Thus in summary it has been shown that meclofenamic acid, an inhibitor of prostaglandin synthesis, abolished the inhibitory action of bradykinin at high control transfer and caused the appearance of a stimulation of transfer at intermediate control transfer. It has also been reported that bradykinin and several prostaglandins appeared to have synergistic effects on the inhibition of water transfer.

The reported phenomena can be explained by considering that bradykinin inhibits water transfer at high control transfer by elaboration of prostaglandins and that bradykinin-induced stimulation at low control transfer occurs by some other mechanism not involving prostaglandins. The lack of observable effect at intermediate control transfer by bradykinin is thought to be due to a cancelling out of these stimulatory and inhibitory effects. Procedures which affect the stimulatory or inhibitory mechanisms could shift the point of net cancellation of effects. Thus meclofenamic acid is thought to have removed the inhibitory action of bradykinin so that a stimulation of water transfer is seen at intermediate control transfer and addition of prostaglandins is also thought to have altered the usual balance and caused the stimulatory action of bradykinin at low control transfer to disappear and even be replaced by inhibition in the case of $PGF_{2\alpha}$.

(d) Role of Cyclic AMP in the Action of Bradykinin on Water Transfer

There is considerable evidence that cyclic AMP affects sodium and water transfer in the small intestine (Field, 1974; Kimberg, 1974; Scratcherd & Case, 1973), abolishing net sodium absorption and replacing it with sodium secretion (Field, 1971). It is also believed that cyclic AMP is involved in the small intestinal secretion caused by prostaglandins (Kimberg, Field, Gershon & Henderson, 1974; Kimberg, Field, Johnson, Henderson & Gershon, 1971; Pierce, Carpenter, Elliott & Greenough, 1971; Sharp & Hynie, 1971) and cholera enterotoxin (Guerrant, Chen & Sharp, 1972; Kimberg, Field, Gershon & Henderson, 1974; Kimberg, Field, Johnson, Henderson & Gershon, 1971; Sharp & Hynie, 1971).

Thus it was considered pertinent to investigate the involvement of cyclic AMP with the inhibitory action of bradykinin on sodium and water transfer.

It was shown that theophylline, which prevents breakdown of cyclic AMP by inhibiting phosphodiesterase (Butcher & Sutherland, 1962), inhibited water transfer across isolated rat jejunum confirming earlier reports (Field, Fromm & Silen, 1969; Field, 1971). A clear dose response effect was seen with theophylline, 10, 1.0 and 0.1 mM. The effect of 1mM theophylline on the action of bradykinin on water transfer was studied and it was seen at high control transfer that the combination of bradykinin and theophylline inhibited water transfer significantly more than either agent alone, although the degree of inhibition was less than the sum of the inhibitions seen with bradykinin and theophylline separately, perhaps suggesting a common mechanism. At intermediate control transfer bradykinin and theophylline inhibited water transfer significantly more than either agent separately and the degree of inhibition was greater than the sum of the effects of both agents. Bradykinin-induced stimulation at low control water transfer was converted to a significant inhibition of water transfer by theophylline which had no effect itself.

Therefore it has been shown that theophylline enhanced the inhibition of water transfer caused by bradykinin at high levels of control water transfer. Raising intracellular cyclic AMP levels with theophylline is thought to have caused an imbalance in the opposing stimulatory and inhibitory effects of bradykinin so that inhibitions of water transfer were seen with bradykinin in the presence of theophylline at low and intermediate levels of control transfer.

As a result of this interesting synergism between the inhibitory actions on water transfer of bradykinin and theophylline it was decided to study the effects of cyclic AMP and dibutyryl cyclic AMP on water transfer and on the action of bradykinin on water transfer. Cyclic AMP, 1mM, inhibited water transfer at high control transfer, confirming earlier reports (Field, 1971; 1974) but dibutyryl cyclic AMP significantly inhibited water transfer at all levels of control water transfer. This was presumably due to the dibutyryl derivative having greater ability to penetrate membranes and to resist degradation by phosphodiesterase (Posternak, Sutherland & Henion, 1962). At high control water transfer bradykinin and db cyclic AMP together produced an inhibition of water transfer which was approximately equal to the arithmetic sum of the inhibitory effects of both agents separately. However at intermediate control transfer there was an inhibition of water transfer which was significantly greater than that seen with both agents separately while at low control transfer the usual stimulatory action of bradykinin was replaced by an inhibition of water transfer in the presence of db cyclic AMP. Thus db cyclic AMP which itself inhibited water transfer was capable of potentiating bradykinin-induced inhibition of water transfer.

These findings with theophylline and db cyclic AMP suggest an involvement of cyclic AMP in the inhibitory action of bradykinin since this inhibitory effect is enhanced in situations where cyclic AMP levels are elevated. However these experiments give little indication of the exact nature of the stimulatory action of bradykinin.

It has been suggested that cyclic AMP might mediate bradykinin-induced stimulation of mitotic activity and DNA synthesis in rat thymocytes (Whitfield, MacManus & Gillan, 1970) and bradykinin-induced inhibition of catalase activity in mouse liver and kidney (Hokama & Yanagihara, 1971) and it has been reported that bradykinin elevated cyclic AMP levels in fibroblast tissue cultures (Schonhofer, Peters, Karzel, Dinnendahl & Westhofen, 1974) and in guinea pig lung (Stoner, Manganiello & Vaughan. 1973). In the latter case the cyclic AMP-elevating action of bradykinin was enhanced by an inhibitor of kininase enzyme, BPP_{5a}, and abolished by indomethacin. However Schwartz, Kimberg, Sheerin, Field & Said (1974) did not observe any effect of bradykinin on cyclic AMP levels in rabbit ileal mucosa after a six minute exposure.

(e) Summary of Discussion of Sodium and Water Transfer Results

It has been shown that bradykinin stimulated sodium and water transfer in rat jejunum at low control water transfer and inhibited sodium and water transfer at high control water transfer. The stimulatory action was only observed when bradykinin was present in the serosal solution whereas the inhibitory action was observed when bradykinin was in either mucosal or serosal solution.

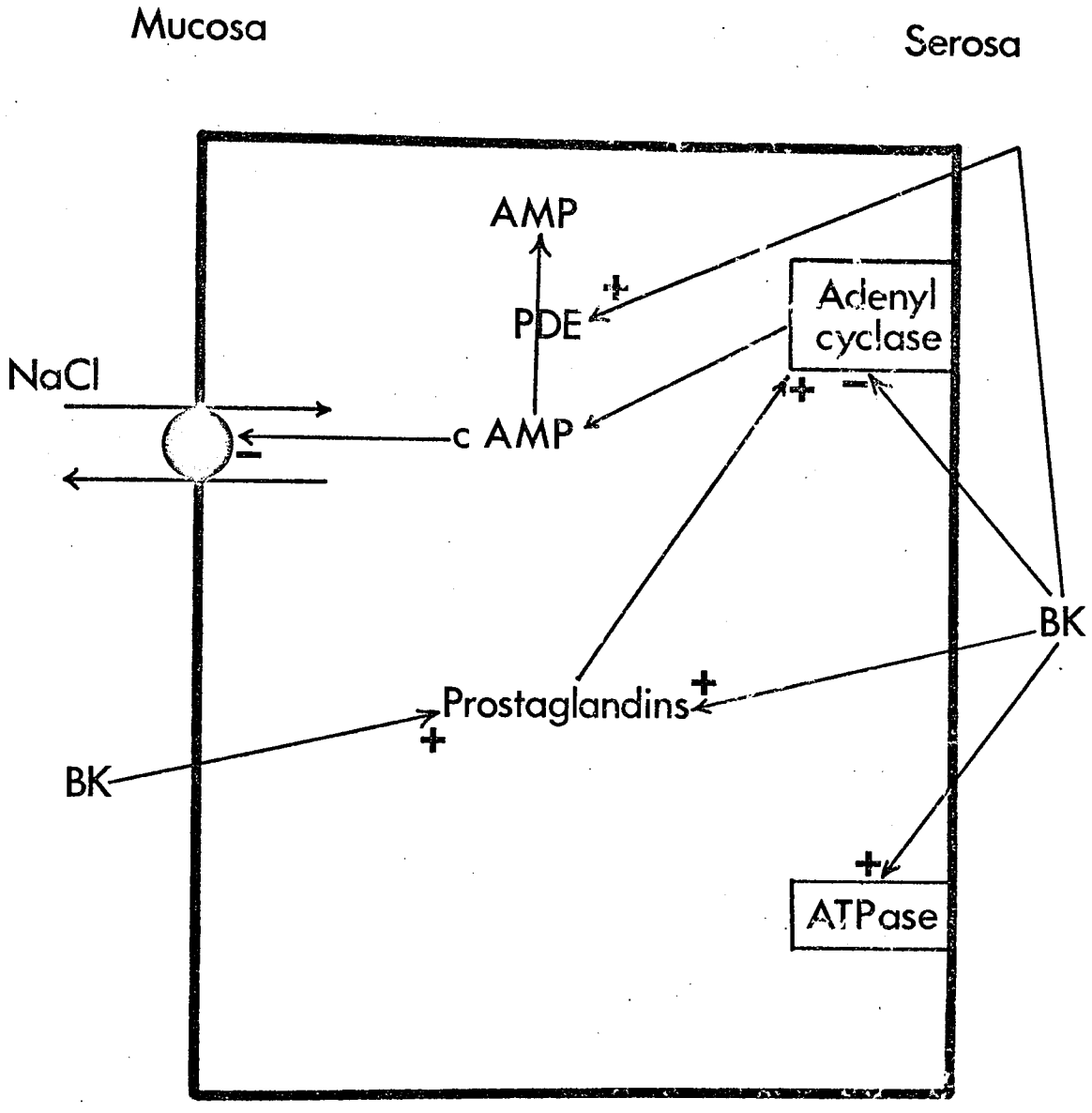
The stimulatory action of bradykinin was shown to be unaffected by procedures which were designed to alter the activity of the renin-angiotensin-aldosterone system such as nephrectomy, adrenalectomy, sodium loading and sodium restriction and the inhibitory action was not affected by sodium restriction. Thus it would appear that the observed actions of bradykinin are not dependent on the activity of the renin-angiotensin-aldosterone system.

Bradykinin-induced inhibition of water transfer was abolished by meclofenamic acid, an inhibitor of prostaglandin synthetase (Flower, 1974) and augmented by prostaglandins E_1, A_1 and $F_{2\alpha}$ suggesting involvement of prostaglandins in this action. The inhibitory action of bradykinin on water transfer was enhanced by theophylline, an inhibitor of phosphodiesterase (Butcher & Sutherland, 1962) and dibutyryl cyclic AMP.

Thus it is considered that the inhibitory action of bradykinin on water transfer involves activation by bradykinin of prostaglandins which in turn increase adenyl cyclase activity to produce cyclic AMP. There is much evidence to suggest that prostaglandins and cyclic AMP are involved in sodium and water movement in the intestine (see Introduction).

Little information has been obtained regarding the stimulatory action of bradykinin at low control transfer although it does appear to be unaffected by the renin- angiotensin-aldosterone system and is only observed when bradykin is present on the serosal surface of the gut. It would appear possible that the stimulatory action of bradykinin could be explained by at least three mechanisms. Thus it is possible that

bradykinin could stimulate Na-K-ATPase, inhibit adenyl cyclase directly or stimulate phosphodiesterase. These possibilities as well as the presumed mechanism of bradykinin-induced inhibition of water transfer are illustrated diagramatically below:-



All three possible mechanisms would tend to increase sodium absorption. It is interesting that bradykinin has been reported to increase rat kidney Na-K-ATPase activity (Chernukh, Yarovaya & Glebov, 1975) but it is premature at this stage to attempt to indicate which of the possible mechanisms of stimulation of water transfer is most likely. Indeed some other mechanism may be operative.

3. CONTRACTILE ACTION OF BRADYKININ ON RAT TERMINAL ILEUM

The previous experiments were concerned with sodium and water transfer in rat jejunum and suggested an involvement of the prostaglandin-cyclic AMP systems in the action of bradykinin on water transfer. However it is difficult to perform adequate controls using this system and it was decided to extend the study to include an investigation of the contractile action of bradykinin on rat terminal ileum, which gives regular, repeatable and dose-related responses to bradykinin.

In these experiments acetylcholine was used as a control agonist since its contractile action is thought to be the result of an increase in passive permeability of smooth muscle to sodium ions (Bolton, 1972) and to potassium ions (Bulbring & Szursweski, 1974).

(a) Contribution of Nerves to Contractile Action of Bradykinin

Khairallah & Page (1961; 1963) who showed that bradykinin-induced contractions of guinea pig ileum and rat uterus were not affected by atropine, neostigmine or ganglion blocking drugs concluded that the contractile action of bradykinin was not mediated via cholinergic nerves. Supporting evidence for this idea was obtained by Day & Vane (1963), and Gershon (1967) although Wiegershausen, Stopp & Eichstadt (1964) observed that bradykinin-induced contractions of guinea pig intestine were inhibited by atropine and potentiated by eserine.

In the present study it was clearly shown that acetylcholine induced contractions were abolished by hyoscine while those of bradykinin were only slightly affected. The effect of hyoscine on the responses to acetylcholine was progressive which was not the case for the small effect on bradykinin responses. It seemed that the small effect of hyoscine on responses to bradykinin might be due to some mechanism other than blockade of muscarinic receptors on rat terminal ileu. Thus the present results support the view that cholinergic nerves are not involved in the contractile action of bradykinin on rat terminal ileum.

(b) Metabolic Requirements for the Contractile Action of Bradykinin on Rat Terminal Ileum

In this part of the study the metabolic requirement for the contractile action of bradykinin was compared with that of acetylcholine. Since acetylcholine induced contractions are thought to be due to a passive increase in sodium ion permeability of smooth muscle (Bolton, 1972; Bulbring & Szursweski, 1974) the metabolic requirement for acetylcholine-induced contractions should just be that of the contractile apparatus itself. However the metabolic requirement of an agonist which increased the activity of one or more intermediate substances before inducing contraction would be greater than that for acetylcholine and thus would be more susceptible to metabolic inhibition. This has been demonstrated for angiotensin by Crocker & Wilson (1974; 1975). Thus if bradykinin-induced contractions were affected more by metabolic inhibition than those of acetylcholine then this might be interpreted as evidence that an active initial interaction was involved in the contractile response to bradykinin.

The effect of removing oxygen from in vitro smooth muscle preparations has been investigated extensively (Coe, Detar & Bohr, 1968; Furchgott & Shorr, 1948; Prasad, 1935a,b) and the reduction in spontaneous and induced mechanical activity has been attributed to an impairment of energy processes within the muscle. Therefore the effect of anoxia was investigated on the contractile actions of acetylcholine and bradykinin.

Replacement of oxygen by nitrogen caused a rapid, progressive impairment of the contractions induced by bradykinin while having relatively little effect on those of acetylcholine. Since glucose was present during the period of anoxia it seemed that although some energy would still be generated by anaerobic glycolysis the reduction of bradykinin responses suggested that aerobic metabolism was necessary to produce sufficient energy to support the bradykinin response. This is supported by the preliminary observation that 2,4 - dinitrophenol, which uncouples oxidative

phosphorylation in isolated preparations (Rangachari, Paton & Daniell, 1972), inhibits bradykinin-induced contractions of rat terminal ileum significantly more than those of acetylcholine.

Since both the synthesis of prostaglandins has been shown to be dependent upon the presence of molecular oxygen (Samuelsson, Granstrom & Hamberg, 1967; Nugteren, Beerthuis & van Dorp, 1967) and the contractile action of prostaglandins upon isolated smooth muscle is dependent upon oxygen (Coceani & Wolfe, 1966) it is possible that the demonstration that bradykinin-induced responses of rat terminal ileum are energy-dependent might be a reflection of prostaglandin involvement in those responses.

(c) Role of Cyclic AMP in the Contractile Response of Rat Terminal Ileum to Bradykinin

Cyclic AMP would appear to play a role in smooth muscle contraction and relaxation (Andersson, 1972; Bar, 1974). Relaxation of smooth muscle has been associated with increased intracellular cyclic AMP levels (Andersson, 1972; McFarland, Guyton & Pfaffman, 1971) and it has been suggested that catecholamines relax smooth muscle by this mechanism (Andersson, 1972; Sutherland & Robison, 1966). However contraction of smooth muscle has not been consistently associated with decreased intracellular cyclic AMP (Andersson, 1972).

In this study theophylline, which is known to inhibit phosphodiesterase (Butcher & Sutherland, 1962) and to potentiate actions of drugs which are mediated by cyclic AMP (Robison, Butcher & Sutherland, 1971), was used to assess the role of cyclic AMP in the contractile action of bradykinin. Theophylline, 1mM, markedly reduced the contractile action of bradykinin whereas acetylcholine-induced contractions of rat terminal ileum were only partially reduced. This preferential reduction of bradykinin responses by theophylline might suggest that cyclic AMP may in some way modulate the contractile action of bradykinin. Theoretically bradykinin-induced contractions ought to be associated with decreased cyclic AMP concentrations since theophylline both inhibits phosphodiesterase and reduces bradykinin responses. However it would be premature to speculate too strongly on the role of cyclic AMP in the contractile action of bradykinin until further experiments have been performed.

Theophylline, 10mM, caused abolition of both acetylcholine- and bradykinin-induced contractions of rat terminal ileum, suggesting either that theophylline was having unspecific effects unrelated to phosphodiesterase inhibition or that increased intracellular cyclic AMP was causing a non specific inhibition of all contractile responses, probably through a reduction in intracellular calcium levels.

(d) Role of Prostaglandins in the Contractile Action of Bradykinin on Rat Terminal Ileum

In these experiments the role of prostaglandins in the bradykinin-induced contractions of rat terminal ileum was investigated by using a series of prostaglandin synthetase inhibitors and prostaglandin receptor blocking drugs.

(i) Prostaglandin Synthetase Inhibitors

Four prostaglandin synthetase inhibitors were used in this part of the study, namely aspirin, indomethacin, meclofenamic acid and flufenamic acid. A loss of smooth muscle tone was observed in most preparations of rat terminal ileum exposed to these agents supporting the view that prostaglandins may contribute to the maintenance of intestinal smooth muscle tone (Bennett & Posner, 1971; Bennett, Eley & Stockley, 1975; Botting & Saltzman, 1974; Davison, Ramwell & Willis, 1972; Eckenfels & Vane, 1972; Ferreira, Herman & Vane, 1972).

It was shown that aspirin in a concentration of 610μM, which has been reported to inhibit the release of prostaglandins from pregnant rat uterus (Aiken, 1972), markedly reduced the contractile ability of bradykinin while responses to acetylcholine and PGE_2 were not affected. There was a slow recovery of responses to bradykinin after removal of aspirin so that they returned to normal after about 90 minutes. It would seem unlikely that aspirin is acting here by inhibition of the energy supply, as suggested by Whitehouse (1962;1964;1965), since prostaglandin responses, which are reported to be energy-dependent in other smooth muscle tissues (Coceani & Wolfe, 1966) were unaffected.

Indomethacin, 2.8μM, partially reduced bradykinin-induced contractions while not affecting those of acetylcholine, and the recovery of bradykinin responses was gradual over a period of 50 minutes. Indomethacin, 28.0μM, markedly and progressively inhibited the contractile responses to bradykinin while those to acetylcholine were unaffected and

those to PGE_2 were slightly reduced although not as much as those to bradykinin. There would seem to be two different actions of indomethacin operating since on its removal the partial reduction of PGE_2 responses was immediately reversed while the reduction of bradykinin-induced responses was slow to recover, reaching normal values after 90 minutes. The slight non specific action of indomethacin on PGE_2 induced responses may be related to an action on energy supply (Whitehouse, 1962;1964;1965) or calcium movement (Northover, 1972;1973).

Meclofenamic acid, 3.4μM, drastically reduced the contractile responses to bradykinin while having only a slight inhibitory effect on responses to PGE_2 and $PGF_{2\alpha}$ and a slight potentiation of acetylcholine-induced responses. There were distinct differences between the effects of meclofenamic acid, 3.4μM, on bradykinin and prostaglandin responses. Inhibition of bradykinin was progressive while that of PG responses was not and on removal of meclofenamic acid the responses to PGE_2 and $PGF_{2\alpha}$ rapidly returned to normal while the responses to bradykinin recovered slowly. Thus it would appear that two mechanisms are operating in the above situation.

Meclofenamic acid, 17.0μM, inhibited responses to bradykinin to a greater extent that 3.4μM although PGE_2, $PGF_{2\alpha}$ and acetylcholine were also significantly affected. It was noticeable that on removal of meclofenamic acid responses to acetylcholine, PGE_2 and $PGF_{2\alpha}$ recovered rapidly while those to bradykinin recovered only slowly. The non specific impairment of acetylcholine responses suggests an interference with the contractile process at higher concentrations of meclofenamic acid. Responses to PGs were more affected than acetylcholine and it is relevant that Collier & Sweatman (1968) reported direct antagonism of prostaglandin receptors by the fenamates.

Flufenamic acid, 3.55μM, drastically reduced responses to bradykinin while not affecting those to PGE_2 or acetylcholine and partially reducing those to $PGF_{2\alpha}$. Removal of flufenamic acid produced a very

slow and partial recovery of bradykinin responses but a quicker recovery of $PGF_{2\alpha}$.

Thus it has been shown that four different prostaglandin synthetase inhibitors, aspirin, indomethacin, meclofenamic acid and flufenamic acid, inhibited the contractile responses of rat terminal ileum to bradykinin. It has been reported elsewhere that aspirin (Collier & Shorley, 1960; Malone & Trottier, 1973) and phenylbutazone (Malone & Trottier, 1973) did not specifically antagonise bradykinin-induced responses of guinea pig ileum but that flufenamic acid (10-30μM) exhibited both competitive and non competitive inhibition and indomethacin (100μM) exhibited non competitive inhibition of bradykinin induced contractions of rat uterus (Malone & Trottier, 1973). In this study the concentrations of these agents used were similar to those used previously in comparable situations and which were observed to diminish prostaglandin synthesis and were lower than those reported to affect other enzymes (see Flower, 1974 for references). It is likely that the drugs used were acting fairly specifically on prostaglandin synthetase since for example indomethacin was reported to affect oxidative phosphorylation (Whitehouse & Haslam, 1962) and calcium movements (Northover, 1972;1973) only at concentrations of 250μM and 300-600μM respectively.

The smooth muscle actions of PGE_2 and $PGF_{2\alpha}$ were reduced by meclofenamic acid and flufenamic acid as reported by Collier & Sweatman (1968), although this effect only became marked at a concentration of 17.0μM meclofenamic acid. It is interesting that $PGF_{2\alpha}$ was more affected than PGE_2 by flufenamic acid and this may possibly indicate the presence of more than one type of receptor for prostaglandins on the longitudinal smooth muscle of rat terminal ileum. This possibility will be discussed later when considering the action of prostaglandin receptor blocking agents. The reductions of bradykinin and the prostaglandins was probably through different mechanisms since the recoveries of responses to bradykinin and prostaglandins were markedly different.

Prostaglandins recovered rapidly after removal of flufenamic acid and meclofenamic acid while bradykinin recovered slowly. This would tend to support the view that inhibition of bradykinin responses was due to synthetase inhibition since prostaglandin synthetase inhibition by antiinflammatory drugs is not quickly reversible (Flower, 1974).

It is the view of Vane & Ferreira (1975) that inhibition of a pharmacological action by low concentrations of aspirin-like drugs, as demonstrated here, is indicative of prostaglandin involvement in that pharmacological action. It is therefore considered that prostaglandins may be involved in the contractile action of bradykinin on the longitudinal muscle of rat terminal ileum.

(ii) Prostaglandin Receptor Antagonists

Several prostaglandin receptor antagonists have become available in recent years and the actions of these substances have been extensively reviewed (Bennett, 1974; Eakins & Sanner, 1972; Sanner, 1974).

Polyphloretin phosphate (PPP), which is a mixture of polyanionic polyesters of phosphoric acid and phloretin (Diczfalusy, Ferno, Fex, Hogberg, Linderot & Rosenberg, 1953), was found to inhibit markedly contractile responses of longitudinal muscle of rat terminal ileum to $PGF_{2\alpha}$ while reducing the responses to bradykinin and PGE_2 to a lesser degree and those to acetylcholine not at all. It has been shown previously that PPP did not antagonise the action of bradykinin on jird colon (Eakins, Karim & Miller, 1970; Eakins, Miller & Karim, 1971) but did on rat uterus (Barabe, Park & Regoli, 1975). However the effect of PPP on the contractile action of bradykinin on intestinal smooth muscle has not been studied extensively. PPP has been observed to inhibit the contractile action of prostaglandins E & F on longitudinal smooth muscle of jird colon and rabbit jejunum (Eakins, Karim & Miller, 1970), guinea pig colon (Bennett & Posner, 1971), rat colon (Gagnon & Sirois, 1972; Somova, 1973), chick rectum (Somova, 1973), human stomach and colon (Bennett & Posner, 1971) and human foetal intestine (Hart, 1974).

PPP has been observed to reduce responses of guinea pig ileum to nicotine, electrical stimulation and acetylcholine to varying degrees (Bennett, Eley & Stockley, 1973) suggesting that PPP might block nicotine receptors or nerve conduction. It is unlikely that the inhibition of bradykinin-induced responses was a result of such an action since it was shown earlier that bradykinin responses in rat terminal ileum do not appear to be mediated through cholinergic nerves.

It is interesting that responses to $PGF_{2\alpha}$ were inhibited significantly more by PPP than those of PGE_2. Similar observations have been reported for jird colon (Eakins, Karim & Miller, 1970), rat stomach (Collier 1973) and rat colon (Gagnon & Sirois, 1972; Somova, 1973) but not in rabbit jejunum and uterus (Eakins, Karim & Millier, 1970), guinea pig colon (Bennett & Posner, 1971), guinea pig ileum (Bennett, Charlier & Szechter, 1973), human stomach and colon (Bennett & Posner, 1971) or human foetal intestine (Hart, 1974). Differential inhibition of prostaglandin responses by PPP suggests the presence of more than one type of receptor on longitudinal smooth muscle of rat terminal ileum.

Eakins (1971) used a gel filtration method to fractionate PPP and found that the most active PG antagonist activity was recovered from the low molecular weight fractions. Eakins, Fex, Fredholm, Hogberg & Veige (1973) found that the dimer di-4-phloretin phosphate (DPP) was a more potent and selective antagonist of $PGF_{2\alpha}$ on jird colon compared with PPP. In the present study DPP did not alter responses to acetylcholine but responses to $PGF_{2\alpha}$ were very strongly inhibited. Responses to bradykinin and PGE_2 were reduced equally by DPP but to a lesser degree than $PGF_{2\alpha}$. The inhibition of responses to bradykinin by DPP is in contrast to the report of Eakins, Fex, Fredholm, Hogberg & Veige (1973) on jird colon although consistent with those reported above for PPP.

As with PPP responses to $PGF_{2\alpha}$ were more strongly affected by DPP than those to PGE_2. Eakins, Fex, Fredholm, Hogberg & Veige (1973) only studied the inhibitory action of DPP on $PGF_{2\alpha}$ while Bennett,

Charlier & Szechter (1973) observed antagonism of PGE_2 and $PGF_{2\alpha}$, although no details are available for this study. Unfortunately, there are few reports of the action of DPP on contractile responses of isolated smooth muscle preparations which is perhaps somewhat surprising in light of its reportedly greater potency and selectivity than PPP (Eakins, Fex, Fredholm, Hogberg & Veige, 1973). In the present study DPP was almost ten times more potent than PPP. The inhibition of responses to bradykinin and PGE_2 in the presence of DPP were almost identical, the only difference being at the lowest concentration of DPP.

SC 19220, a derivative of a series of dibenzoxazepine compounds synthesised by Coyne & Cusic (1968), was first shown to antagonise prostaglandins by Sanner (1969). In the present study SC 19220 was shown to inhibit the responses of rat terminal ileum to both bradykinin and PGE_2, the effect being very marked at the highest concentrations of SC 19220. The degree of inhibition of both these agonists was very similar at all concentrations of SC 19220 and were greater than that observed for acetylcholine and $PGF_{2\alpha}$. The present observations are in contrast with those of Sanner (1969, 1972) who reported that bradykinin-induced responses of guinea pig ileum were not affected by SC 19220.

SC 19220 has been reported to antagonise PGE_2 and $PGF_{2\alpha}$ equally in guinea pig ileum (Bennett & Posner, 1971; Sanner, 1969;1972) and rat stomach (Bennett & Posner, 1971; Splawinski, Nies, Sweetman & Oates, 1973) but on jird colon PGE_1 and PGE_2 were reported to be more sensitive to block by SC 19220 than $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (Eakins & Miller, 1970). The present findings would suggest that there are separate prostaglandin receptors for PGE_2 and $PGE_{2\alpha}$ and is consistent with findings reported above with PPP and DPP.

7 oxa-13-prostynoic acid (7 OPA) was seen to reduce the responses of rat terminal ileum to acetylcholine, bradykinin and PGE_2. The non specific depressant action of 7 OPA has been reported previously (Bennett & Posner, 1971; Ehrenpreis, Greenberg & Belman, 1973; Flack, 1970)

although selective inhibition of responses to PGE and PGF has been observed on jird colon (Fried, Santhana Krishnan, Himizu, Lin, Ford, Rubin & Grigas, 1969) and rat colon and chick rectum (Somova, 1973). Thus there would appear to be some doubt about the specificity of 7 OPA as a prostaglandin receptor antagonist and its usefulness as such would appear limited.

In summary it has been shown that the contractile action of bradykinin on longitudinal smooth muscle of rat terminal ileum was antagonised by three prostaglandin receptor antagonists, namely PPP, DPP and SC 19220 while having little or no effect on the contractile ability of the smooth muscle, as indicated by the lack of effect on responses to acetylcholine. Responses to $PGF_{2\alpha}$ and PGE_2 were affected differently by these compounds which suggests that there may be separate receptors for each prostaglandin. Furthermore the inhibition of responses to PGE_2 by PPP, DPP and SC 19220 was very similar to that for bradykinin. These findings support the previous observations with prostaglandin synthetase inhibitors and are consistent with the view that prostaglandins may be involved in the contractile action of bradykinin on rat terminal ileum. Furthermore the similarity between the effects of PPP, DPP and SC 19220 on responses to bradykinin and PGE_2 suggest that a prostaglandin of the E series is the most likely candidate for such a role.

(e) Release of Prostaglandin-like Substance from Rat Terminal Ileum

Bradykinin has been shown to increase the release of prostaglandins from a number of tissues (see Introduction).

(i) Prostaglandin Bioassay

It was seen that there was a basal release from rat terminal ileum of a substance which contracted rat stomach strip and that this was increased by bradykinin in the presence of the peptide SQ 20881, which has been shown to inhibit kininase (Greene, Camargo, Krieger, Stewart & Ferreira, 1972). Inclusion of hyoscine, mepyramine, methysergide, propranolol and phentolamine in the Tyrode solution surrounding the assay tissue served to exclude the possibility that the smooth muscle contracting substance released by bradykinin could have been acetylcholine, histamine, 5-HT, adrenaline or noradrenaline. Indomethacin was also included to increase the sensitivity of the assay tissue to prostaglandins (Eckenfels & Vane, 1972).

It was also noted that the characteristics of smooth muscle stimulation by the released substance was different from that usually observed with acetylcholine and histamine, being slower in action. The contracting substance was unlikely to be bradykinin since there was no detectable extraction of bradykinin from solutions stronger than those encountered in the organ bath.

The observation that release of smooth-muscle-contracting substance from rat terminal ileum was significantly reduced by aspirin, indomethacin, meclofenamic acid and flufenamic acid at concentrations used previously lends support to the idea that the smooth-muscle-contracting substance might be a prostaglandin-like substance. Since the previous experiments suggested a similarity between the action of bradykinin and PGE_2 on rat terminal ileum the prostaglandin-like material was bioassayed against standard PGE_2.

(ii) Characterisation of the Released Prostaglandin-like Substance

It was shown that the acidic ether extract of organ bath fluid which contained prostaglandin-like material produced three spots when chromatographed on silica gel plates. The observation that incubation of the tissue with indomethacin caused the disappearance of two of the spots suggested that these two spots may have been prostaglandin-like material. However neither of these spots showed similar chromatographic activity to standard PGE_1, PGE_2, $PGF_{2\alpha}$ or PGA_1. Thus it was decided to investigate if the prostaglandin-like material was a pharmacologically active metabolite of the primary prostaglandins. It has been reported that the gastrointestinal tract may be an important site of prostaglandin metabolism (Kunze, 1970; Pace-Asciak, Morawska & Wolfe, 1970; Parkinson & Schneider, 1969). Since it is known that 15-keto-PGE_2 is pharmacologically active on gastrointestinal preparations (Anggard, 1966; Anggard & Samuelsson, 1966; Crutchley & Piper, 1975) and that the intestine contains prostaglandin dehydrogenase enzyme (Anggard, Larsson & Samuelsson, 1971) which is the principal metabolising enzyme for prostaglandins (Samuelsson, 1970), the chromatographic activity of this substance was studied. 15-keto-PGE_2 showed a similar Rf value to the most prominent spot in the ether extract.

Thus it is considered that the substance responsible for prostaglandin-like activity in the extract of organ bath fluid might be tentatively identified as 15-keto-PGE_2. If this were so it might explain the relatively low levels of prostaglandin-like substance released into the organ bath fluid when compared with similar studies elsewhere (Ferreira, Herman & Vane, 1972) since 15-keto-PGE_2 is reported to be about one tenth as active as PGE_2 (Anggard, 1966; Anggard & Samuelsson, 1966; Crutchley & Piper, 1975).

CONCLUSIONS

The kallikrein-kinin system has been implicated in the control of sodium and water homeostasis (Adetuyibi & Mills, 1972; Marin Grez, Cottone & Carretero, 1972). This system has been shown to occur in the kidney and urine (Kraut, Frey & Werle, 1930; Nustad, 1970a,b), to produce diuresis and natriuresis (Barraclough & Mills, 1965) and to be correlated with sodium and water excretion (Adetuyibi & Mills, 1972; Marin Grez, Cottone & Carretero, 1972). However the mechanism by which the kallikrein-kinin system produces its natriuretic response has not been clarified although it would appear that bradykinin could affect renal haemodynamics (Willis, Ludens, Hook & Williamson, 1969), or have a direct action on tubular reabsorption of sodium (Alzamora & Capelo, 1973) or have both effects. It was the purpose of the first part of this study to investigate if bradykinin could affect transepithelial transfer of sodium and water in an in vitro preparation where haemodynamic factors were eliminated.

It was observed that bradykinin could have an effect on sodium and water transfer in rat jejunum which was dependent on the initial control level of water transfer. Thus when control water transfer was high bradykinin caused an inhibition of water transfer whereas when control transfer was low bradykinin stimulated transfer. An attempt was made to identify the mechanism operating in the inhibitory action of bradykinin on water transfer since this is the action which might explain the renal action of bradykinin.

It was decided to investigate the involvement of prostaglandins, which have been shown to inhibit sodium and water absorption in mammalian intestine (Al-Awqati & Greenough, 1972; Kimberg, Field, Johnson, Henderson & Gershon, 1971; Pierce, Carpenter, Elliott & Greenough, 1971), in the inhibitory action of bradykinin. Meclofenamic acid abolished this action of bradykinin, suggesting prostaglandin involvement. Prostaglandins A_1, E_1 and $F_{2\alpha}$ produced a significantly greater degree of inhibition of

water transfer when in combination with bradykinin than when either agent was used alone, again suggesting a possible relationship between the two substances.

Prostaglandins increase intestinal adenyl cyclase activity (Kimberg, Field, Johnson, Henderson & Gershon, 1974) and hence cyclic AMP which is known to inhibit intestinal transfer of sodium and water (Field, 1974; Kimberg, 1974). It was considered pertinent to investigate the involvement of cyclic AMP in the action of bradykinin on water transfer. The inhibitory action of bradykinin was potentiated by theophylline and dibutyryl cyclic AMP.

Therefore it was thought that the inhibitory action of bradykinin might involve both prostaglandins and cyclic AMP. Insufficient data is available to suggest a mechanism for the stimulatory action of bradykinin although some possibilities are considered above.

As an extension of the study it was decided to investigate the possible involvement of the prostaglandin-cyclic AMP systems in the contractile action of bradykinin on rat terminal ileum. The contractile action of bradykinin was shown to be independent of parasympathetic nerves as shown previously (Day & Vane, 1963; Khairallah & Page, 1961, 1963) but to be oxygen-dependent, which although not conclusive is at least consistent with prostaglandin involvement since prostaglandin synthesis is oxygen-dependent (Nugteren, Beerthuis & van Dorp, 1967; Samuelsson, Granstrom & Hamberg, 1967).

The contractile action was markedly reduced by four different prostaglandin synthetase inhibitors, namely aspirin, indomethacin, meclofenamic acid and flufenamic acid while responses to acetylcholine, PGE_2 and $PGF_{2\alpha}$ were either not effected or less affected than bradykinin.

Four prostaglandin receptor antagonists were used, namely, polyphloretin phosphate, diphloretin phosphate, SC 19220 and 7 oxa-13-prostynoic acid, to clarify the contractile action of bradykinin.

PPP, DPP and SC 19220 inhibited bradykinin, PGE_2-and $PGF_{2\alpha}$-induced contractions but not those of acetylcholine and there was a noticeable difference between the inhibitions of PGE_2 and $PGF_{2\alpha}$ by these agents. The inhibition of bradykinin was very similar to that of PGE_2 by PPP, DPP and SC 19220. Thus these experiments support the idea that prostaglandins may participate in the contractile action of bradykinin and indeed suggest a closer relationship between bradykinin and PGE_2 than between bradykinin and $PGF_{2\alpha}$.

Theophylline inhibited bradykinin-induced contractions but not those of acetylcholine. The role of cyclic AMP in smooth muscle contraction has not been clarified completely (Andersson, 1972; Bar, 1974) and it is premature to speculate on the significance of this observation.

Bradykinin in the presence of SQ 20881, an inhibitor of kininase (Greene, Camargo, Krieger, Stewart & Ferreira, 1972) caused an increase in the output of prostaglandin-like material from rat terminal ileum which was prevented by aspirin, indomethacin, meclofenamic acid and flufenamic acid. Attempts were made to characterise this activity by TLC but there was no activity corresponding to standard PGE_2, $PGF_{2\alpha}$ or PGA_1. However, indomethacin pretreatment caused disappearance of a spot which had similar mobility to 15-keto-PGE_2, which is the major metabolite of PGE_2. Incubation of rat terminal ileum with PGE_2 caused the appearance on TLC plates of a spot other than that for PGE_2 which had the same mobility as 15-keto-PGE_2.

Thus it is concluded that prostaglandins are involved in the contractile action of bradykinin although the exact nature of this involvement requires some more clarification

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