THE INTERRELATIONSHIP BETWEEN BRADYKININ AND CYCLO-OXYGENASE PRODUCTS IN THE RAT SMALL INTESTINE

BY

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Ph.D. Thesis
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The effect of bradykinin on the release of prostaglandins from the rat isolated terminal ileum has been investigated. The release of prostaglandins from the ileum, into the surrounding bathing fluid, was measured by radioimmunoassay and by bioassay. Under basal conditions more PGE-like than PGF-like material was released from the ileum. When the ileum was incubated with bradykinin, an increase in the release of PGF-like, but not PGE-like material was observed.

The release of prostaglandin-like material from the rat isolated perfused terminal ileum was assayed on the rat fundus strip. Bradykinin perfused over either surface of the ileum contracted the rat fundus strip more than when the ileum was absent. No increase was observed in the presence of indomethacin. However, it was found difficult to quantify the release of prostaglandin-like material after exposure to bradykinin, since contractions of the assay tissue to bradykinin appeared to be potentiated by the release of prostaglandin-like material from the ileum.

The effect of the cyclo-oxygenase inhibitor indomethacin, was investigated upon contractions of the rat isolated perfused ileum to bradykinin. Contractions of the ileal longitudinal muscle to bradykinin during mucosal, but not serosal perfusion of the ileum, were reduced in the presence of indomethacin. Furthermore, in the ileum from rats pretreated with indomethacin, contractions to bradykinin were also only reduced during mucosal perfusion. A similar interrelationship on the mucosal surface, between bradykinin and cyclo-oxygenase products, was demonstrated in the relaxation of the rat isolated perfused duodenum to bradykinin. The additional involvement of cyclic nucleotides and a neuronal mechanism in the response of the mucosal perfused rat small intestine to bradykinin is suggested.

It is concluded that cyclo-oxygenase products are involved in the response of the mucosal, but not the serosal perfused rat small intestine to bradykinin, although the exact nature of this involvement is unclear.

Key words: Bradykinin Cyclo-Oxygenase Products Intestine

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GENERAL INTRODUCTION

The present study has involved an investigation of the interrelationship between bradykinin and cyclo-oxygenase products, in particular the prostaglandins, in the rat intestinal tract. Thus, it is considered relevant to review the biochemistry of both bradykinin and prostaglandins, and to briefly outline their role, actions and interactions, with particular reference to the gastrointestinal tract.

This study was stimulated by the report of Crocker and Willavoys (1976), that prostaglandins participate in the contractile action of bradykinin on the longitudinal muscle of rat isolated terminal ileum. Since bradykinin had been previously demonstrated to stimulate the release of prostaglandins from several organs including the dog kidney (McGiff et al., 1972), cat isolated spleen (Ferreira et al., 1973a) and guinea-pig isolated lungs (Palmer et al., 1973), the existence of a similar bradykinin-induced prostaglandin release was investigated in the rat ileum. The study was extended to investigate the role of cyclo-oxygenase products, and the possible additional involvement of cyclic nucleotides or a neuronal mechanism, in the response of the longitudinal muscle of rat small intestine to bradykinin. Thus, the mode of action of bradykinin on isolated gastrointestinal smooth muscle has also been reviewed, together with the possible involvement of prostaglandins with cyclic AMP and nerve activity.

When this study was initiated it was believed that prostaglandins of the E and F series were the main products of the ubiquitously distributed membrane-bound enzyme

cyclo-oxygenase. These primary prostaglandins were therefore the subject of the initial experiments performed. However, as this study progressed, there was mounting evidence in the literature that endoperoxides were not always converted to the classical prostaglandins and in fact were often converted almost entirely to thromboxanes (Hamberg et al., I975b) or prostacyclin (Moncada et al., I976a). Facilities were not available to assess the direct involvement of these compounds in the studies performed. However, where relevant, an indication of their possible involvement is outlined in the literature review. Subsequent studies were concerned with the involvement of cyclo-oxygenase products in general, and not simply with the primary prostaglandins. The involvement of cyclo-oxygenase products was indirectly assessed with the use of inhibitors of cyclo-oxygenase activity. Thus, it is also considered relevant to briefly review the literature on inhibitors of cyclo-oxygenase activity.

SECTION I PROSTAGLANDINS

A. Biochemistry

Several recent reviews have dealt with the biochemistry of prostaglandins: ElAttar (I978); Flower (I978); Samuelsson et al., (I978a,I978b); Vane (I978); and Vapaatalo and and Parantainen (I978).

(i) History

The biological actions of extracts from accessory genital glands were described in I906 by Japelli and Scafa. Similar studies were made by other workers (see von Euler I936) but received little attention since the experimental data did not permit conclusions to be made regarding the nature of the active component. The first report of a biologically active and specific compound from human seminal fluid was by Kurzrok and Lieb (I930). This was followed by the independent observations of Goldblatt (I933) and von Euler (I934) that human seminal fluid and secretions from the prostate and seminal vesicles, altered the tone of smooth muscle preparations in vitro, and lowered the blood pressure of experimental animals. This active substance was given the name prostaglandin by von Euler (I935), since he believed the new factor was produced in the prostate glands.

Research into prostaglandins received little attention until Bergstrom and Sjovall (I957,I960a,I960b) isolated the first pure prostaglandins from freeze-dried sheep prostate glands. Subsequent rapid technological development permitted Bergstrom et al. (I963) to elucidate the structure of prostaglandin E_I, $F_{I\alpha}$ and $F_{I\beta}$ as oxygenated C_{20} fatty acids.

Consequently, prostaglandins became more readily available and work was centred on the mechanism of prostaglandin biosynthesis. Samuelsson (I965) proposed that an endoperoxide structure was formed as an intermediate in the biosynthesis of prostaglandins, but it was not until I973 that the first unstable endoperoxide PGH_2, initially called compound X, PGR_2 or LASS, was isolated by Hamberg and Samuelsson. This was followed by the isolation of an additional endoperoxide, PGG_2 (Hamberg et al., I974a; Nugteren and Hazelhof, I973). The metabolism of endoperoxides to a I7-C fatty acid HHT (I2-hydroxy-heptadecatrienoic acid), and malondialdehyde was also described (Hamberg and Samuelsson, I974a, I974b; Hamberg et al., I974b). Subsequently, it was observed that endoperoxides could be readily metabolized to give thromboxane A_2 and thromboxane B_2, which was initially called PHD (Hamberg et al., I975b).

In the search for a thromboxane synthesizing enzyme, it was found that endoperoxides were converted to a substance with opposite biological properties to those of thromboxanes (Bunting et al., I976; Gryglewski et al., I976; Moncada et al., I976a, I976b). The structure of this new unstable product, initially called PGX, was elucidated in I976 by Johnson et al., and was named prostacyclin.

Incubation of platelets with arachidonic acid revealed an alternative to the classical cyclo-oxygenase pathway which produces the endoperoxides, thromboxanes, prostacyclin and prostaglandins described above. It was demonstrated that arachidonic acid could also be converted by a lipoxygenase enzyme, to the noncyclized I2-hydroperoxy derivative of arachidonic acid (HPETE) and its corresponding I2-hydroxy acid

(HETE) (Hamberg and Samuelsson, I974a; Nugteren, I975). Recently, an additional lipoxygenase pathway was described which results in the formation of 5-hydroperoxy derivatives of arachidonic acid; a group of compounds which have been termed the leucotrienes (Samuelsson, I979). One member of this group, leucotriene C, is the compound formerly known as slow-reacting substance or SRS, which was first described by Feldberg and Kellaway in I938.

(ii) Biosynthesis

It has been shown that prostaglandins are formed by the action of a membrane-bound multienzyme complex, called cyclo-oxygenase, which catalyses the oxidation of polyunsaturated fatty acids to endoperoxides and their subsequent transformation to prostaglandins. The predominant precursor fatty acids have been demonstrated to be arachidonic acid (5,8,II,I4-eicosatetraenoic acid) and dihomo-ɣ-linolenic acid (8,II,I4-eicosatrienoic acid), which form prostaglandins of the 2 and I series, respectively (Bergstrom et al., I964; van Dorp et al., I964; Samuelsson, I965). Only non-esterified fatty acids were found to be substrates for the enzyme (Lands and Samuelsson, I968; Vonkeman and van Dorp, I968) and these were shown to originate from a number of intracellular lipid pools; cholesterol esters, phosphatides, mono, di, or triglycerides, under the influence of the enzyme phospholipase A_2 (see Flower, I978). From these precursors a cyclo-oxygenase was shown to form cyclic endoperoxides (PGG and PGH), which were isolated and characterized by Nugteren and Hazelhof (I973), Hamberg and Samuelsson (I973, I974a, I974b), Hamberg et al. (I974a).

PGG_2 and PGH_2 have been shown to possess considerable

biological activity and cause smooth muscle contraction (Hamberg et al., I975a), induction of platelet aggregation (Hamberg et al., I974a) and modification of adenyl cyclase levels (Gorman et al., I975). The endoperoxides are unstable in aqueous media and spontaneously decompose ($t_{\frac{1}{2}}$ 4-6min) to a mixture of PGE_2 and PGD_2.

After the formation of PGG by the cyclo-oxygenase, the synthesis of the primary prostaglandins has been postulated to proceed in at least two directions. Samuelsson and Hamberg (I974) suggested an isomerase converted PGG to I5-hydroperoxy PGE, whilst Nugteren and Hazelhof (I973) suggested a peroxidase enzyme converted PGG to PGH. Both I5-hydroperoxy PGE and PGH were then converted to PGE. The favoured route is the reaction of PGH to the more stable PGE, catalysed by a glutathione-dependent isomerase (Ogino et al., I977). PGF is synthesized from the endoperoxide by an enzyme endoperoxide reductase, although Nugteren and Hazelhof (I973) have suggested this conversion could be non-enzymatic. Nugteren and Hazelhof (I973) further reported the conversion of PGH compounds into PGD, by a PGD isomerase.

It was initially believed that prostaglandins of the E series were not converted to the F series in the body. However, Hamberg and Samuelsson (I972) demonstated that in the guinea-pig, the main urinary metabolite of PGE_2 was an F_{β} derivative.The prostaglandin-9-ketoreductases were shown to convert PGE to PGF, and required either NADH or NADPH as cofactors (Lee and Levine, I974; Kaplan et al., I975; Stone and Hart, I975) or were active in the absence of added cofactors (Hensby, I974). The presence of these enzymes was subsequently detected in many tissues from several species,

including human, sheep, chicken, monkey, rat, pigeon, swine, rabbit and horse (see Granstrom, I977). Hassid and Levine (I977) isolated prostaglandin-9-ketoreductase from chicken kidney in multiple forms and found it reversibly transformed the functional group at C-9 and also the oxidation or reduction of the C-I5 functional group. Thus, prostaglandin-9-ketoreductases may have a dual role in vivo. They could regulate the relative levels of E_2 and $F_{2\alpha}$ in a physiological process, or function in metabolism by oxidizing the prostaglandins at C-I5.

Hamberg et al. (I975b) found that PGG_2 was converted enzymatically in human platelets via the unstable thromboxane A_2, to thromboxane B_2, its stable derivative. Thromboxane A_2 was determined to have a half-life of 32 secs at 37°C in aqueous medium at pH 7.4. It has recently been shown that the formation of thromboxanes is not limited to platelets, but may also occur in several tissues including lung, spleen, kidney, leucocytes, umbilical artery and brain (see Samuelsson et al., I978a).

The biosynthesis of prostacyclin from arachidonic acid or endoperoxides (PGG_2 or PGH_2) was first described in rabbit and pig aortas (Moncada et al., I976a), and rat fundus strip (Pace-Asciak, I976). More recent work has revealed that prostacyclin synthetase is widespread in the body and its formation has been reported in several tissues, including bull and sheep seminal vesicles, guinea-pig lung, rat, monkey and human uterus, rat and guinea-pig heart, human kidney, rabbit lung and rat small intestine (see Weeks, I978). It has been shown that prostacyclin is a potent vasodilator, a relaxant of isolated vascular tissue, and an

inhibitor of platelet aggregation (Bunting et al., I976; Moncada et al., I976a, I976b; Gryglewski et al., I976). The half-life of prostacyclin in aqueous media at pH 7.4 and at either 4°C or 38°C was estimated to be I4.5 min (Cho and Allen, I978) and 3-5 min (Levy, I978a), respectively.

Thus, the endoperoxide PGH_2, serves as a common intermediate for the enzymatic production of prostaglandins (PGEs, PGFs, and PGDs), thromboxanes and prostacyclin. Furthermore, the products have different biological actions which may be either complementary or antagonistic. Thus the response of an organ to activation of cyclo-oxygenase, will represent the sum of a number of complex interactions.

(iii) Metabolism

A large number of prostaglandin degradation pathways, widely distributed throughout the animal body, have been described and reviewed: Hamberg and Samuelsson (I97I); Samuelsson et al. (I97I); Hansen (I976); Granstrom (I977).

Enzymic mechanisms have been shown to exist, at least for the PGE and PGF series, whereby the biological activity of the molecule is rapidly destroyed and the metabolite is excreted in the urine. It has been demonstrated that the most important pathways for the metabolism of the prostaglandins are:

(a) Dehydrogenation of the I5-hydroxyl group to the corresponding I5-keto compound, under the influence of the enzyme prostaglandin-I5-hydroxydehydrogenase (PGDH), (Anggard and Samuelsson, I964, I966; Hamberg and Samuelsson, I97I). I5-keto prostaglandins can be formed by two different enzymes. PGDH I catalyzes the dehydrogenation with NAD^+ as substrate,

whereas PGDH II can only use $NADP^+$ (Lee and Levine, I975; Lee et al., I975).

(b) Reduction of the C-I3 double bond by the enzyme prostaglandin Δ^{I3} reductase (Anggard and Samuelsson, I964, I966; Anggard et al., I97I). This reduction follows the dehydrogenation in most situations.

(c) ω-oxidation of the alkyl side chain following hydroxylation at C-I9 or C-20. This precedes oxidation of the β-side chain and yeilds di-nor or tetra-nor compounds, which result in the formation of a dicarboxylic acid (Israelsson et al., I969; Nakano and Morsy, I97I; Samuelsson et al., I97I).

Reactions (a) and (b), in which the prostaglandins base their biological activity, are relatively rapid and catalyzed by prostaglandin specific enzymes. Whilst those reactions in (c) are relatively slow, and oxidized by enzymes responsible for β- and ω-oxidation of fatty acids in general.

The metabolism of the short-lived thromboxane A_2 and prostacyclin to their relatively biologically inactive metabolites, thromboxane B_2 and 6-keto $F_{I\alpha}$ respectively, has been shown to occur non-enzymatically. However, it has been demonstrated in the rabbit lung (Wong et al.,I978), and in studies of rat urinary metabolites (McGuire and Sun, I978), that some prostacyclin is metabolized by PGDH to I5-keto-prostacyclin, prior to its hydrolysis to the 6-keto-derivative.

B. Inhibitors Of Cyclo-Oxygenase Activity

Drugs which inhibit the cyclo-oxygenase enzyme have made a significant contribution to prostaglandin research. Several comprehensive reviews have dealt with non-steroidal

anti-inflammatory drugs as inhibitors of cyclo-oxygenase activity: Ferreira and Vane (I974); Flower (I974); Lands and Rome (I976); Ferreira (I977).

The discovery that aspirin and indomethacin could inhibit cyclo-oxygenase activity was made in I97I (Ferreira et al., I97I; Smith and Willis, I97I; Vane I97I). This effect was presumed to play a major role in the action of these drugs, and provided an important tool for the determination of the role of intrinsic cyclo-oxygenase products in numerous systems.

The non-steroidal anti-inflammatory drugs have been shown to be inhibitors of cyclo-oxygenase activity in tissue homogenates, isolated organs, and whole animals, although the situation is often complicated (see Flower, I974, and Needleman, I978) by;

(a) the presence of enzymes which metabolize the cyclo-oxygenase products;

(b) the ability of drugs to reach the cyclo-oxygenase enzyme;

(c) the differential sensitivity of the tissues to the inhibitors;

(d) the differences in the duration of action of the cyclo-oxygenase inhibitors;

(e) the fact that one often relies on the release of cyclo-oxygenase products to indicate cellular synthesis; and

(f) the blockade of the synthesis of all cyclo-oxygenase products including those unrelated to the mechanism studied.

It has now been demonstrated that, with the exception of gold, penicillamine and colchicine, all of the many non-

steroidal anti-inflammatory drugs inhibit prostaglandin biosynthesis in vitro (Ferreira and Vane, I974; Flower, I974; Vane, I978). However, the mechanism of inhibition of cyclo-oxygenase activity by the non-steroidal anti-inflammatory drugs is poorly understood. Ku and Wasvary (I973) and Lands et al. (I973) showed that inhibition by these drugs was of a dual nature,termed "competitive-irreversible." According to this concept, the drug interacts, in a time-dependent fashion, with a binding site, sufficiently close to the active centre to reduce the catalytic activity of the enzyme. While in combination with the substrate, the enzyme cannot bind the inhibitor and this gives rise to a competitive effect. However, since there is always a fraction of the enzyme which is not in combination with the substrate and which is therefore free to combine (irreversibly) with the inhibitor, the substrate concentration cannot influence the ultimate inhibition of the cyclo-oxygenase by these drugs.

More recently, it has been demonstrated that aspirin inhibits cyclo-oxygenase activity by covalently acetylating the active site of cyclo-oxygenase (Roth et al., I975). When aspirin modified this enzyme from human platelets, the effect was permanent, persisting for the lifespan of the aspirin-treated platelets (Roth and Majerus, I975). Presumably, in other tissues in which protein synthesis occurs, new enzyme synthesis could restore cyclo-oxygenase activity. In contrast, indomethacin, a potent inhibitor of cyclo-oxygenase, does not covalently modify the enzyme (Stanford et al., I977). Saeed and Cuthbert (I977), suggested that indomethacin inhibited cyclo-oxygenase by interfering with co-factor induced stimulation of the enzyme.

In addition to inhibition of cyclo-oxygenase, the non-steroidal anti-inflammatory drugs have been shown to affect other enzymes and cellular systems (see Flower, I974), although the concentration required was often much higher than that required to inhibit cyclo-oxygenase. Tolman and Partridge (I975) indicated the possibility of a direct interaction between indomethacin and other non-steroidal anti-inflammatory drugs, with PGE_I, at a common receptor site in rat epididymal adipocytes. In rat kidney homogenates, indomethacin has been shown to act as an inhibitor of the prostaglandin metabolizing enzymes 9-hydroxy dehydrogenase, Δ^{I3} reductase, and I5-hydroxy dehydrogenase at concentrations of 2, I0, and 50µcg/ml, respectively (Pace-Asciak and Cole, I975). Smith (I975,I978) challenged the role of inhibition of cyclo-oxygenase as the site of action of non-steroidal anti-inflammatory drugs and suggested that interference with leucocyte emigration was an equally important role, at least in the anti-inflammatory actions of indomethacin. Furthermore, a correlation between a calcium antagonistic action and anti-inflammatory action, particularly for indomethacin, has been proposed (see Northover, I977).

Recently, the existence of an endogenous inhibitor of cyclo-oxygenase has been demonstrated in canine mucosal microsomes (LeDuc and Needleman, I979), human plasma (Saeed et al., I977; Collier et al., I979) and pig renal cortex (Terragno et al., I978). However, the existence of an endogenous inhibitor of cyclo-oxygenase in plasma has been disputed (Whittle, I978b). Nevertheless, the possible existence of such an inhibitor provides the attractive possibility of an additional regulatory site of action for non-steroidal anti-inflammatory drugs.

C. Prostaglandins and the Gastrointestinal Tract

(i) Role of Prostaglandins in the Gastrointestinal Tract

The possible physiological roles for prostaglandins in the gastrointestinal tract are too numerous to discuss in detail. Among the postulated roles are regulation of intestinal motility, gastric secretion, salivary secretion, intestinal secretion and absorption, and insulin secretion, all of which have been adequately reviewed elsewhere (see Bennett, I976a, I977a). Similarly, prostaglandins have been implicated in various diseases of the gastrointestinal tract including diarrhoea, carcinoma and inflammation, which have also been reviewed by Bennett (I976a, I976b). The literature review presented covers those actions of prostaglandins involved in intestinal smooth muscle contractility. The possible involvement of nerve activity or cyclic nucleotides, in the smooth muscle response to prostaglandins, will also be discussed.

(ii) Occurrence and Distribution of Prostaglandins in the Gastrointestinal Tract

Prostaglandin-like material has been found in the gastrointestinal tract of all species studied. Prostaglandin E-like and F-like material was initially demonstrated to be readily released by drug, nerve or hormone stimulation, or the presence of biosynthetic substrates, from the intestinal tract of the guinea-pig and rabbit (Ambache et al., I966), frog (Vogt et al., I966), rat (Bennett et al., I967), man (Bennett et al., I968b) and pig (Miyazaki, I968). Subsequent

studies have shown a similar release from numerous other species (see Bennett, I976a). The formation of PGD by gastrointestinal tissue was first demonstrated by Nugteren and Hazelhof (I973). Recently, Knapp et al. (I978) made the first formal identification, by gas chromatography-mass spectrometry, of the products from whole cell preparations of rat stomach corpus, jejunum and colon and identified PGE_2, $PGF_{2\alpha}$ and PGD_2. There are no reports of the presence of the A and B series of prostaglandins in the intestinal tract, although PGA_2-like material was found in human saliva and gastric juice (Peskar et al., I974). Prostacyclin has been shown to be generated by the gastric mucosa and muscle of several species (Moncada et al., I978) and to be the predominant product of PGH_2 in the rat small intestine (Sun et al., I977).

Most of the primary prostaglandins found in human and rat stomach were thought to occur in the mucosa rather than the muscle (Bennett et al., I967, I968b), with largest amounts near to the inner surface in human gastric mucosa (Bennett, I972). More recently Bennett et al. (I977) demonstrated that production of PGE-like and PGF-like material in the human ileal mucosa was greater than that of ileal muscle, whilst production in the colon mucosa was less than that in colon muscle. Sanders and Ross (I978) demonstrated that muscle layers of dissected segments of cat intestine contained twice as much PGE-like material compared to the mucosal layers. Eley et al. (I977) suggested that in the guinea-pig isolated ileum PGE_2 was generated in the longitudinal muscle (and mucosa), whereas $PGF_{2\alpha}$ alone or together with PGE_2 was generated in the circular muscle and/or enteric plexus.

LeDuc and Needleman (I979) demonstrated that muscularis microsomes from all regions of the dog gastrointestinal tract quantitatively converted PGH_2 to 6-keto $PGF_{I\alpha}$. This was different from arachidonic acid metabolism which resulted in the formation of both PGE_2 and 6-keto $PGF_{I\alpha}$. Furthermore, LeDuc and Needleman (I979) also demonstrated, using PGH_2 as substrate, that the mucosa possessed active thromboxane and prostacyclin synthetases.

The ability of the gastrointestinal tract to inactivate prostaglandins in vitro and in vivo has been demonstrated (Kunze, I970; Pace-Asciak et al., I970; Parkinson and Schneider, I969). However, few studies have been concerned with the distribution of the prostaglandin metabolizing enzymes. Peskar (I978) recently performed such a study on the human gastrointestinal tract, and found the prostaglandin metabolizing enzymes PGDH, Δ^{I3} reductase and PGE 9-ketoreductase in all regions.

(iii) Effect of Prostaglandins on Gastrointestinal Smooth Muscle

Several reviews have dealt exclusively with the effect of prostaglandins on the gastrointestinal tract and these include: Bennett and Fleshler (I970); Bennett (I972, I976a, I976b, I977a, I977b); Wilson (I972, I974); Main (I973); Waller (I973); Robert (I974, I976, I977); and Karim and Ganesan (I974).

Only prostaglandins of the E and F series were thought to affect the contractility of gastrointestinal tissue (Bennett and Fleshler, I970; Bennett, I972). Adaikan and Karim (I976) confirmed that prostaglandins of the A and B series were less potent than PGE_2 or $PGF_{2\alpha}$ on the gastrointestinal

smooth muscle of various species in vitro. Nugteren and Hazelhof (I973) reported that PGD_I and PGD_2, in contrast to PGE_I, possessed negligible biological activity. However, Horton and Jones (I974) demonstrated that PGD_2 had significant pharmacological activity on rat fundus strip and isolated rabbit jejunum. Furthermore, it was found that PGG_2 and PGH_2 had comparable activity to PGE_2 and $PGF_{2\alpha}$ on the gerbil colon and rat stomach, in vitro (Hamberg et al., I975a).

Prostaglandins of the E and F series contract the longitudinal muscle of the gastrointestinal tract of many species in vitro, and these include the cat, dog, guinea-pig, hamster, man, rabbit and rat (see Bennett, I972). However, PGE was reported to relax the longitudinal muscle of human gastric antrum (Bennett et al., I968b) and rat duodenum (Khairallah et al., I967), whilst PGF was found to relax the longitudinal muscle of human distal colon (Bennett and Fleshler, I970). The circular muscle of the gastrointestinal tract, in vitro, generally respond to PGF with a contraction and to PGE with a relaxation as demonstrated in the dog, guinea-pig, man and rat (see Bennett, I972). However, a relaxation to PGF has been demonstrated in the circular muscle of human proximal colon (Bennett and Posner, I97I) and human stomach (Adaikan and Karim, I976); whilst a contraction to PGE has been reported in the circular muscle of rat stomach (Bennett and Fleshler, I970) and human ileum and distal colon (Bennett et al., I968a). Furthermore, Eley and Bennett (I979) demonstrated that although PGE_I and PGE_2 relaxed circular muscle strips of rat colon in vitro, studies performed in vivo showed that PGE_2 caused circular muscle contraction, whilst PGE_I caused a relaxation. The action of

PGD_2, on the circular muscle of guinea-pig isolated ileum and colon, was investigated by Bennett and Sanger (I978) and shown to be predominantly excitatory "$PGF_{2\alpha}$-like" in the colon, and inhibitory "PGE_2-like" in the ileum.

Therefore, it appears that the response of gastrointestinal smooth muscle to prostaglandins is dependent upon the prostaglandin, the species, the tissue and the muscle studied. Furthermore, even if all these parameters are identical the response in vivo may be different to that observed in vitro.

(iv) Prostaglandins and Nerve Activity in the Gastrointestinal Tract

There is considerable evidence indicating an involvement of prostaglandins in neurotransmission in the autonomic nervous system, since;

(a) prostaglandins are released in the vicinity of autonomic neuroeffector junctions by various stimuli;

(b) prostaglandins modulate transmitter release from nerve terminals and the response to the transmitter; and

(c) inhibition of cyclo-oxygenase produces opposite effects to the prostaglandins, (see Hedqvist, I97I, I976, I977; Starke, I977; Westfall, I977).

(a) Prostaglandins and Cholinergic Neurotransmission

In the gastrointestinal tract prostaglandins may have either, a pre-synaptic or a post-synaptic effect on cholinergic transmission, or both. Evidence for a pre-synaptic role of the prostaglandins in cholinergic transmission has been indicated

by measurement of acetylcholine release. Hall et al. (I975) demonstrated that acetylsalicylic acid inhibited the release of acetylcholine from the guinea-pig ileum and this inhibition could be restored by PGE_I. Kadlec et al. (I975) obtained similar results with PGE_2 but not with $PGF_{2\alpha}$. In a further investigation (Kadlec et al., I978), it was demonstrated that the effect of PGE_2 on the output of acetylcholine, both at rest and during stimulation, was inversely related to the initial level of this output. Schulz and Cartwright (I976) found only a partial restoration with PGE_I of the acetylcholine release reduced by the administration of morphine. Evidence which indicated a pre-synaptic site of action for prostaglandins, but in which acetylcholine release was not measured, was reported by Ehrenpreis et al. (I973,I976a, I976b) and Ehrenpreis and Greenberg (I975). However, studies on the guinea-pig isolated ileum by Botting and Salzmann (I974), Illes et al.(I974) and Hazra (I975) failed to reveal facilitation of acetylcholine release by PGE_2, or inhibition by indomethacin.

Prostaglandins have a post-synaptic action on cholinergic neurotransmission. This action involves the ability of prostaglandins to enhance the response of an agonist, an action first observed on smooth muscle by Hall and Pickles (I963). Enhancement by prostaglandins of guinea-pig ileum contractions, has been shown for the electrically induced twitch (Harry, I968; Famaey et al., I975; Hall et al., I975) and tetanic contractions (Ambache and Freeman, I968) as well as for exogenous acetylcholine (Famaey et al., I975; Schulz and Cartwright, I976). Recently, Laekeman and Herman (I978) demonstrated that PGE_I, PGE_2, $PGF_{2\alpha}$, and arachidonic acid,

partially increased the hyoscine inhibited twitch, and the tetanic response of the guinea-pig ileum to electrical stimulation. They suggested that although their results indicated a post-synaptic sensitization effect, a pre-synaptic facilitatory activity on transmitter release could operate simultaneously, and that according to the tissue or experimental parameters used, one system might give an erroneous view of physiological effects. Hedqvist and Gustafsson (I979) studied the isolated longitudinal muscle of guinea-pig ileum and determined that whilst acetylcholine release was not altered by PGE_2, indomethacin did decrease acetylcholine release and this effect was partially antagonized by PGE_2. They suggested that both PGE_2 and cyclo-oxygenase inhibition, affect cholinergic transmission mainly, if not exclusively, at a post-synaptic level.

Alternatively, some workers have investigated the action of exogenous prostaglandins on nerves. It has been demonstrated that low concentrations of PGE_I, PGE_2, $PGF_{I\alpha}$, and $PGF_{2\alpha}$ contract the longitudinal muscle of guinea-pig isolated ileum, in part by a direct action on the muscle cells, and partly through stimulation of nervous elements. In the ileum the nerves stimulated were cholinergic irrespective of the prostaglandin, but in the colon, $PGF_{I\alpha}$ and $PGF_{2\alpha}$ stimulated cholinergic nerves, whereas PGE_I and PGE_2 stimulated non-cholinergic excitatory nerves (Bennett et al., I968c, I975; Harry, I968). In an extension of this work on the guinea-pig isolated ileum, Stockley (I979) proposed that endogenous prostaglandin synthesis was necessary to maintain maximal cholinergic transmission.

(b) Prostaglandins and Sympathetic Neurotransmission

Prostaglandins have been shown to modulate sympathetic neurotransmission in different tissues including the gastro-intestinal tract (see Hedqvist, I977). In the rabbit jejunum and ileum, relatively small amounts of PGE_I inhibited the effect of sympathetic nerve stimulation at a pre-synaptic site (Illes et al., I973, I974; Abdel-Aziz, I974; Hedqvist, I974; Hedqvist and Persson, I975). Similar results were reported by Bartho (I978) from studies on the guinea-pig ileum. In the same paper, Bartho also demonstrated the existence of a post-synaptic site of action, since the action of exogenous noradrenaline was reduced by PGE_I or PGE_2 and potentiated by indomethacin. In contrast, Ehrenpreis et al. (I973) reported that in the guinea-pig isolated ileum, prostaglandins failed to reverse the action of electrically induced contractions blocked by noradrenaline, an observation in direct conflict to that of Gintzler and Musacchio (I974). However, in a more recent paper Ehrenpeis et al. (I978) have reported a reversal of noradrenaline blocked electrical contractions with prostaglandins. They attributed this effect to a prostaglandin-induced enhancement of noradrenaline autoxidation. Sakato and Shimo (I976) reported indirect evidence that, in the guinea-pig taenia caecum, adrenergic neurotransmission was modulated by PGE_I. In an extension of this work Ishii et al. (I977) confirmed the observation by measurement of a stimulation-evoked increased overflow of noradrenaline, at low frequencies, in the presence of indomethacin.

Some workers favour an interrelationship between cholinergic nerves, adrenergic nerves and prostaglandins.

Kadlec et al. (1974) suggested endogenous PGE_2 inhibited the release of noradrenaline from sympathetic nerve endings in the guinea-pig isolated ileum. The blockade of cyclo-oxygenase prevented this inhibition, with a subsequent increase of noradrenaline release. This could in turn decrease the release of acetylcholine from cholinergic nerve terminals. A similar hypothesis was also proposed by Botting (1977), based upon studies on the same tissue. He suggested that prostaglandins reduced the release of noradrenaline, with consequent removal of the inhibition of acetylcholine release produced by the action of endogenous noradrenaline on pre-synaptic α-receptors. Recently, Masek et al. (1979) have demonstrated that in the myenteric plexus-longitudinal muscle preparation of guinea-pig ileum, neuronally released noradrenaline may affect the function of the cholinergic system through an effect upon prostaglandin release.

Therefore, there is considerable evidence to implicate prostaglandins, particularly of the E series, as modulators of intestinal motility through effects on both cholinergic and adrenergic neurotransmission. The possible involvement of prostacyclin in intestinal neurotransmission is unclear at present. However, Kadowitz et al. (1979), demonstrated prostacyclin could modulate the effect of the sympathetic nervous system in the cat intestinal vascular bed, but this was by an effect on the vascular smooth muscle and not on nerve terminal release.

(v) Prostaglandins and Cyclic Nucleotides

There have been several excellant reviews on prostaglandin-cyclic nucleotide interactions in mammalian tissue

(Kuehl, I974; Kuehl et al., I976; Samuelsson et al., I975, I978b).

The involvement of prostaglandins with the function of the intracellular adenyl cyclase-cyclic AMP-phosphodiesterase was suggested by Steinberg et al., (I964). However, the mechanism of this involvement remains unclear. Nevertheless, the majority of prostaglandin effects have been explained by their ability to regulate cyclic nucleotide metabolism. In this respect, prostaglandins of the E series have generally been found to be more potent than those of the F series (see Curtis-Prior, I976 and Kuehl et al., I976). However, the recently discovered prostacyclin appears to be a more potent stimulus of adenyl cyclase activity in platelets (Best et al., I977; Gorman et al., I977; Tateson et al., I977) and of comparable activity in human colonic mucosa (Simon and Kather, I978; Simon et al., I978) to the most potent primary prostaglandin. However, not all prostaglandin effects are associated with an increased level of cyclic AMP. In adipose tissue PGE_I has been shown to inhibit cyclic AMP, raising the possibility that it may form part of a feedback regulating system (Ramwell and Shaw, I970).

Kuehl et al. (I976) suggested that prostaglandins of the F series may stimulate the synthesis of cyclic GMP and this has since been confirmed by several workers (see Horrobin, I978). Recently it has been suggested that the prostaglandin endoperoxides may be the most potent stimulators of guanylate cyclase (Glass et al., I977; Goldberg and Haddox, I977; Hidaka and Asano, I977).

The contractile response of various tissues to prostaglandins has been suggested to be mediated by cyclic

nucleotides. Studies on myocardial preparations have demonstrated the positive inotropic action of prostaglandins may be mediated by the adenyl cyclase-cyclic AMP system (see Vapaatalo et al., I978). Reports on the stimulatory effect of PGE_I on adenyl cyclase in the uterus and various parts of the oviduct have appeared (Harbon and Clauser, I97I; Beatty et al., I973; Lerner et al., I973). Furthermore, PGE_2 has been shown to contract the gall bladder and relax the sphincter of Oddi (Andersson et al., I973). These effects were accompanied by a decrease in gall bladder and an increase in sphincter cyclic AMP concentration.

In the gastrointestinal tract both cyclic GMP and cyclic AMP are reported to be present, although the role of both cyclic nucleotides, and particularly that of cyclic GMP, is unclear. Ong et al. (I975) reported distinct differences in the localization of cyclic AMP and cyclic GMP in the rat small intestine. Cyclic GMP was found in the villus brush border membrane as well as in the crypt areas, whilst cyclic AMP was found in the villus tip, lamina propria and smooth muscle. Furthermore, Yamamoto (I977) has also shown in the rat small intestine, three different forms of independent and specific phosphodiesterases involved in the hydrolysis of cyclic GMP and cyclic AMP. The role of cyclic AMP in the small intestine is probably as an important regulator of ion and fluid transport (see Kimberg, I974). Evidence that prostaglandins may exert their effect on ion transport through an interaction with the cyclic AMP system has also been reported (see Matuchansky and Bernier, I976 and Matuchansky and Coutrot, I978).

The possibility that cyclic AMP acts as a modulator of

prostaglandin biosynthesis must also be considered. Cyclic AMP has been shown to stimulate prostaglandin biosynthesis in cultured mammalian cells, Graafian follicles, thyroid cells, adrenal cortex and adipocytes (see Samuelsson et al., I978b). Furthermore, Minkes et al. (I977) have demonstrated that cyclic AMP levels regulate phospholipase activity in platelets. It has also been suggested that cyclic GMP can modulate prostaglandin synthesis by stimulating the enzyme PGE 9-ketoreductase in blood vessels (Wong et al., I977a).

SECTION 2 THE KALLIKREIN-KININ SYSTEM

A. Biochemistry

The general aspects of the kallikrein-kinin system will be discussed. Several reviews have dealt with the biochemistry of the kallikrein-kinin system and the reader is directed to them for a more comprehensive discussion: Erdos (I970, I976); Rocha e Silva (I970); Pisano (I975); Pisano and Austen (I976); Garcia Leme (I978); Movat (I978).

(i) History

In I909 Abelous and Bardier reported that human urine contained a substance which reduced blood pressure when injected intravenously into dogs. Frey (I926) demonstrated that the active substance was destroyed by boiling and was non-dialyzable. Frey and Kraut (I928) found a similar vasodepressor in human blood, and in the pancreas (Kraut et al., I930). They assumed the active substances to be identical and named the principle kallikrein (Gk: kallikreas = pancreas).

Werle et al. (I937) demonstrated that a mixture of plasma and kallikrein contracted an isolated segment of guinea-pig ileum. The spasmogen was referred to as "darmkontrahierende Substanz" or gut-contracting substance. Werle and Berek (I948) substituted the name kallidin for "darmkontrahierende Substanz" and called the precursor kallidinogen.

Rocha e Silva et al. (I949) isolated a polypeptide from the pseudoglobulin fraction of plasma, with trypsin and certain snake venoms. This polypeptide was a potent vasodilator and smooth muscle contractor, which induced a slower contractile response on the guinea-pig isolated ileum

than either histamine or acetylcholine. Hence, Rocha e Silva named it bradykinin, from the Greek word meaning slow, and referred to the precursor as bradykininogen. It is now clear that the trypsin-bradykininogen-bradykinin system is closely related to the kallikrein-kallidinogen-kallidin system.

Bradykinin was isolated from ox blood by Elliot et al. (I960) and synthesized by Boissonnas et al. (I960). Kallidin was obtained by Pierce and Webster (I96I) from human plasma treated with human urinary kallikrein and found to be lysylbradykinin. Met-lys-bradykinin was described by Elliot et al. (I963) in ox blood, and its synthesis performed by Schroder (I964) and Merrifield (I964). Bradykinin, kallidin and met-lys-bradykinin are now known as plasma kinins, and are derived from a precursor protein, kininogen, by various protease kallikreins.

(ii) Biosynthesis

The activation sequence of the plasma kinin system, which leads to the formation of bradykinin involves a series of enzymes. The initial step involves activation of Hageman factor (blood coagulation factor XII), which can be achieved by either of two mechanisms in vitro. Firstly, there may be a solid-phase activation resulting from contact with a wide variety of negatively charged surfaces such as glass, kaolin and celite (see Margolis, I966 and Ratnoff, I966); secondly, fluid phase activation may occur after proteolytic cleavage of factor XII, with resultant production of active fragments of the molecule (Burrowes et al., I97I; Kaplan and Austen, I97I; Cochrane et al., I973). In vivo, various tissue components

have been shown to be capable of absorbing and activating factor XII in plasma, and these include collagen, elastin and basement membrane (see Movat, I978). These components initiate the activation of factor XII, which then releases kallikrein. Further activation of factor XII is performed enzymatically, by plasma kallikrein, through a positive feedback.

The resulting active factor XII initiates blood clotting in vitro (Kaplan and Austen, I970), and converts prekallikrein to kallikrein (Kaplan and Austen, I970, I97I). It can also activate a prekallikrein in the colon wall (Nakajima et al., I973).

Kallikreins are ubiquitous in the body and can be derived from plasma (plasma kallikreins) or from glandular sources (glandular kallikreins). Fritz et al. (I967) found that the molecular weights of kallikreins from pancreas, urine and submaxillary gland were relatively similar, ranging from 33,000 to 36,000. Compared with the glandular kallikreins, plasma kallikrein was found to have a higher molecular weight of 97,000 (Habermann and Klett, I966), and to differ from them physicochemically and immunologically (see Erdos, I976). Plasma kallikrein was demonstrated to be synthesized as an inactive precursor, prekallikrein, by the liver (Werle et al.,I955; Eisen and Vogt, I970). Plasma kallikrein liberates bradykinin, and glandular kallikreins release kallidin when acting on kininogen precursors (Webster and Pierce, I963; Habermann and Blennemann, I964). The general features of the glandular kallikrein-kinin system have recently been reviewed (Nustad et al., I978).

Kallikrein inhibitors have been found in the blood

serum of all species studied, in several organs of ruminants, in egg white, colostrum and in plants (see Vogel and Werle, I970). Plasma kallikrein has been shown to be inhibited by the naturally occurring protease inhibitors, $C\bar{1}$ esterase inhibitor (Ratnoff et al., I969), α_2 macroglobulin (Harpel, I970), α_I-antitrypsin (McConnel, I972) and antithrombin III (Lahiri et al., I974).

It has been suggested that human plasma contains at least two classes of kininogens. Pierce and Webster (I966) isolated from human plasma two kininogens, I and II, of molecular weight 50,000. Carboxypeptidase B destroyed only the activity of I, suggesting the bradykinin sequence of amino acids was located in different parts of the molecule. The so called high molecular weight kininogen (molecular weight $\simeq$ 200,000) was considered to be a polymer of either of the two low molecular weight kininogens (Colman et al., I97I). The high molecular weight kininogen represents approximately 20% of the total kininogen content of plasma. Plasma kallikrein has a higher affinity for high molecular weight kininogen than low molecular weight kininogen, whereas glandular kallikreins act rapidly on both types (Pierce and Guimaraes, I976).

(iii) Metabolism

Blood and tissues contain enzymes, collectively called kininases, which rapidly inactivate kinins. The most important kininases were named kininase I and II (see Erdos and Yang, I970). Kininase I (Erdos, I96I), is a carboxypeptidase called carboxypeptidase N (arginine carboxypeptidase), and is primarily responsible for the hydrolysis of plasma

kinins by removal of the C-terminal arginine. Carboxypeptidase N probably originates from the liver (Oshima et al., I974). Kininase II is a peptidyldipeptide hydrolase (Yang and Erdos, I967) which cleaves Phe^{8}-Arg^{9} from bradykinin. It appears that kininase I functions mostly in blood, while in tissues kininase II terminates the actions of kinins (see Erdos, I976).

B. The Kallikrein-Kinin System and the Gastrointestinal Tract

(i) Role of the Kallikrein-Kinin System in the Gastrointestinal Tract

The physiological function of the kallikrein present in the wall of the intestinal tract is unclear. Dennhardt and Haberich (I973) showed that kallikrein could affect the transfer of salt, water and hexoses across rat jejunum and colon in vivo. Haberland and Rohen (I973) and Haberland et al. (I975), suggested the kallikrein in the intestinal tract may have an effect on absorption of glucose and amino acids through the gastrointestinal epithelium. However, Moriwaki and Fujimari (I975) suggested the effect of kinins on membrane transport could be secondary to their vasodilator action.

An alternative theory for the role of the kallikrein-kinin system in the intestine was proposed by Fasth and Hulten (I973). They suggested that at least in the cat intestine, a kinin-like mechanism appeared to be involved in the regulation of colonic motility and blood flow.

A role for the kallikrein-kinin system has been

implicated in several diseases involving the gastrointestinal tract, which include the carcinoid and postgastrectomy dumping syndromes, and septic shock (see Vogel and Zickgraf -Rudel, I970, and Colman et al., I976). Furthermore, Bell (I975) postulated that the kallikrein-kinin system mediated food and chemical sensitivities.

(ii) Occurrence and Distribution of the Kallikrein-Kinin System

The first observation of kallikrein in the gastrointestinal tract was by Werle (I960), who reported the presence of a trypsin activated hypotensive activity in the gastrointestinal tract of rats. Kallikrein has since been detected along the length of the gastrointestinal tract both in man and animals (Amundsen and Nustad, I965; Burger et al., I968; Seki et al., I970; Zeitlin, I970; Zeitlin and Smith, I973; Frankish and Zeitlin, I977). In the gastrointestinal tract of rats all the tissues studied were found to contain kallikrein, with least in the stomach and most in the caecum (Frankish and Zeitlin, I977). However, Seki et al. (I972) were unable to find kallikrein or its precursor in the small intestine of rat, dog and swine, although it was detected in colon tissue from these animals after activation by tryptic digestion. The molecular weight of the kallikrein from rat jejunum, ileum, caecum and colon was found to be 33,000, which suggested that it was a glandular kallikrein (Zeitlin et al., I976). In addition, the gastrointestinal tract of rats was found to contain negligible kininase activity (Frankish and Zeitlin, I977).

(iii) Effect of the Kallikrein-Kinin System on Gastrointestinal Smooth Muscle

It has been demonstrated that kallikrein contracts dog, pig and rat intestine (Werle, I936), cat intestine (Werle et al., I937) but not rabbit intestine (Laborit et al., I964).

Bradykinin and kallidin contract the cat jejunum (Erspamer and Erspamer, I962) and terminal ileum (Ferreira et al., I973c), guinea-pig ileum (Rocha e Silva et al., I949), hen rectal caecum (Bisset and Lewis, I962), longitudinal human ileum (Fishlock, I966), and rat stomach strip (Vane, I964). Ferreira and Vane (I967a) suggested that the cat jejunum was the most sensitive tissue to contract in response to bradykinin, although only about half the tissues showed a response.

A relaxation to bradykinin has been reported in the circular muscle of human ileum (Fishlock, I966), rabbit intestine (Rocha e Silva, I962) and rat duodenum and colon (Horton, I959; Gaddum and Horton, I959). Ferreira and Vane (I967a) reported that the rat duodenum was the most sensitive of the tissues which relaxed to bradykinin, but they could not use the tissue for bioassay since it did not maintain a a stable relaxation.

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

The mode of action of bradykinin on smooth muscle has not been established conclusively. Many early studies concentrated upon an investigation into the role of the parasympathetic and sympathetic nerves in the response of intestinal preparations to bradykinin. It was shown that

contractions to bradykinin in the cat jejunum and terminal ileum, guinea-pig ileum and rat stomach strip were not affected by anticholinesterases, antihistamines, anoxia, BOL I48, cooling, denervation, ganglion blockers, muscarinic receptor blockers, phenoxybenzamine or tetrodotoxin (see Walaszek, I970). These results led several workers (Khairallah and Page, I96I; Day and Vane I963; Gershon, I967) to conclude that the contractile effect of bradykinin was a direct one on the smooth muscle. However, Wiegershausen et al. (I964) demonstrated that the response of the guinea-pig ileum to bradykinin was inhibited by atropine and morphine, and potentiated by eserine, thus implicating a possible cholinergic mechanism. Furthermore, Potter and Walaszeck (I972) showed that the potentiation of bradykinin responses on guinea-pig ileum by cysteine, was inhibited by atropine, morphine and tetrodotoxin but not by chlorpheniramine, mecamylamine, hexamethonium, BOL I48 or phentolamine. They postulated the involvement of a cholinergic mechanism in this potentiation.

A possible involvement of prostaglandins in the contractile response of the rat ileum to bradykinin was postulated by Crocker and Willavoys (I976). They demonstrated that the response to bradykinin, but not to acetylcholine, was significantly reduced in the presence of indomethacin. However, Barabe et al. (I975) demonstrated that the interaction between bradykinin and its receptors in the cat terminal ileum and jejunum was a bi-molecular reaction, and the receptors for bradykinin were specific since they were not influenced by inhibitors of neurotransmitters and autocoids or by indomethacin. In a later paper by the same workers

(Barabe et al., I977),these specific receptors in the cat ileum were found to be stable components of the smooth muscle membrane and were classified as type B_2. This was in contrast to type B_I receptors which were generated de novo during incubation and found in rabbit aortae (Regoli et al., I977).

Conflicting results have been obtained in an attempt to determine the mechanism of bradykinin-induced relaxation of smooth muscle. Some authors using α and β receptor blockers, favoured a sympathicomimetic mechanism for the bradykinin induced relaxation of the rat duodenum (Turker et al., I964; Montgomery, I968), and circular muscle of human ileum (Fishlock, I966). However, Ohashi et al. (I967), Antonio (I968), Hall and Bonta (I973) and Ufkes and van der Meer (I975), concluded that the bradykinin induced relaxation of rat duodenum was due to a direct effect on smooth muscle cells, since relaxations were not affected by α and β adrenergic blockers, or by catecholamine depletion.

Therefore, 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 possibility of an involvement of prostaglandins in the contractile and relaxatory response to bradykinin appears to warrant further investigation.

SECTION 3 BRADYKININ-PROSTAGLANDIN INTERACTIONS

The blockade of bradykinin responses by aspirin and other non-steroidal anti-inflammatory drugs, was demonstrated long before the action of these drugs had been elucidated, and included;

(a) antagonism of the inflammatory action of bradykinin in the rabbit (Lecomte and Troquet, I960) and rat (Lisin and Leclercq, I963);

(b) antagonism of the nociceptive action of bradykinin in the dog (Guzman et al., I964), guinea-pig (Gjuris et al., I964), mouse (Collier et al., I968) and rat (Gilfoil et al., I964);

(c) antagonism of the bradykinin-induced bronchoconstriction in the guinea-pig (Collier, I960); and

(d) the duration, but not the degree, of the bradykinin induced hypotension in dog, rabbit and rat (Collier and Shorley, I960; Collier et al., I968; Vargaftig, I966).

Collier (I969) suggested that aspirin antagonized the action of bradykinin indirectly rather than directly, possibly by the blockade of an intermediary mechanism, such as the release of another mediator. This was supported by Piper and Vane (I969) who demonstrated that bradykinin liberated a rabbit aorta contracting substance from guinea-pig isolated lungs, and aspirin and other non-steroidal anti-inflammatory drugs blocked this release. The observation that non-steroidal anti-inflammatory drugs were potent inhibitors of prostaglandin synthetase (Ferreira et al., I97I; Smith and Willis, I97I; Vane, I97I), implicated prostaglandins as the mediators in the response to bradykinin.

In fact the non-steroidal anti-inflammatory drugs are now frequently employed to assess the contribution of prostaglandins to the biological actions of bradykinin.

The first direct evidence that bradykinin could release prostaglandins came from Piper and Vane (I969) who demonstrated the release of PGE_2, $PGF_{2\alpha}$ and a rabbit aorta contracting substance from guinea-pig isolated lungs, when challenged with bradykinin. Rabbit aorta contracting substance was shown to consist mainly of thromboxane A_2 (Hamberg et al., I975b) and to a minor part, of prostaglandin endoperoxides (Svensson et al., I975). Bradykinin has since been demonstrated to stimulate the release of prostaglandins from several organs including dog kidney (Mc Giff et al., I972), dog spleen in vitro and in vivo (Moncada et al., I972; Ferreira et al., I973b), cat isolated spleen (Ferreira et al., I973a), dog knee joint (Moncada et al., I975), guinea-pig and rat isolated lungs (Palmer et al., I973; Damas and Deby, I976), rabbit isolated heart (Needleman et al., I976) and rabbit isolated perfused ear (Juan and Lembeck, I976). The possibility that bradykinin might activate the release of prostacyclin in these organs must also be considered, since a release into the circulation has been demonstrated in the anaesthetized cat (Gryglewski et al., I979) and dog (Moncada et al., I978).

The fact that bradykinin induced prostaglandin biosynthesis in several tissues, suggested a common biochemical mechanism. Vargaftig and Dao Hai (I972) pinpointed the mechanism by measuring the release of rabbit aorta contracting substance from guinea-pig isolated lungs. The release induced by bradykinin was suppressed by mepacrine, a drug which has

been shown to inhibit phospholipase A (Markus and Ball, I969), whereas, the release caused by arachidonic acid was unaffected by mepacrine. This suggested that bradykinin might activate an acylhydrolase which caused the release of prostaglandin precursors. Activation of phospholipase A_2 by bradykinin became the accepted mechanism. Hseuh et al. (I977) demonstrated, using several radioactive fatty acids, that bradykinin only stimulated the release of arachidonic acid from rabbit isolated hearts. They suggested that bradykinin induced a selective release of only the prostaglandin precursor acid. However, it was not until recently that Antonello et al. (I978) demonstrated, in the rat kidney in vivo, a dose related increase in phospholipase A_2 activity by bradykinin with the subsequent release of arachidonic acid. Similar results were reported by Hong and Deykin (I979) who demonstrated, in methylcholanthrene-transformed mouse fibroblasts, that bradykinin activated a phospholipase that specifically hydrolyzed arachidonyl and eicosatrienoyl phosphatidylinositol and phosphatidylcholine.

However, there is a lack of information as to whether the action of bradykinin on phospholipase is direct or indirect. Bradykinin might activate phospholipase A_2 by mobilization of calcium ions which are required for maximal activity of phospholipase A_2 (De Haas et al., I968). Alternatively bradykinin could promote the release of cell membrane phospholipase A_2, or it might convert a pro-phospholipase A_2 into the active form as suggested by Picket et al. (I976) with regard to trypsin as activator. Furthermore, some pulmonary (Stoner et al., I973) as well as vascular (Clyman et al., I975) actions of bradykinin have been shown

to be cyclic GMP dependent and involve stimulation of prostaglandin biosynthesis. Therefore, the action of bradykinin on phospholipase activity might be mediated by this cyclic nucleotide.

Wong et al. (I977b) demonstrated that bradykinin contracted the bovine mesenteric vein not only by promoting prostaglandin biosynthesis, but also by virtue of its capacity to regulate the amount of PGF formed from PGE. This latter action depended on the ability of the kinin to augment the activity of PGE 9-ketoreductase, which enzymically reduced PGE to PGF (Leslie and Levine, I973). The existence of a similar mechanism had been postulated for the kidney (see Mc Giff et al., I976). Wong et al. (I977a) further suggested that those vascular actions of bradykinin mediated by PGF, formed via PGE 9-ketoreductase, may occur through a mechanism involving cyclic GMP. Hassid and Levine (I977) demonstrated bradykinin did not affect the activity of any of the three PGE 9-ketoreductases found in chicken kidney, thus implicating a tissue and species variation.

In the gastrointestinal tract an interrelationship between bradykinin and prostaglandins has been suggested by several workers. Crocker and Willavoys (I976) demonstrated that aspirin, indomethacin, or polyphloretin phosphate antagonized the contractile response to bradykinin in the longitudinal muscle of rat isolated terminal ileum, thus implicating an involvement of prostaglandins. Liebmann and Arold (I978) demonstrated bradykinin increased the production of PGE_2 and $PGF_{2\alpha}$ in microsomal fractions from rat duodenum, the formation of PGE_2 being stimulated to a higher degree. Hardcastle et al. (I978) showed that bradykinin increased

the potential difference across the jejunum and colon of the rat. This effect was significantly reduced by indomethacin, suggesting it too was mediated by prostaglandins.

In conclusion, it has been shown that an interaction between bradykinin and prostaglandins exists in several systems, including the intestinal tract. This interaction involves the ability of bradykinin to stimulate prostaglandin biosynthesis and to modulate the type of prostaglandin released.

METHODS

SECTION I RADIOIMMUNOASSAY

(i) Tissue Preparation

Male Wistar rats (180g-250g) were killed by a blow on the head and a 2cm section of terminal ileum, cleaned of mesentery and fat, was removed and flushed through with Tyrode solution. Ileal preparations were suspended, under a load of 1g, in 10ml organ baths containing aerated Tyrode solution at 37°C. The composition of the Tyrode solution was (mM) : NaCl, 137; KCl, 2.7; $NaH_2PO_4 \cdot 2H_2O$, 0.42; $NaHCO_3$, 11.9; $MgSO_4 \cdot 7H_2O$, 1.1; $CaCl_2 \cdot 2H_2O$, 1.8; glucose, 5.6. The bathing fluid was changed at 5min intervals during the first 30min. Thereafter, the tissue was left for 60min either alone, or in the presence of indomethacin (28μM), bradykinin (3.2×10^{-12}M) or potassium chloride (10^{-5}M). Concentrations of bradykinin and potassium chloride were selected to produce contractions approximately equal to the 50% maximal bradykinin response. At the end of the incubation period the bathing fluid was extracted for prostaglandins.

(ii) Extraction Procedure

The bathing fluid was collected into siliconized glass flasks over ice. The fluid was acidified with 1N hydrochloric acid to pH 3.5 and extracted twice, for 4min with vortex mixing, into equal volumes of ethyl acetate. The ethyl acetate extracts were centrifuged at 2,500 r.p.m. and 4°C for 5min to seperate out residual water which had formed an emulsion. The extract was then evaporated to dryness under

reduced pressure at 40°C on a rotary evaporator, and stored at -2I°C for no longer than 24hr prior to thin-layer chromatography.

To determine the percentage recovery of prostaglandins 25,000 c.p.m. of (^{3}H)$PGF_{2\alpha}$ or (^{3}H)PGE_2 were added to the bathing fluid immediately prior to extraction.

(iii) Thin-Layer Chromatography

Prostaglandin extracts were redissolved in 0.5ml of ethanol and applied as bands (60mm x 2mm) onto thin-layer silica gel plates (Eastman Chromagram No I3I8I). Chromagram plates were activated at II0°C for 30min prior to use. The plates were developed to a solvent front height of I5cm from the origin, in the AI solvent system (benzene:dioxan:acetic acid; 20:20:I) of Green and Samuelsson (I964). After development the plate was air dried, cut, and marker prostaglandins visualized, according to the method of Kiefer et al. (I975), by spraying with anisaldehyde-ethanol-sulphuric acid (I:9:I) and heating at 90°C for I0min. This resulted in the appearance of reference $PGF_{2\alpha}$ and PGE_2, as greyish purple and brown spots respectively. The marker section was re-aligned with the original plate and the silica gel corresponding to the reference prostaglandins was scraped off and eluted into 2ml ethanol. The ethanol was evaporated off under a stream of nitrogen and the dried extract was stored at -2I°C for no longer than 6 days. Extracts were redissolved in 3ml of assay buffer immediately prior to the radioimmunoassay.

(iv) Assay Procedure

All solutions and dilutions were made in 0.05M Tris

buffer which contained 0.I% gelatin, pH 7.4. Preliminary studies were performed with phosphate buffered saline as the assay buffer, but this was found to give high non-specific binding values (> I4% of control binding).

Rabbit antisera with affinities for either PGE_2 or $PGF_{2\alpha}$ were used at a final dilution of I in 500 and I in I000 respectively. The dilutions employed were such that 0.Iml of antisera resulted in 50% binding of 8,000 c.p.m. of its respective standard prostaglandin.

The radioimmunoassay was performed in IOmm x 75mm plastic tubes, previously rinsed in bi-distilled water. Standard curves of unlabelled PGE_2 and $PGF_{2\alpha}$ (0.05ng to 3.2 or 6.4ng, respectively) appropriately diluted in 0.Iml of assay buffer, or 0.Iml of the test extract, were pre-incubated for IOmin at $4^{\circ}C$ with 0.Iml of the respective antiserum. 8,000c.p.m. in 0.Iml of $(^3H)PGE_2$ or $(^3H)PGF_{2\alpha}$ were then added to their respective tubes, and after gentle vortex mixing the contents were allowed to equilibrate for I6hr at $4^{\circ}C$.

The separation of antibody bound, from unbound labelled prostaglandin, was accomplished by the addition of 0.Iml of a solution of bovine-γ-globulins (25mg/ml) in Tris-gelatin buffer to each tube followed by gentle vortex mixing. The protein complex in each tube was precipitated by the addition of 0.4ml of saturated ammonium sulphate solution at $4^{\circ}C$ and gentle vortex mixing, followed by centrifugation for 20min at 2,500 r.p.m. and $4^{\circ}C$. The supernatant was discarded and the process repeated using 0.8ml aliquots of 50% w/v ammonium sulphate. The residual pellet was resuspended in Iml bi-distilled water and transferred to counting vials which

contained IOml of NE 260 scintillation fluor. Each vial was counted in a Beckman counter (Type MS 230) for IOmin. The counting efficiency for tritium in the system used was 47%.

Each assay contained three blanks:

(a) the diluent blank, containing labelled prostaglandin and assay buffer. This gave information about non-specific binding of labelled hormone.

(b) the antibody blank, containing labelled prostaglandin, antibody and buffer. This provided information concerning the reaction between labelled prostaglandin and antibody, and represented, after correction for non-specific binding, the IOO% control binding.

(c) the method blank, containing extracted Tyrode in which no tissue had been incubated, labelled prostaglandin and antibody. This provided information about interfering material introduced during the extraction and chromatographic separation.

(v) Statistical Analysis

All results were characterized, except where stated, by a mean $\pm$ standard error of the mean (s.e.m.), and statistical analysis was performed by application of Student's t-test.

The standard curve was expressed as the percentage control binding of $(^3H)PGF_{2\alpha}$ or $(^3H)PGE_2$, corrected for non-specific binding, on the ordinate, plotted against the log concentration of the respective unlabelled prostaglandin on the abscissa. Levels of $PGF_{2\alpha}$ and PGE_2 equivalents in the extracts were interpolated from the standard curve and adjusted by the appropriate recovery, dilution and tissue weight factors. All extracts and points on the standard curve

were done in triplicate.

The precision of the radioimmunoassay was presented as the intra- and inter-assay variation and expressed as coefficients of variation.

The accuracy of the radioimmunoassay was determined by plotting the known concentration of $PGF_{2\alpha}$ or PGE_2 added to a sample on the abscissa, against the amount of the respective prostaglandin measured in the radioimmunoassay, corrected for recovery, on the ordinate. The regression lines constructed were calculated by the method of least squares and the degree of linear relationship presented as correlation coefficients.

SECTION 2 BIOASSAY

A. Bioassay to Validate Radioimmunoassay

Extracts of $PGF_{2\alpha}$-like or PGE_2-like material released from the rat isolated terminal ileum, prepared as described (see pp. 39-40), were bioassayed within 24hr of their respective radioimmunoassay. Extracts were assayed, using the "dose-bracket" method, by cascade superfusion as described by Naylor (I977).

(i) Tissue Preparation

Two assay tissues were used to bioassay the extracts;

(a) The rat fundus strip. This tissue was described by Vane (I957). A longitudinal muscle strip 2mm wide was prepared from the greater curvature of the rat gastric fundus pouch. The tissue was assembled in the superfusion jacket and placed under a 2g tension.

(b) The rat colon. A Icm section of colon was removed approximately 6cm from the caecum. The tissue was assembled in the superfusion jacket and placed under a I.5g tension.

The assay tissues were superfused with Krebs solution at 37°C, previously gassed with 5% CO_2 in O_2, at 6ml/min by a Watson Marlow pump (Type MHRE 7). The composition of the Krebs solution was (mM): NaCl, 94; KCl, 4.69; $CaCl_2 \cdot 2H_2O$, I.9; KH_2PO_4, I.I6; $NaHCO_3$, 24.9; $MgSO_4 \cdot 7H_2O$, 4.46; glucose, II. A stabilization period of 90min was used prior to commencing the assay. All contractions were recorded on isometric transducers (Type UFI) coupled to a Devices (Type M2) recorder.

(ii) Statistical Analysis

All results were characterized by a mean ± s.e.m. The effect of experimental treatments were analysed by the Student's t-test.

B. Bioassay of Prostaglandin-like Material Released from the Rat Isolated Perfused Ileum

The prostaglandin-like material released from the isolated perfused rat ileum was bioassayed on the rat fundus strip by means of the apparatus shown in Fig.I (described below).

(i) Donor Tissue Preparation

Male Wistar rats (I80g-250g) were killed by a blow on the head and a 4cm section of terminal ileum, cleaned of mesentery and fat, was carefully removed and placed in Krebs solution at 37°C. After approximately I5min the ileum had cleared itself of faecal material and was ready for introduction into the apparatus. The proximal end of the ileum was fastened to polypropylene tubing (2mm internal diameter) and mounted horizontally in a heated water jacket (internal diameter Icm) at 37°C. The caecal end of the ileum was connected by thread, over a pulley, to an isometric transducer and the tissue was placed under a Ig tension. The ileum was perfused through its lumen, at 3ml/min by a Watson Marlow pump (Type MHRE 7), with Krebs solution (K_I) at 37°C previously gassed with 5% CO_2 in O_2. When the action of bradykinin was studied during serosal perfusion, the ileum was everted over a glass rod prior to introduction into the apparatus.

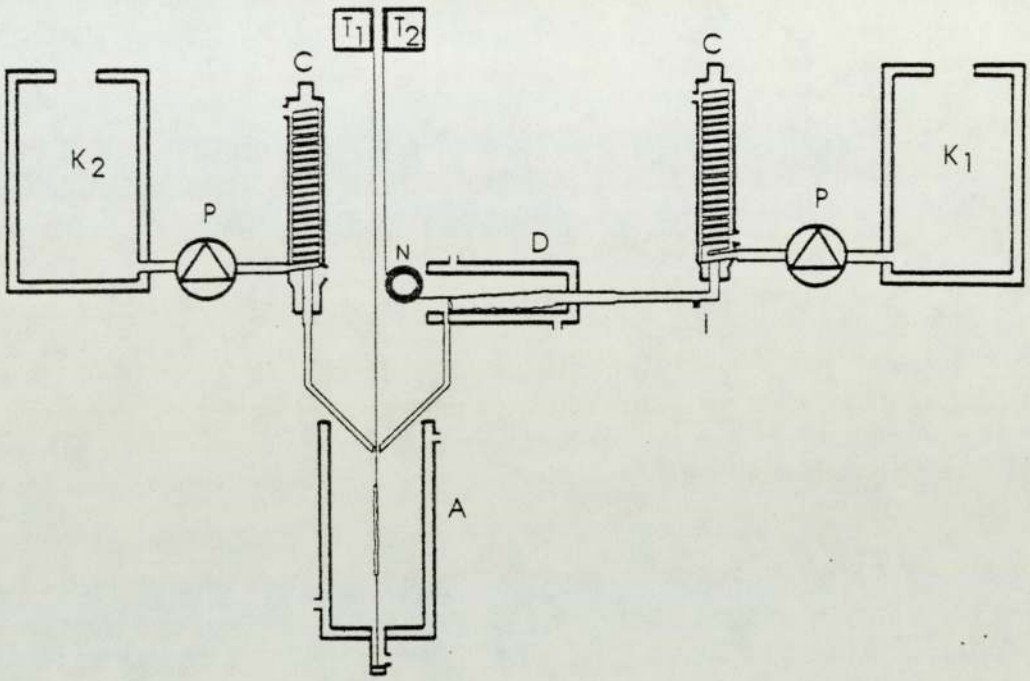

FIG.I Apparatus used to bioassay the prostaglandin-like material released from the isolated perfused ileum.

(A) Heated assay tissue jacket containing rat fundus strip.

(C) Heating coils.

(D) Heated donor tissue jacket containing perfused ileum.

(I) Injection port.

(K_I) Krebs solution reservoir.

(K_2) Krebs solution reservoir containing antagonists and indomethacin.

(N) Pulley.

(P) Peristaltic pump.

(T_I) Isometric transducer for assay tissue.

(T_2) Isometric transducer for perfused ileum.

(ii) Assay Tissue Preparation

Prostaglandin-like activity was assayed on the rat fundus strip prepared as described (see p.44), which was superfused with the effluent (3ml/min) from the ileum. The assay tissue was simultaneously superfused, also at 3ml/min, with a Krebs solution (K_2) which contained antagonists and indomethacin to increase the specificity and sensitivity of the assay tissue to prostaglandins. To achieve the recommended concentration of antagonists (Gilmore et al., I968) and indomethacin (2.8μM) in the final solution superfusing the rat fundus strip, adjustments were made to the Krebs solution (K_2) reservoir to compensate for dilution by the effluent from the ileum.

Bradykinin and potassium chloride were diluted in Krebs solution immediately before use, and injected at a constant volume (0.2ml) into the Krebs solution stream prior to perfusion of the ileum.

Isometric contractions and recordings were as described previously (see p.44).

(iii) Statistical Analysis

All results were characterized by a mean ± s.e.m. and analyzed by Student's t-test.

The effect of bradykinin or potassium chloride on the release of prostaglandin-like material from the perfused rat ileum was studied by direct bioassay on the rat fundus strip. The contractions of the rat fundus strip to increasing concentrations of bradykinin or potassium chloride were represented as regression lines, calculated by the method of least squares. The regression lines were constructed from

those concentrations of bradykinin or potassium chloride which gave contractions in the range 20% to 80% of the maximal contraction for the agonist. The displacement of regression lines to bradykinin or potassium chloride caused by experimental treatment, were analyzed, after showing parallelism between regression lines, by the t-test for the difference between regression lines (Mood et al., I974). The difference between regression lines was calculated at the concentration of bradykinin or potassium chloride which gave a contraction of 50% maximal in their respective control experiment (EC_{50}).

Experiments were performed to quantify, on the rat fundus strip, the release of prostaglandin-like material from the perfused rat ileum by bradykinin or potassium chloride. The concentrations of bradykinin or potassium chloride used gave similar contraction heights on the rat fundus strip, which were approximately 40% to 50% of the maximal contraction for the agonists. The contraction of the rat fundus strip to either agonist during perfusion with $PGF_{2\alpha}$ or PGE_2, was expressed as a percentage of the maximal contraction height to the agonist in the absence of prostaglandin.

SECTION 3 SMOOTH MUSCLE CONTRACTION

A. Experiments to Investigate the Mode of Action of Bradykinin on Isolated Intestinal Smooth Muscle

(i) Tissue Preparation

(a) Rat terminal ileum (see p.45).

(b) Rat duodenum. The tissue preparation for the rat duodenum was similar to that described for the rat ileum. A 2cm section of proximal duodenum, adjacent to the pyloric sphincter, was removed and placed in Krebs solution at 37°C to clean itself of faecal material. The duodenum was then introduced into the apparatus as described (see p.45) and placed under a Ig tension.

(c) Cat terminal ileum. Two male cats weighing 2.4Kg and 2.9Kg were starved for 24hr prior to use. The cats were anaesthetized with halothane and killed by intravenous air injection. Four sections of terminal ileum, approximately 5cm long, were removed from each cat. The tissues were washed in Krebs solution, cleaned of mesentery and fat, and stored at 4°C in Krebs solution for I8hr before use, as recommended by Erspamer and Erspamer (I962). The tissues were introduced into apparatus similar to that used and described for the rat ileum (see p.45), and placed under a 2g tension. The heated water jacket used had an internal diameter of I.5 cm to accommodate the cat ileum.

In all experiments on perfused intestine, bradykinin, potassium chloride or isoprenaline were diluted in Krebs solution immediately prior to use, and injected at a constant volume (0.2ml) into the perfusion stream of the tissue under

study.

(ii) Statistical Analysis

All data in this section was characterized by a mean ± s.e.m. and analyzed by Student's t-test.

The contractions of the cat or rat isolated perfused terminal ileum, to increasing concentrations of bradykinin, or potassium chloride, were expressed as a percentage of their maximum contraction on the surface of the ileum studied.

In experiments that were concerned with the role of cyclic nucleotides, or a neuronal mechanism, in contractions of the rat isolated perfused ileum to bradykinin or potassium chloride, a single concentration of agonist was studied. The concentration of bradykinin or potassium chloride used, gave a 50% maximal contraction (EC_{50}) on the surface of the ileum studied. The contraction height elicited by the respective concentration (EC_{50}) of agonist, was referred to as the 100% control response on the surface studied. All contractions were expressed as a percentage change from the respective 100% control response.

The relaxations of the rat isolated perfused duodenum to increasing concentrations of bradykinin, or isoprenaline, were expressed as a percentage of their maximum relaxation on the surface of the duodenum studied. When the role of cyclic nucleotides was investigated on the bradykinin or isoprenaline-induced relaxation, the effect upon a single concentration of agonist was studied. The concentration of isoprenaline used gave a 50% maximal relaxation on the mucosal and serosal surface. However, since there was only a small

concentration range over which there was a graded relaxation of the duodenum to bradykinin, the threshold concentrations for graded relaxations on the respective ileal surface were used. The relaxation induced by the respective concentration of agonist was referred to as the 100% control relaxation on the surface studied. All relaxations were expressed as a percentage change from the respective 100% control relaxation.

B. Experiments to Investigate the Action of Indomethacin on the Contractile Response to Bradykinin

All experiments in this section were performed on the rat isolated perfused ileum. The methods employed for assembly of the apparatus and setting up the tissue, were as described previously (see p.45).

(i) Time-Course for Inhibition of Bradykinin Contractions by Indomethacin

In this study only the mucosal perfused ileum was used. The effect of indomethacin (2.8μM or 28μM), added to the ileal perfusate, was studied on contractions of the longitudinal muscle to the same concentration of bradykinin (EC_{50}) injected at 10min intervals, for 3hr. The contraction height induced by this concentration (EC_{50}) of bradykinin was referred to as the 100% control response. All contractions were expressed as a percentage change from the 100% control response.

(ii) Effect of Indomethacin on the Mucosa of the Perfused Ileum

This study also used only the mucosal perfused ileum.

Two 4cm sections of ileum from the same rat were introduced into heated water jackets as previously described (see p.45). One tissue was perfused with Krebs solution alone, whilst the second tissue was perfused with Krebs solution which contained either indomethacin, or indomethacin and PGE_2. Each tissue was perfused at 3ml/min for 3hr. The effluent from each ileum was filtered to collect any mucosal material removed during perfusion of the ileum. At the end of the perfusion period the filtrate, together with the respective section of ileum, was dried and weighed.

The results were presented as percentage mucosal loss. This was the difference between the weight of filtrate obtained after perfusion of the ileum with Krebs solution which contained either indomethacin or indomethacin and PGE_2, and the weight of filtrate obtained after perfusion of the other section of ileum with Krebs solution alone. The weight of each filtrate was expressed as a percentage of the weight of the respective section of ileum.

A preliminary histological investigation was undertaken to determine whether the mucosal loss was associated with any significant change in the morphology of the tissue. At the end of the 3hr perfusion period the ileum was frozen in liquid nitrogen and mounted for cryostat sectioning. All sections were stained with haemotoxylin and eosin.

(iii) Inhibition of Prostaglandin Synthesis in the Rat Ileum by Pretreatment with Indomethacin

In this study the tension of a perfused section of ileum was increased from Ig to 6g, for Imin. This increased tension was associated with the release of a prostaglandin-

like material from the ileum, which was detected by direct bioassay on the rat fundus strip. The release of prostaglandin-like material from the perfused ileum was quantified by comparison with authentic PGE_2. The release of PGE_2-like material from a 4cm section of ileum from an untreated rat, assessed 20min after mounting the tissue in the perfusion jacket, was regarded as the 100% control release for the ileal surface studied. The control release of PGE_2-like material, was compared to that of a section of ileum removed from a rat injected subcutaneously with indomethacin, 2hr prior to killing the animal and removal of the tissue. The release of PGE_2-like material from the mucosal or serosal perfused ileum of a pretreated rat, following an increase in the tissue tension, was expressed as a percentage of the respective control release.

SECTION 4 DRUGS AND CHEMICALS

With the exception of bradykinin, tetrodotoxin and prostaglandins, all drug solutions were freshly prepared immediately prior to use. Bradykinin and tetrodotoxin were dissolved in bi-distilled water (8.06×10^{-4}M and 3.2×10^{-2}M respectively), sealed in ampoules and stored at -21°C for no longer than 4 and 2 weeks respectively, before use.

Prostaglandins E_1, E_2, A_2 and $F_{2\alpha}$ were dissolved in absolute alcohol (10^{-3}M to 10^{-5}M), sealed in ampoules and stored at -21°C.

Indomethacin was dissolved in 5% sodium bicarbonte immediately prior to use, whilst propranolol and SC I9220 were made up in absolute alcohol. Further dilutions in either Tyrode or Krebs were made as required. Care was taken to ensure the final concentration of solvent had no effect on the response being measured.

The following were generous gifts:

Indomethacin	Merck, Sharp and Dohme Ltd.
Methysergide Hydrogenmaleinate	Sandoz Ltd.
Propranolol	I.C.I.
Prostaglandins E_1, E_2, A_2 and $F_{2\alpha}$	Dr J.E.Pike, Upjohn Co.
PGE_2 and $PGF_{2\alpha}$ antibodies	Dr L.Levine, Brandeis University.
SC I9220	Dr J.Sanner, Searle & Co.

The following drugs and chemicals were also used:

Absolute alcohol	B.D.H., Analar.

Acetylcholine hydrochloride	Sigma
Anisaldehyde	B.D.H.
Aspirin	Sigma
Bradykinin triacetate	Sigma
Benzene	B.D.H., Analar
Bovine-γ-globulins	Sigma
Dioxan	B.D.H., Analar
Ethyl acetate	B.D.H., Analar
Gelatin	B.D.H., Analar
Hydrochloric acid	B.D.H., Analar
5-Hydroxytryptamine creatinine sulphate	Sigma
Hyoscine hydrobromide	B.D.H.
Imidazole	B.D.H.
Isoprenaline sulphate	Sigma
Mepyramine maleate	May & Baker
Morphine sulphate	May & Baker
NE 260	Nuclear Enterprises
Pempidine tartrate	May & Baker
Phentolamine mesylate	Sigma
(5,6,8,II,I2,I4,I5(n) ^{3}H) $PGF_{2\alpha}$ (I50 Ci/m.mol)	Radiochemical Centre, Amersham
(5,6,8,II,I2,I4,I5(n) ^{3}H) PGE_{2} (I60 Ci/m.mol)	Radiochemical Centre, Amersham
Sulphuric acid (98%)	B.D.H.
Tetrodotoxin	Sigma
Theophylline	B.D.H.
Tris buffer	Sigma

All salts used to make Krebs and Tyrode were of Analar grade (B.D.H.).

RESULTS

SECTION I EXPERIMENTS TO DETERMINE THE EFFECT OF BRADYKININ ON PROSTAGLANDIN RELEASE FROM THE RAT ISOLATED ILEUM

Bradykinin has been demonstrated to stimulate the release of prostaglandins from several organs including the dog kidney (McGiff et al.,I972), rabbit isolated perfused ear (Juan and Lembeck, I976) and rat isolated lungs (Damas and Deby, I976). In the present study the possible existence of such a bradykinin-induced prostaglandin release has been investigated in the rat isolated ileum. The rat ileum is a tissue in which prostaglandins have previously been suggested to participate in the contractile response to bradykinin (Crocker and Willavoys, I976).

The release of prostaglandins from the rat isolated ileum, into the surrounding bathing fluid, was quantified using both radioimmunoassay and bioassay. The concentrations of $PGF_{2\alpha}$ and PGE_2 were measured, since both prostaglandins were considered to be present in the rat gastrointestinal tract (see Collier, I974).

A. Measurement by Radioimmunoassay of Prostaglandin Release from the Rat Isolated Terminal Ileum

Radioimmunoassay of prostaglandins was first described for the measurement of PGE_I and $PGF_{2\alpha}$ by Levine and van Vunakis (I970). Numerous methods have since been developed upon the principles of radioimmunoassay, as the technique

offers a precise and rapid means of quantitating prostaglandins, prostaglandin analogues, prostaglandin metabolites and thromboxanes.

This study was concerned with the development of a reliable radioimmunoassay to measure the release of PGE_2 and $PGF_{2\alpha}$ from the rat isolated ileum, and to study the effect of bradykinin on this release.

(i) Evaluation of the Radioimmunoassay

The criteria of reliability which are applied to the radioimmunoassay of prostaglandins include specificity, sensitivity, precision and accuracy. Initially, experiments were performed to determine these values for the radioimmunoassay used in this study.

(a) Standard Curve

Typical standard curves for $PGF_{2\alpha}$ and PGE_2 are presented (Fig. 2 and 3). The dried extracts of $PGF_{2\alpha}$ or PGE_2, obtained after thin-layer chromatography, were redissolved in 3ml Tris-gelatin buffer and assayed in 0.1ml aliquots. It was found in parallelism studies that extracts of either $PGF_{2\alpha}$ or PGE_2 assayed in volumes which varied from 0.025ml to 0.20ml, gave a linear relationship between the volume of extract added and the measured amount of prostaglandin. The correlation coefficients for $PGF_{2\alpha}$ and PGE_2 were 0.94 and 0.90, respectively.

(b) Specificity

The cross reactivity between the antisera raised against $PGF_{2\alpha}$ or PGE_2 and structurally related prostaglandins, was supplied by Dr L.Levine. These values were verified where studied (Tables I and 2).

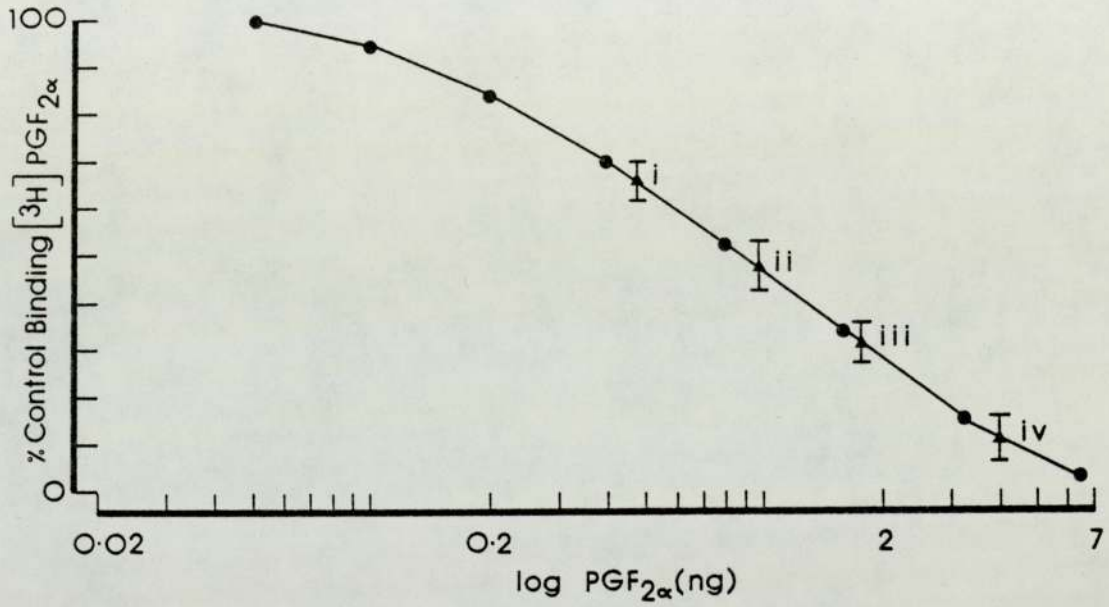

FIG.2 A typical standard curve for the radioimmunoassay of $PGF_{2\alpha}$ where each point (●) is the mean of 3 values. Parallelism studies demonstrated (i) 0.025ml; (ii) 0.05ml; (iii) 0.Iml; and (iv) 0.2ml of the same bathing fluid extract contained 0.48, 0.98, I.8, and 4.0ng of $PGF_{2\alpha}$ respectively. Each point is the mean ± s.e.m. (n=4).

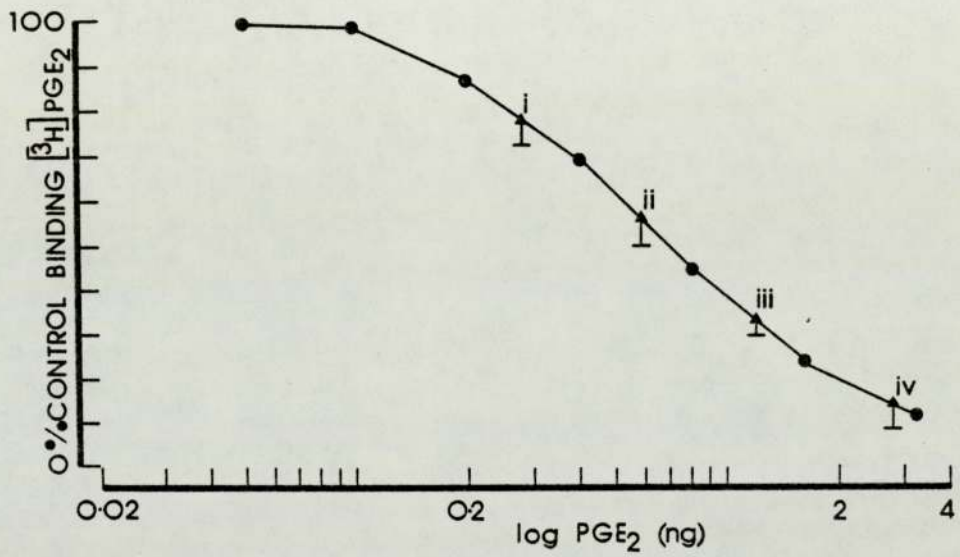

FIG.3 A typical standard curve for the radioimmunoassay of PGE_2 where each (●) point is the mean of 3 values. Parallelism studies demonstrated (i) 0.025ml; (ii) 0.05ml; (iii) 0.Iml; and (iv) 0.2ml of the same bathing fluid extract contained 0.28, 0.59, I.20, and 2.80ng of PGE_2 respectively. Each point is the mean ± s.e.m. (n=4).

PROSTAGLANDIN	CROSS REACTIVITY (a)	(b)
$PGF_{2\alpha}$	I00%	I00%
$PGF_{I\alpha}$	80%	
$PGF_{2\beta}$	5.8%	
PGE_2	0.9%	0.8%
I5-keto $PGF_{2\alpha}$	0.2%	
I3,I4,Dihydro-I5-keto $PGF_{2\alpha}$	<0.I%	
I3,I4,Dihydro $PGF_{2\alpha}$	0.4%	
PGA_2	(N.S)	0.7%
Arachidonic acid	<0.I%	

TABLE I. Cross reactivity of $PGF_{2\alpha}$ antiserum with various prostaglandins. Results are expressed as the percent cross reaction calculated as the weight (ng) of prostaglandin required to give a 50% displacement of the bound (^3H)$PGF_{2\alpha}$ from the antiserum, compared to the weight of $PGF_{2\alpha}$ required for the same displacement (=I00% cross reaction). (a) data provided; (b) data verified; (N.S) data not supplied.

PROSTAGLANDIN	CROSS REACTIVITY (a)	(b)
PGE_2	I00%	I00%
PGA_2	96%	93%
PGB_2	89%	
PGE_I	67%	
I3,I4,Dihydro-I5-keto PGE_2	3.6%	
$PGF_{2\alpha}$	I%	0.9%
Arachidonic acid	<0.I%	

TABLE 2. Cross reactivity of PGE_2 antiserum with various prostaglandins. Results are expressed as the percent cross reaction calculated as the weight (ng) of prostaglandin required to give a 50% displacement of the bound (^3H)PGE_2 from the antiserum, compared to the weight of PGE_2 required for the same displacement (= I00% cross reaction). (a) data provided; (b) data verified.

The marked cross reactivity of $PGF_{2\alpha}$ antiserum with $PGF_{I\alpha}$, and PGE_2 antiserum with PGA_2, PGB_2 and PGE_I, necessitated the expression of all results as either PGF-like or PGE-like.

(c) Sensitivity

The radioimmunoassay for both $PGF_{2\alpha}$ and PGE_2 had detection limits of 0.10ng, as measured in the 0.1ml aliquots, which corresponded with a 5% and 1% inhibition of binding respectively. However, the method blank accounted for a 5% to 14% inhibition of binding for $PGF_{2\alpha}$ (equivalent to 0.10ng to 0.16ng) and a 3% to 12% inhibition for PGE_2 (equivalent to 0.13ng to 0.19ng). Therefore, the overall detection limits, given by those values which were significantly different ($p < 0.05$) from the method blank, were 0.20ng for $PGF_{2\alpha}$ and 0.24ng for PGE_2.

(d) Precision

The intra-assay variation was assessed for $PGF_{2\alpha}$ or PGE_2 on 8 duplicate determinations of a bathing fluid extract performed in the same assay. Values ranging from 0.60ng to 0.84ng for $PGF_{2\alpha}$ and 0.69ng to 0.90ng for PGE_2, produced coefficients of variation of 9.6% and 7.4% respectively.

The inter-assay variation for $PGF_{2\alpha}$ and PGE_2 was determined using a pooled extract from which samples in duplicate were assayed in 7 separate runs. Values ranging from 0.72ng to 0.90ng for $PGF_{2\alpha}$ and 0.95ng to 1.30ng for PGE_2 produced coefficients of variation of 14.6% and 13.4% respectively.

(e) Accuracy

The accuracy of the radioimmunoassay for $PGF_{2\alpha}$ or PGE_2 was determined by studying the recovery of 2.5ng to 10ng $PGF_{2\alpha}$ or PGE_2 added to the Tyrode solution in which a tissue had previously been incubated. The solution then underwent

extraction and thin-layer chromatography in the usual manner. The amount of $PGF_{2\alpha}$ or PGE_2 added to the Tyrode incubate was plotted against the amount of the respective prostaglandin, corrected for recovery, measured in the radioimmunoassay. This gave correlation coefficients of 0.98 with $PGF_{2\alpha}$ and 0.90 with PGE_2 (Figs. 4 and 5).

The accuracy of the radioimmunoassay was also evaluated by comparison of the results with those obtained by a bioassay procedure (results presented later).

(f) Recovery

The total recovery of 25,000 c.p.m. (^3H)$PGF_{2\alpha}$ or (^3H) PGE_2, added to the Tyrode solution after removal of the tissue, was 57.0 ± 3% (n=9) and 58.I ± 4% (n=I2) respectively. The major site of loss was during thin-layer chromatography which accounted for 34.3 ± 6% (n=5) (^3H)$PGF_{2\alpha}$ and 3I.9 ± 6% (n=5) (^3H)PGE_2 of the original activity.

(^3H)$PGF_{2\alpha}$ or (^3H)PGE_2 were added to the Tyrode solution bathing the ileum, for the duration of the 60min incubation period. There was no significant difference in the total recovery of either (^3H)$PGF_{2\alpha}$ (55.5 ± 6%) or (^3H)PGE_2 (54.5 ± 5%), both n=4, $p > 0.05$, compared with values obtained in the absence of the ileum. This suggested that there was no significant metabolism of exogenous (^3H)$PGF_{2\alpha}$ or (^3H)PGE_2 by the ileum.

(ii) Assay of Prostaglandin Release from the Rat Isolated Terminal Ileum

(a) Determination of Prostaglandin Release Rate

Ferreira et al. (I976) demonstrated that prostaglandin output from segments of rabbit jejunum declined during the

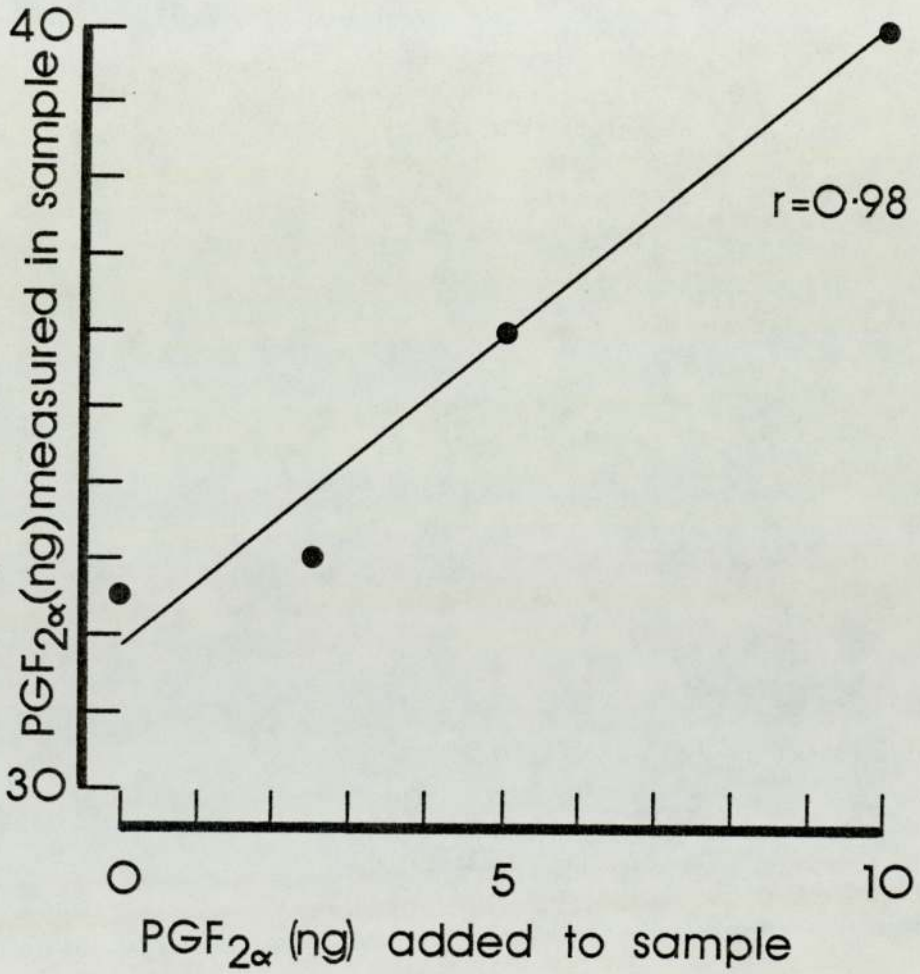

FIG.4 Accuracy of the radioimmunoassay to $PGF_{2\alpha}$, determined by assay of Tyrode incubates (sample) after the addition of increasing amounts of standard $PGF_{2\alpha}$. Measured values are corrected for recovery. r= correlation coefficient. Each point is the mean of 3 values.

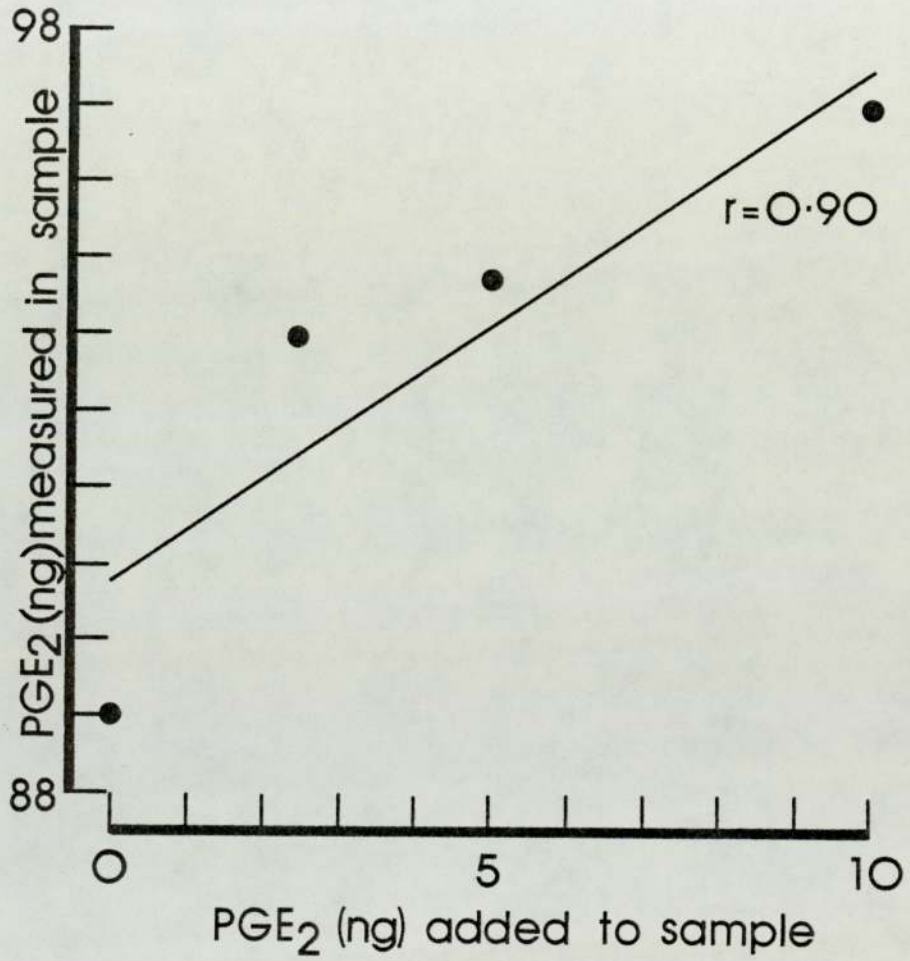

FIG.5 Accuracy of the radioimmunoassay to PGE_2, determined by assay of Tyrode incubates (sample) after the addition of increasing amounts of standard PGE_2. Measured values were corrected for recovery.
r= correlation coefficient. Each point is the mean of 3 values.

first 2hr after removal from the animal, and then increased. It was the purpose of this study to determine, for later work on the rat isolated terminal ileum, a period during which prostaglandin release was at a stable rate.

The organ bath fluid bathing the ileum was not extracted during the first 30min, since it was considered that prostaglandin release would be variable due to tissue manipulation. The amount of PGF-like or PGE-like material released during the first 60min collection period (30min to 90min) was 59 ± 8ng/g.tissue/hr (n=7) and I87 ± 39ng/g.tissue /hr (n=8) respectively, all values corrected for recovery. The amounts released during the second 60min collection period (90min to I50min) were 64 ± 7ng/g.tissue/hr (n=5) of PGF-like material and I80 ± 32ng/g.tissue/hr (n=6) of PGE-like material, which were not significantly different from the amounts released during the first collection period. Therefore, the first 60min collection period was used in subsequent studies to determine the basal release.

(b) <u>The Effect of Indomethacin, Bradykinin or Potassium Chloride on Prostaglandin Release</u>

The effect of an incubation for 60min in the presence of either indomethacin (28μM), bradykinin ($3.2 \times I0^{-I2}$M) or potassium chloride ($I0^{-5}$M), was investigated upon the basal release of PGF-like and PGE-like material from the rat isolated terminal ileum (Fig.6). Potassium chloride was used as the control spasmogen since it is a non-receptor stimulant which has been shown to cause a fast contraction due to calcium influx associated with membrane depolarization (Goodman and Weiss, I97I; van Breeman et al., I972; Marshall

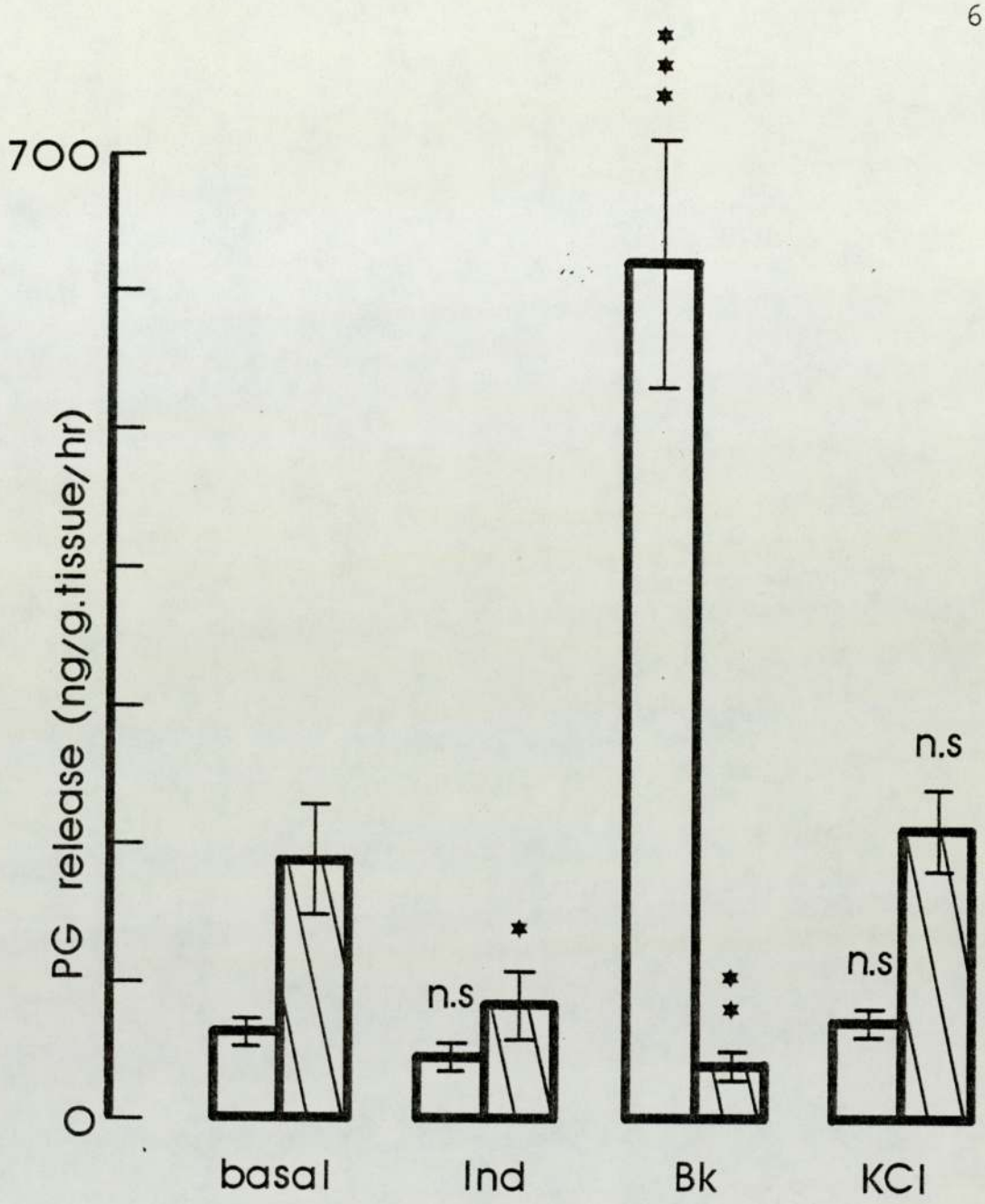

FIG.6 Effect of 28μM indomethacin (Ind); 3.2 x 10^{-12}M bradykinin (Bk); and 10^{-5}M potassium chloride (KCl) on the release of PGF-like (□) and PGE-like (▨) material, measured by radioimmunoassay, from the rat terminal ileum. All values corrected for recovery. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n.s, not significant compared with basal release. Vertical lines represent s.e.m. (n=8-15).

and Kroeger, I973).

The release of PGF-like material during incubation with indomethacin (28μM) was 47 ± IOng/g.tissue/hr (n=I5, $p > 0.05$), compared to the basal release of 65 ± IOng/g.tissue/hr (n=I5). The release of PGE-like material was significantly reduced during incubation with indomethacin (28μM) to 82 ± 27ng/g.tissue/hr (n=I2, $p < 0.05$) from the basal value of I89 ± 4Ing/g.tissue/hr (n=I5). Incubation with bradykinin ($3.2 \times IO^{-I2}M$) produced a significant increase in the release of PGF-like material to 626 ± 96ng/g.tissue/hr (n=8, $p < 0.00I$), but a significant reduction in the release of PGE-like material to 45 ± 8ng/g.tissue/hr (n=I2, $p < 0.0I$), compared to basal levels. In contrast, potassium chloride ($IO^{-5}M$), at a concentration which produced a similar contractile response to bradykinin ($3.2 \times IO^{-I2}M$), had no significant effect on the release of either PGF-like material (76 ± I2ng/g.tissue/hr) or PGE-like material (2I3 ± 27ng/g.tissue/hr) both n=8, $p > 0.05$ compared to basal levels.

It therefore appeared that bradykinin increased the release of PGF-like material from the rat ileum, but reduced the release of PGE-like material. This effect was probably specific to the bradykinin-induced contraction, since potassium chloride had no significant effect upon the release of prostaglandins, compared to basal values. To ensure that the effect of bradykinin was a direct stimulation of prostaglandin release and was not due to non-specific interference with the antigen/antibody reaction, it was decided to evaluate the accuracy of the results by bioassay.

B. Measurement by Bioassay of Prostaglandin Release from the Rat Isolated Terminal Ileum

Bioassay has been developed as a relatively simple, sensitive and specific technique for the measurement of prostaglandins (see Moncada et al., I978). In the present study it was used to confirm the results obtained by radioimmunoassay. The bioassay was performed within 24hr of the respective radioimmunoassay using the same extracts. The extracts were bioassayed by cascade superfusion on the rat fundus strip and rat colon, tissues known to be sensitive to PGE_2 and $PGF_{2\alpha}$ respectively (see Moncada et al., I978).

(i) The Effect of Indomethacin, Bradykinin and Potassium Chloride on Prostaglandin Release

Preliminary studies demonstrated that after application of prostaglandin extracts to thin-layer chromatography plates and the removal of Icm wide bands, biological activity was only present in bands with rf values of 0.46 and 0.60, which corresponded to the bands produced by $PGF_{2\alpha}$ and PGE_2 standards respectively.

Results presented as PGF-like material were assayed on the rat colon by comparison with authentic $PGF_{2\alpha}$, whilst PGE-like material was assayed on the rat fundus strip by comparison with authentic PGE_2. A sensitivity range of I0ng to I80ng $PGF_{2\alpha}$ was determined on the rat colon. The only detectable PGF-like activity (530 $\pm$ 60ng/g.tissue/hr) was after incubation with $3.2 \times I0^{-I2}M$ bradykinin. A sensitivity range of 0.5ng to I00ng PGE_2 was determined on the rat fundus strip.

Fig.7 shows the effect of a 60min incubation in the presence of either indomethacin (28μM), bradykinin (3.2 x 10^{-12}M) or potassium chloride (10^{-5}M) upon the release of PGE-like material from the rat ileum. All values were corrected for recovery. There was no significant change in the release of PGE-like material during incubation with either 3.2 x 10^{-12}M bradykinin (99 ± 27ng/g.tissue/hr) n=10, $p > 0.05$ or 10^{-5}M potassium chloride (199 ± 32ng/g.tissue/hr) n=8, $p > 0.05$ compared to the basal release of 170 ± 50ng/g.tissue/hr (n=10). However, incubation with indomethacin (28μM) significantly reduced the production of PGE-like material from the basal value to 40 ± 29ng/g.tissue/hr (n=10,$p < 0.05$).

The results obtained by bioassay demonstrated that bradykinin increased the release of PGF-like material from the rat ileum, since only after incubation with bradykinin was there a detectable level of PGF-like activity. This confirmed those results obtained with PGF-like material measured by radioimmunoassay. However, after incubation with bradykinin, no significant reduction of PGE-like material could be demonstrated by bioassay. This was contrary to the findings with the radioimmunoassay.

The results did not indicate whether the measured change in prostaglandin release was associated with the contractile response to bradykinin, which would probably have occurred during the first 5min, or with some alternate mechanism which occurred during the 60min incubation. Therefore, the study was extended to measure, by direct bioassay on the rat fundus strip, the release of prostaglandin-like material from the isolated perfused rat terminal ileum after injection of bradykinin.

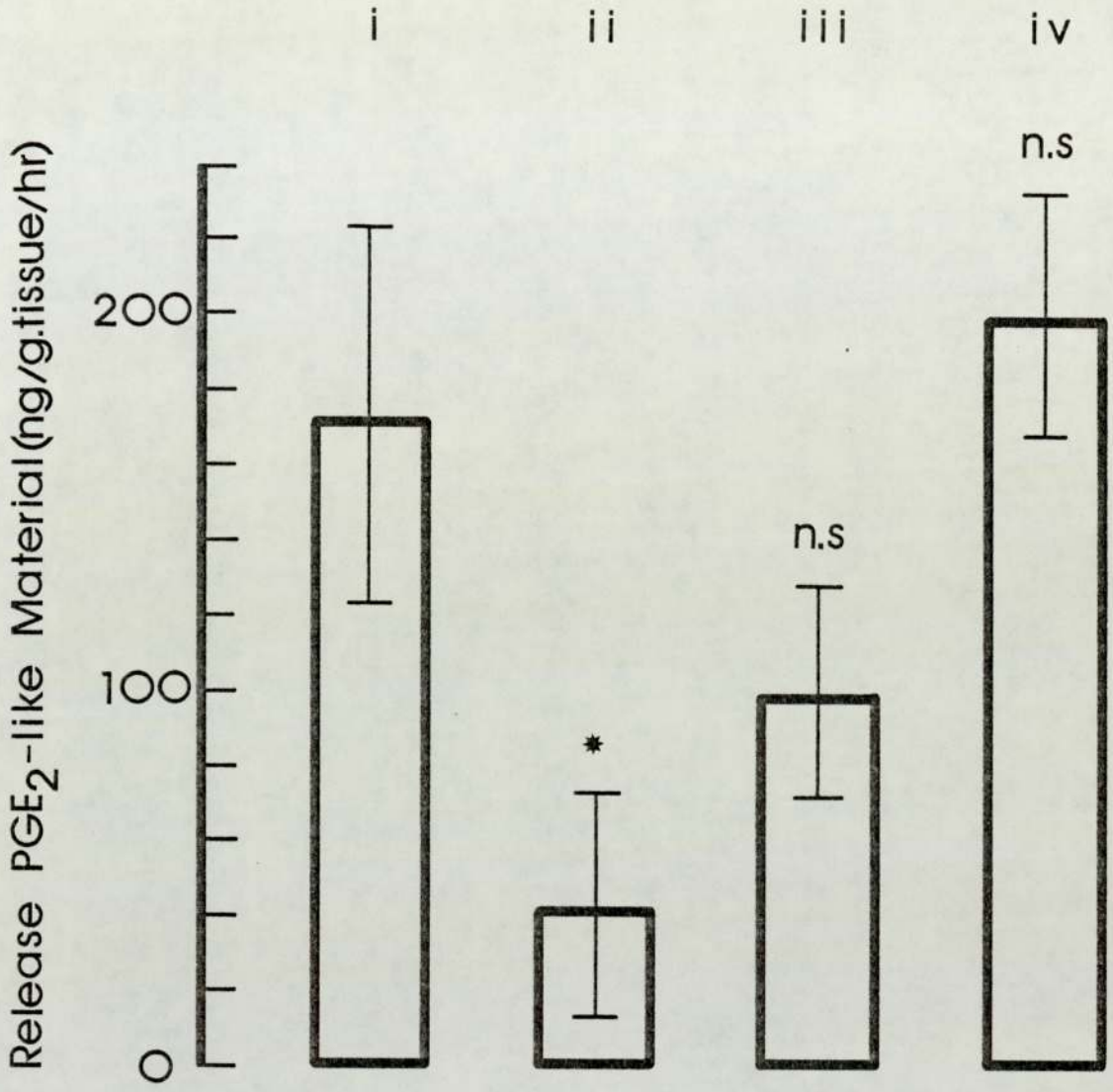

FIG.7 The effect of 28μM indomethacin (ii); 3.2 x 10^{-12}M bradykinin (iii); and 10^{-5}M potassium chloride (iv) on the basal release (i) of PGE_2-like material from rat terminal ileum, measured by comparison to authentic PGE_2. All values corrected for recovery. *: $p < 0.05$; n.s, not significant compared with basal release. Vertical lines represent s.e.m. (n=8-10).

C. Bioassay of the Prostaglandin-like Material Released from the Rat Isolated Perfused Terminal Ileum

In this study the effluent from a 4cm section of ileum, perfused with Krebs solution, was allowed to superfuse a rat fundus strip. The objective was to correlate the contractile response of the ileum to increasing concentrations of bradykinin, with the appearance of prostaglandin-like material in the effluent. The effect of bradykinin during mucosal and serosal perfusion of the ileum was studied. Additionaly, it was hoped to quantify the prostaglandin-like material released from the ileum by bradykinin.

(i) The Effect of the Effluent from the Perfused Ileum on the Tone of the Rat Fundus Strip

The effluent from 4cm sections of terminal ileum perfused with Krebs solution, increased the resting tension of the rat fundus strip. During either mucosal or serosal perfusion of the ileum the resting tension of the rat fundus strip was significantly increased by 0.60 $\pm$ 0.Ig and 0.56 $\pm$ 0.I3g respectively, (both n=35, $p < 0.00I$), and this was maintained throughout the experiment (approx. 3hr). This increase was abolished in all cases by the addition of either indomethacin (28μM) or aspirin (6I0μM) to the mucosal or serosal perfusate (all n=I2). The resting tension of the rat fundus strip returned to the original baseline tension after 33 $\pm$ 4min (n=I2) or 36 $\pm$ 4min (n=I2) following the addition of indomethacin (28μM) to the mucosal or serosal perfusate respectively.

These results demonstrated a continuous basal release

of prostaglandin-like material during perfusion of either surface of the ileum. Therefore, any attempt to quantify a bradykinin-induced release of prostaglandin-like material from the ileum must take into account this basal release.

(ii) The Effect of Bradykinin and Potassium Chloride on the Release of Prostaglandin-like Material from the Rat Perfused Ileum

(a) Mucosal Perfused Ileum

The contractions of the rat fundus strip to bradykinin following perfusion over the mucosal surface of the ileum were greater than control contractions to bradykinin injected in the absence of the ileum. Fig.8 represents the bradykinin contractions on the rat fundus strip presented as regression lines constructed from the concentrations of bradykinin within the linear part of the log dose-response relationship. Perfusion of bradykinin over the mucosal surface of the ileum caused a displacement of the regression line to the left of the control line with a significant difference at the EC_{50} of I4.0 $\pm$ 6.8% (n=I74, $p < 0.05$). In the presence of either indomethacin (28μM) or aspirin (6I0μM), contractions of the rat fundus strip to bradykinin were reduced towards the control values. No significant difference was observed between the regression lines at the EC_{50} in the presence of either 28μM indomethacin, I0.5 $\pm$ 5.3% (n=97, $p > 0.05$) or 6I0μM aspirin, 7.0 $\pm$ 4.I% (n=I02, $p > 0.05$) compared with the control. Indomethacin (28μM) or aspirin (6I0μM) perfused in the absence of the ileum had no effect upon contractions of the rat fundus strip to bradykinin.

Perfusion of potassium chloride over the mucosal

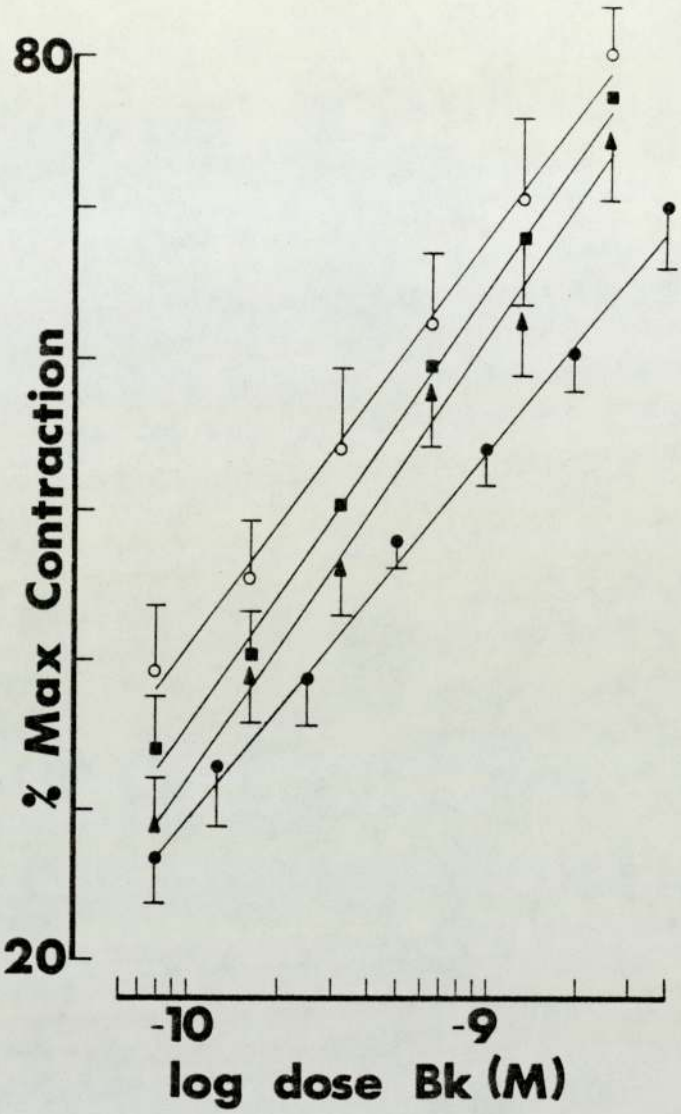

FIG.8 Log dose-response regression lines for contractions of rat fundus strip to bradykinin (Bk) following mucosal perfusion of the rat ileum. Contractions to Bk injected in the absence of the ileum ● (n=10), to Bk injected into the mucosal perfused ileum O (n=19), and to Bk injected into the ileum mucosally perfused with either 28μM indomethacin ■ (n=6) or 610μM aspirin ▲ (n=6). Each point represents the mean ± s.e.m.

surface caused no significant displacement of the regression line from the control line obtained in the absence of the ileum (Fig.9). Addition of indomethacin (28μM) to the perfusate also had no significant effect.

(b) Serosal Perfused Ileum

The contractions of the rat fundus strip to bradykinin following perfusion over the serosal surface of the ileum were of similar size to those obtained during mucosal perfusion. The regression lines for the response of the rat fundus strip to bradykinin after perfusion over either ileal surface are shown in Fig.10. Perfusion of bradykinin over the serosal surface displaced the regression line to the left of the control with a significant displacement at the EC_{50} of 10.3 ± 4.5% (n=201, $p < 0.05$). In the presence of either 28μM indomethacin or 610μM aspirin (Fig.11) contractions of the rat fundus strip to bradykinin were reduced towards the control values and there was no significant displacement calculated at the EC_{50}, 8.3 ± 5.6% (n=106, $p > 0.05$) and 6.5 ± 4.9% (n=144, $p > 0.05$) respectively.

It was of interest to note that the contractile response shape of the rat fundus strip to bradykinin, after injection over either ileal surface, appeared to be the same as the response shape obtained in the absence of the ileum.

Perfusion of potassium chloride over the serosal surface of the ileum caused no significant displacement of the regression line from the control line obtained in the absence of the ileum (Fig.12). Addition of indomethacin (28μM) to the perfusate also had no significant effect upon the response of the rat fundus strip to potassium chloride.

These results demonstrated that the response of the

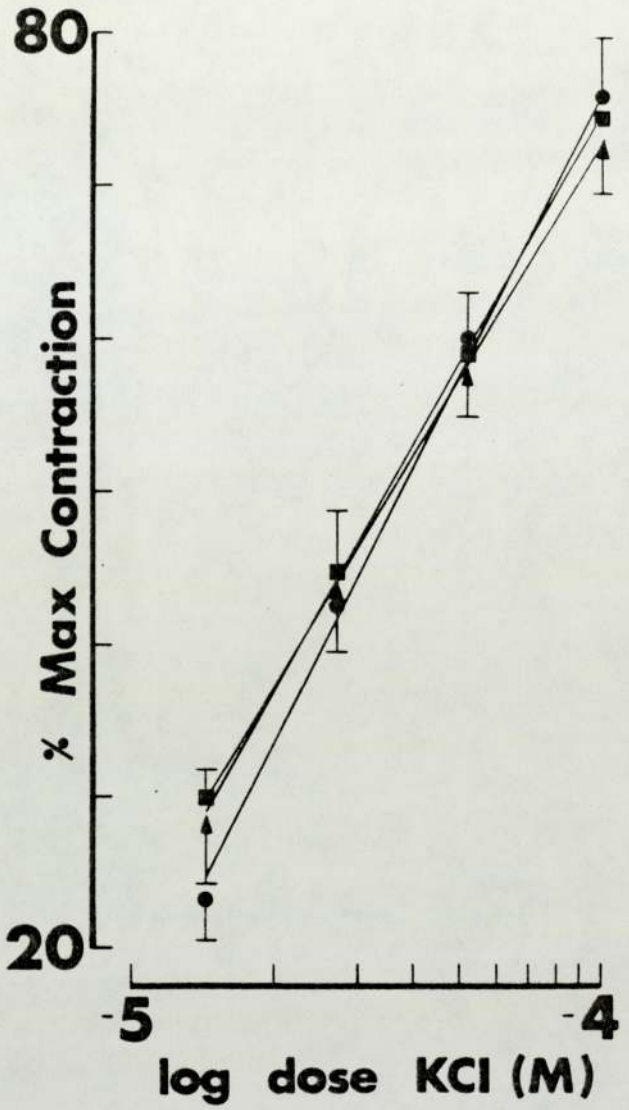

FIG.9 Log dose-response regression lines for contractions of rat fundus strip to potassium chloride (KCl) following mucosal perfusion of the rat ileum. Contractions to KCl injected in the absence of the ileum ● (n=10), to KCl injected into the mucosal perfused ileum ▲ (n=15), and to KCl injected into the ileum mucosally perfused with 28μM indomethacin ■ (n=10) added to the perfusate. Each point represents the mean ± s.e.m.

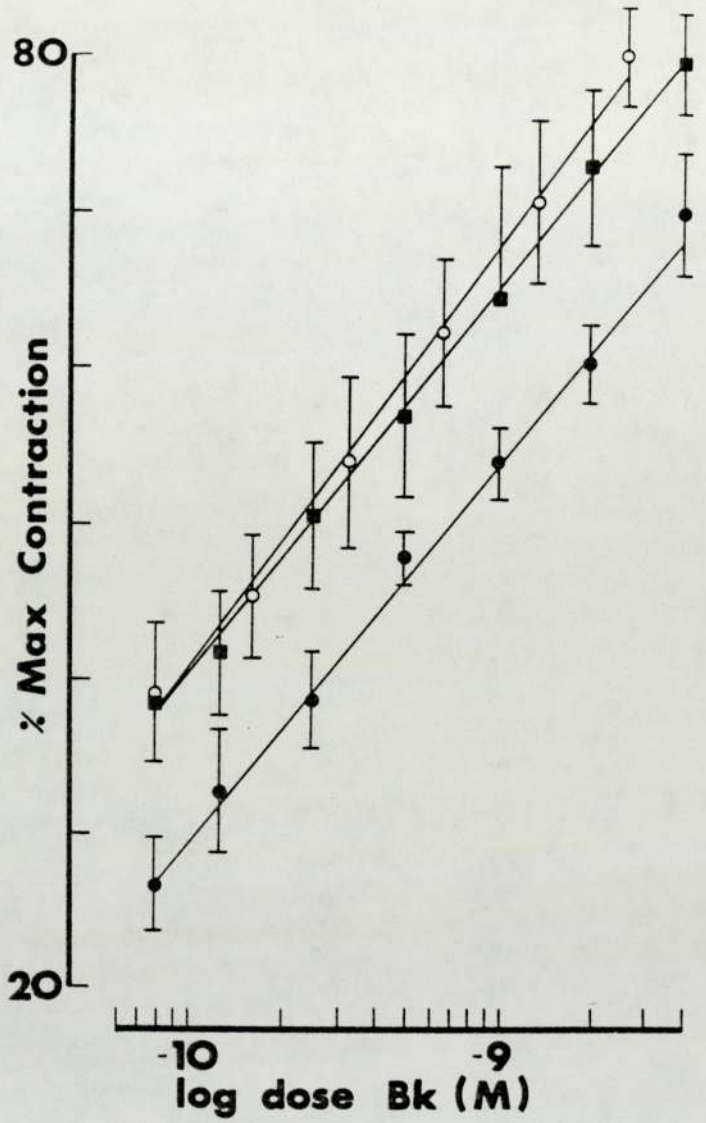

FIG.IO Log dose-response regression lines for contractions of rat fundus strip to bradykinin (Bk). Contractions to Bk injected in the absence of the ileum ● (n=IO), and to Bk injected into the serosal ■ (n=20) or mucosal O (n=I9) perfused ileum. Each point represents the mean ± s.e.m.

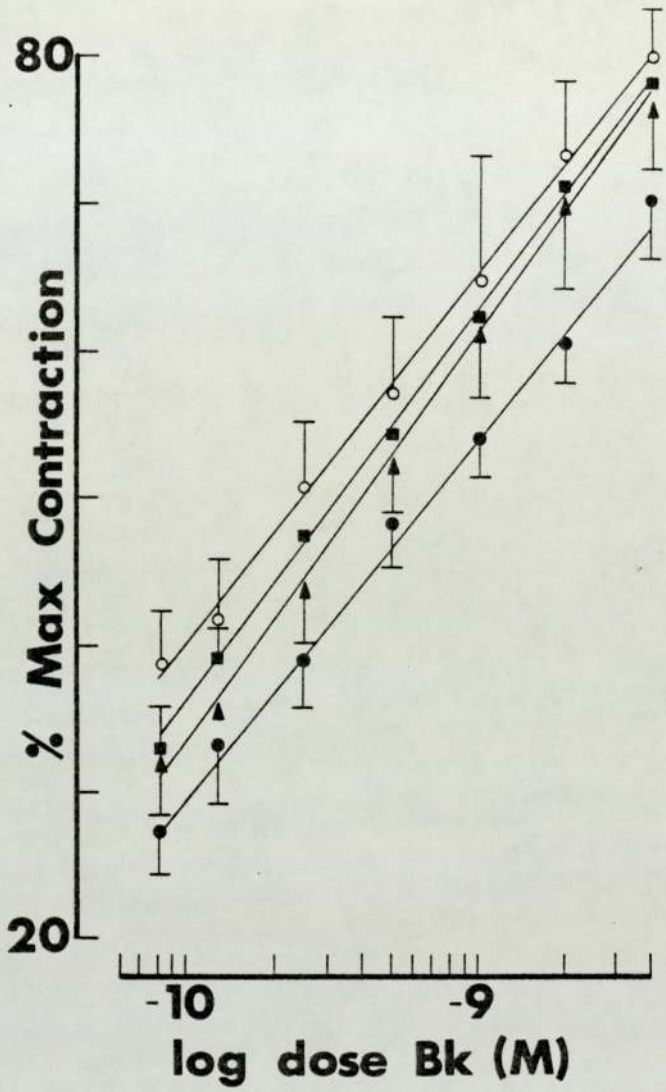

FIG.II Log dose-response regression lines for contractions of rat fundus strip to bradykinin (Bk) following serosal perfusion of the rat ileum. Contractions to Bk injected in the absence of the ileum ● (n=IO), to Bk injected into the serosal perfused ileum O (n=20), and to Bk injected into the ileum serosally perfused with either 28μM indomethacin ■ (n=6) or 6IOμM aspirin ▲ (n=I4). Each point represents the mean ± s.e.m.

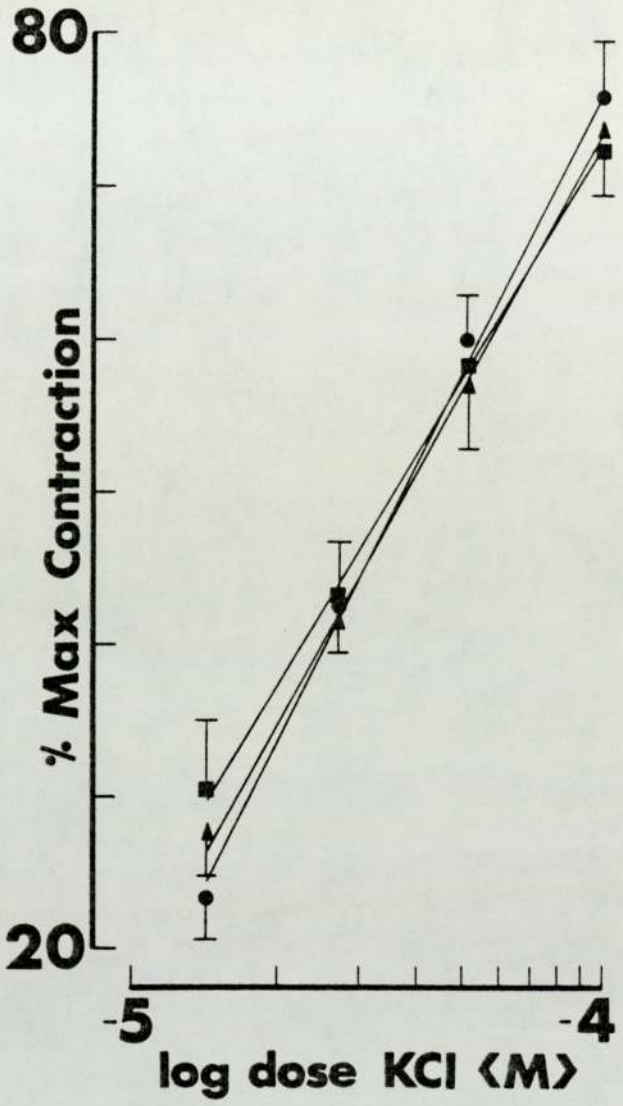

FIG.I2 Log dose-response regression lines for contractions of rat fundus strip to potassium chloride (KCl) following serosal perfusion of the rat ileum. Contractions to KCl injected in the absence of the ileum ● (n=I0), to KCl injected into the serosal perfused ileum ▲ (n=I5), and to KCl injected into the ileum serosally perfused with 28μM indomethacin ■ (n=I0) added to the perfusate. Each point represents the mean ± s.e.m.

rat fundus strip to bradykinin, but not to potassium chloride, was increased after perfusion over either surface of the ileum. This increased response was probably due to the release of a prostaglandin-like material from the ileum.

To verify that a prostaglandin-like material had increased the response of the rat fundus strip to bradykinin, the effect of the prostaglandin receptor antagonist SC I9220 (Sanner, I969) was studied.

(iii) The Effect of SC I9220 on the Response of the Rat Fundus Strip to Bradykinin Perfused through the Rat Ileum

The prostaglandin receptor antagonist SC I9220 ($IO^{-5}M$) was added to the Krebs (K_2) reservoir (see pp.45-47). This Krebs (K_2) solution superfused the rat fundus strip simultaneously with the effluent from the ileum. The regression lines for responses of the rat fundus strip to bradykinin in the presence of SC I9220, and after perfusion over either surface of the ileum were not significantly displaced from the control line (Fig.I3). No significant difference was observed between the regression lines at the EC_{50} after mucosal (4.0 $\pm$ 6.2%) or serosal (0.I $\pm$ 5.9%) perfusion of the ileum, both n=I20, $p > 0.05$ compared with the control line. The control line represents the response of the rat fundus strip to bradykinin injected in the presence of SC I9220 ($IO^{-5}M$) and in the absence of the ileum.

There was no significant difference between the regression lines for bradykinin on the rat fundus strip, obtained in the absence of the ileum and in the absence or presence of SC I9220 ($IO^{-5}M$), 0.9 $\pm$ 5% (n=I54, $p > 0.05$) at

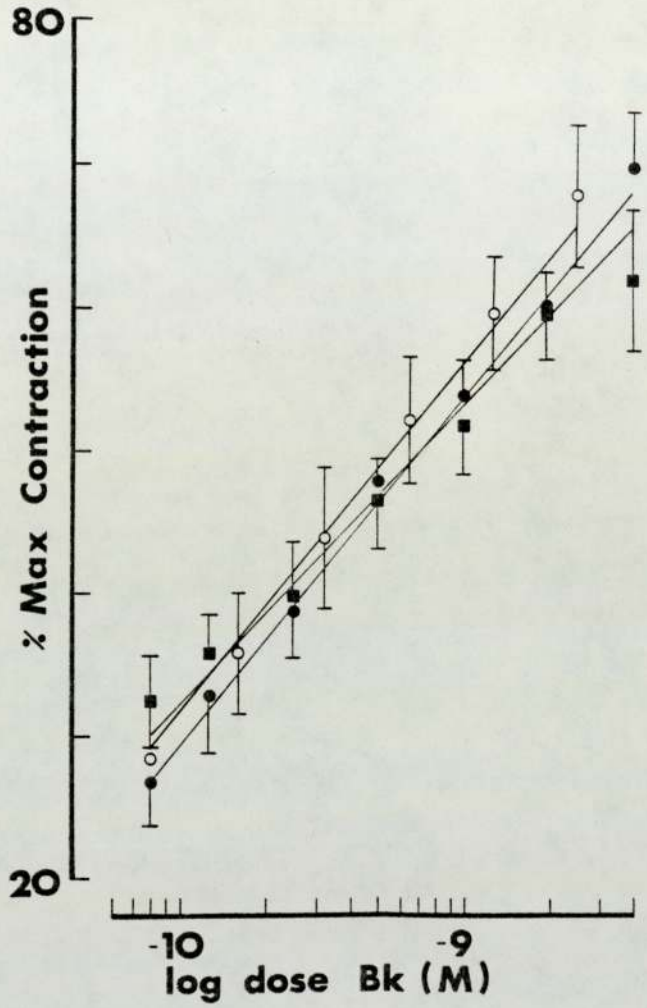

FIG.13 Log dose-response regression lines for contractions of rat fundus strip to bradykinin (Bk) in the presence of SC 19220 (10^{-5}M). Contractions to Bk injected in the absence of the rat ileum ● (n=12), to Bk injected into the mucosal perfused ileum ○ (n=6) and to Bk injected into the serosal perfused ileum ■ (n=6). Each point represents the mean ± s.e.m.

the EC_{50}.

These results demonstrated that the response of the rat fundus strip to bradykinin, in the absence of the ileum, was unaffected by the presence of SC I9220, which suggested the absence of any non-specific depression of bradykinin responses. Furthermore, the responses of the rat fundus strip to bradykinin, perfused over either surface of the ileum, were not increased in the presence of the prostaglandin receptor antagonist SC I9220. This provided further support for the release of a prostaglandin-like material from the perfused ileum.

Thus, these experiments with cyclo-oxygenase inhibitors and the prostaglandin receptor antagonist SC I9220, suggested there was a release of prostaglandin-like material from the ileum. It was therefore decided to investigate whether it was possible to quantify this release.

(iv) Quantification of the Prostaglandin-like Material Released from the Perfused Ileum

The existence of a continuous basal release of prostaglandin-like material from the perfused ileum has been demonstrated (see pp.72-73). Initially the objective was to assess the effect of this basal release on the response of the superfused rat fundus strip to bradykinin. This was essential since the effect of bradykinin and a possible bradykinin-induced release of prostaglandin-like material, would be superimposed on this basal release.

The contribution of the basal release of prostaglandin-like material, on the response of the rat fundus strip to bradykinin, was assessed by removal of the ileum from the

apparatus. PGE_2 and/or $PGF_{2\alpha}$ were added to the Krebs (K_I) reservoir (see pp.45-46) and allowed to continuously superfuse the rat fundus strip together with the Krebs (K_2) solution, which contained antagonists (Gilmore et al., I968) and indomethacin (2.8μM). Prostaglandin concentrations were selected to produce an increase in tone of the rat fundus strip similar to that observed during superfusion with the effluent from the ileum. PGE_2 0.7 and I.4 x $IO^{-I2}M$, and $PGF_{2\alpha}$ I.05 and 2.I x $IO^{-I2}M$ were found to be suitable.

(a) The Effect of PGE_2 on the Response of the Rat Fundus Strip to Bradykinin or Potassium Chloride

Fig.I4a and I4b demonstrate the effect of four concentrations of PGE_2 (0.35, 0.7, I.4 and 2.8 x$IO^{-I2}M$), added to the Krebs (K_I) reservoir, upon the contractions of the rat fundus strip to bradykinin (2.4 x $IO^{-IO}M$) and potassium chloride (3.2 x $IO^{-5}M$) respectively. All contractions were measured from the lowest tension recorded immediately prior to the response and were expressed as a percentage of the maximal response to the agonist studied. The concentrations of bradykinin (2.4 x $IO^{-IO}M$) and potassium chloride (3.2 x $IO^{-5}M$) used, gave similar contraction heights which were 40% to 50% maximal contractions. The increase in resting tension caused by the prostaglandins are shown by the narrow hatched histobars in Fig.I4(a and b). All results have been expressed as a percentage of the maximal contraction of the agonist studied.

Contractions to bradykinin in the presence of the lowest concentration of PGE_2 (0.35 x $IO^{-I2}M$) were 40 ± 6% (n=IO) of maximum and were not significantly different from

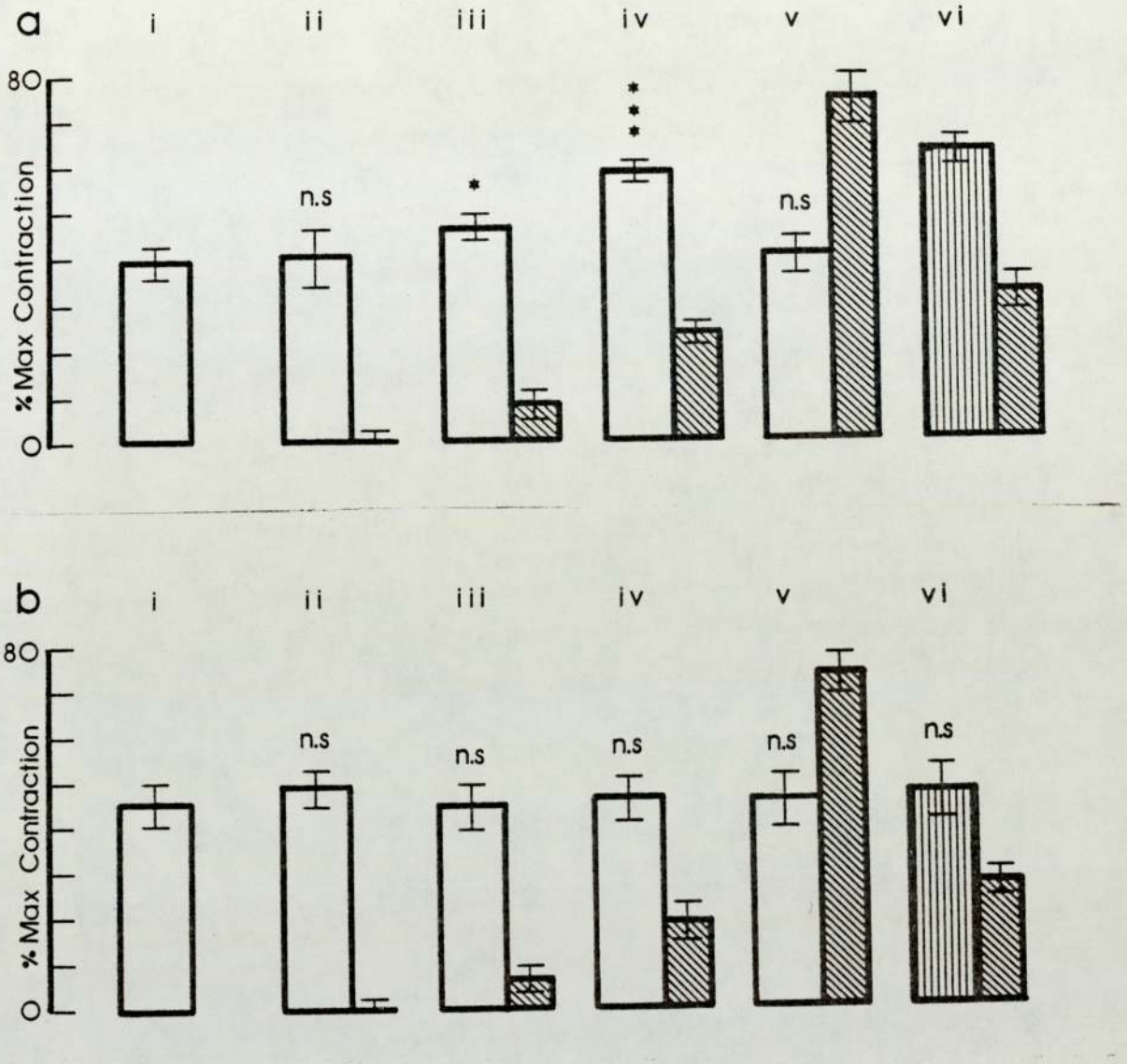

FIG.I4 Effect of continuous superfusion with PGE_2 on contractions of the rat fundus strip to bradykinin or potassium chloride. □ represents contractions to 2.4 x 10^{-10}M bradykinin (FIG.I4a) or 3.2 x 10^{-5}M potassium chloride (FIG.I4b) of the rat fundus strip superfused with Krebs solution alone (i); or with Krebs containing 0.35 (ii); 0.70 (iii); I.40 (iv); and 2.80 (v) x10^{-12}M PGE_2; or a combination of I.40 x 10^{-12}M PGE_2 and I.05 x 10^{-12}M PGF_2 (vi). ▨ represents changes in baseline tension of the rat fundus strip during prostaglandin superfusion. All contractions are expressed as a percentage of the maximal contraction to the agonist in the absence of prostaglandins. *: $p < 0.05$; ***: $p < 0.001$; n.s, not significant compared with control response (i). Vertical lines represent s.e.m. (n=10-14).

the control contractions of 39 ± 3% (n=I4, $p > 0.05$) obtained in the absence of prostaglandins. PGE_2 (0.35 x $IO^{-I2}M$) increased the tone of the tissue by 0.5 ± 2%. However, with 0.7 x $IO^{-I2}M$ and I.4 x $IO^{-I2}M$ PGE_2, the tone of the tissue was increased by 8 ± 4% and 23 ± 2% respectively, and the response of the rat fundus strip to bradykinin was increased to 45 ± 2% (n=IO, $p < 0.05$) and 58 ± 3% (n=I4, $p < 0.00I$) respectively of maximum. There was no significant difference between contractions to bradykinin in the presence of the highest concentration of PGE_2 (2.8 x $IO^{-I2}M$), 40 ± 4% (n=IO, $p > 0.05$) and the control value. However, at this concentration of PGE_2 (2.8 x $IO^{-I2}M$) the tone of the rat fundus strip was 74 ± 6% of maximum and may have masked any increased contractile response to bradykinin. The simultaneous addition of PGE_2 (I.4 x $IO^{-I2}M$) and $PGF_{2\alpha}$ (I.05 x $IO^{-I2}M$) to the Krebs (K_I) reservoir (Fig.I4a) increased the tone of the tissue by 3I ± 5% of maximum. This combination augmented by 5% the increase in the bradykinin contraction produced by PGE_2 (I.4 x $IO^{-I2}M$) alone, to 63 ± 3% (n=I2, $p < 0.00I$).

There was no significant increase in the contractions of the rat fundus strip to potassium chloride (3.2 x $IO^{-5}M$) in the presence of 0.35, 0.7, I.4 and 2.8 x $IO^{-I2}M$ PGE_2 (Fig.I4b), although there was an increase in the tone of 0.4 ± 2%, 7 ± 4%, 20 ± 2% and 65 ± 5% respectively, of maximum. The combination of I.4 x $IO^{-I2}M$ PGE_2 and I.05 x $IO^{-I2}M$ $PGF_{2\alpha}$ increased the tone of the assay tissue by 27 ± 4% of maximum, but had no effect upon contractions to potassium chloride (3.2 x $IO^{-5}M$).

(b) The Effect of $PGF_{2\alpha}$ on the Response of the Rat Fundus Strip to Bradykinin or Potassium Chloride

Fig.I5a and I5b show the effect of four concentrations of $PGF_{2\alpha}$ (0.26, 0.53, I.05 and 2.I x IO^{-I2}M), added to the Krebs (K_I) reservoir, upon contractions of the rat fundus strip to bradykinin (2.4 x IO^{-IO}M) and potassium chloride (3.2 x IO^{-5}M) respectively. Again, all contractions and increases in tone were expressed as a percentage of the maximal contraction of the agonist studied. At all the concentrations of $PGF_{2\alpha}$ studied there was no significant increase in the response of the rat fundus strip to either bradykinin or potassium chloride, compared to their respective control responses. Furthermore, the increase in the tone of the rat fundus strip with $PGF_{2\alpha}$ was less than that seen with PGE_2. Only at concentrations of I.05 and 2.I x IO^{-I2}M $PGF_{2\alpha}$ was there an increase in tone of the rat fundus strip, I2 $\pm$ 2% (n=IO) and 37 $\pm$ 3% (n=IO) respectively, of a maximal bradykinin contraction (Fig.I5a).

These results suggested that in the presence of PGE_2 and/or $PGF_{2\alpha}$ the contractions of the rat fundus strip to bradykinin, but not to potassium chloride, were dependent upon the prostaglandin(s) used and the concentration. A continuous basal release of prostaglandin-like material from the perfused ileum has already been demonstrated. Therefore, the increased contractions of the rat fundus strip to bradykinin could have been due to a potentiation by this basal release, a stimulated release of prostaglandin-like material, or to a combination of these effects. Thus, it did not appear possible to quantify, on the rat fundus strip,

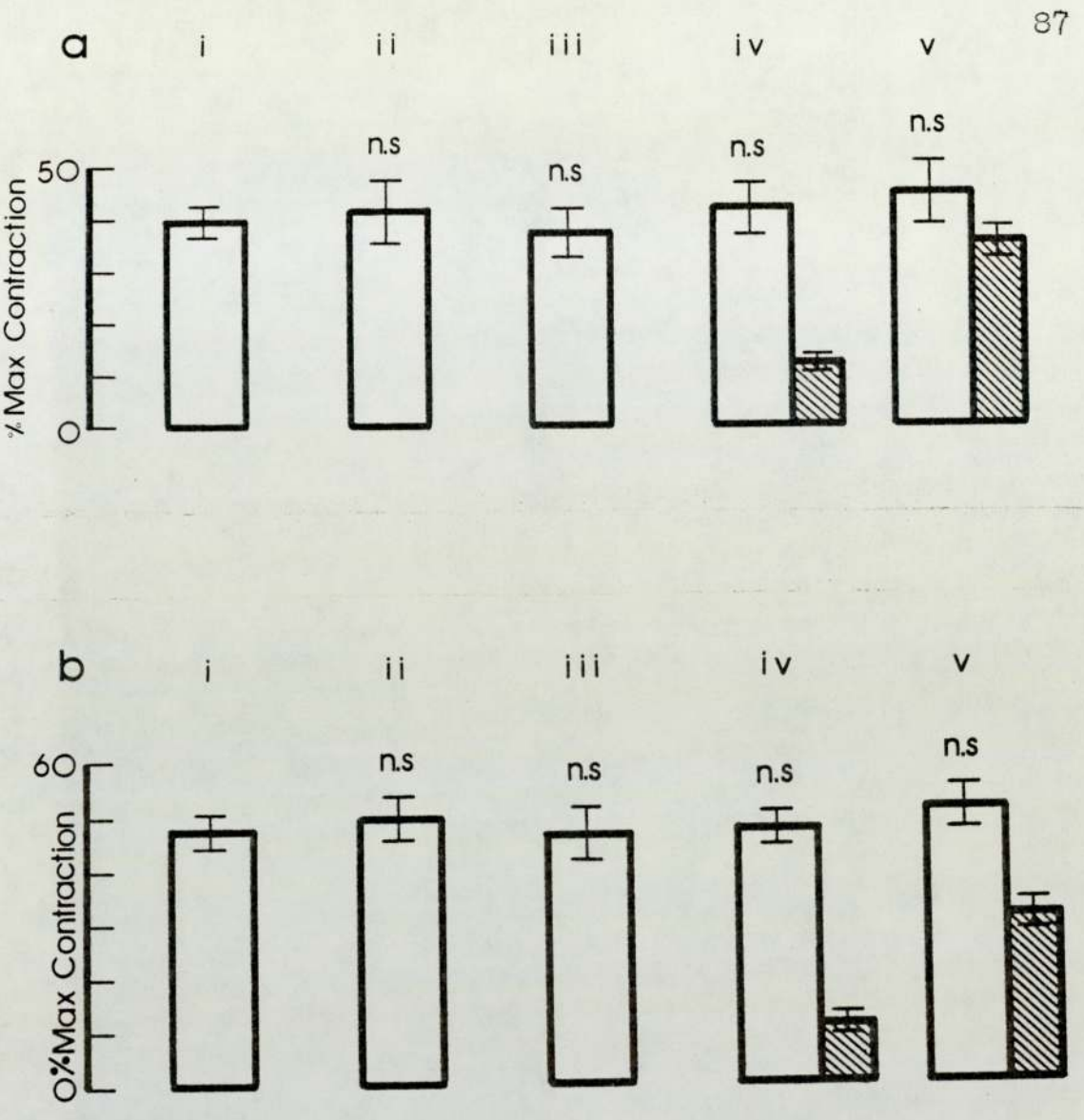

FIG.I5 Effect of continuous superfusion with $PGF_{2\alpha}$ on contractions of the rat fundus strip to bradykinin or potassium chloride. □ represents contractions to 2.4 x 10^{-10}M bradykinin (FIG.I5a) or 3.2 x 10^{-5}M potassium chloride (FIG.I5b) of the rat fundus strip superfused with Krebs solution alone (i); or with Krebs containing 0.26 (ii); 0.53 (iii); I.05 (iv); and 2.I0 (v) x10^{-12}M $PGF_{2\alpha}$. ▨ represent changes in baseline tension of the rat fundus strip during prostaglandin superfusion. All contractions are expressed as a percentage of the maximal contraction to the agonist in the absence of prostaglandins. n.s, not significant compared with control response (i). Vertical lines represent s.e.m. (n=I0-I4).

the prostaglandin-like material released from the perfused ileum by bradykinin.

SECTION 2 Experiments to Investigate the Involvement of Cyclo-Oxygenase Products in the Response of Intestinal Smooth Muscle to Bradykinin

In the previous section the effect of bradykinin was investigated on the release of prostaglandin-like material from the perfused ileum. During these experiments, the contraction of the longitudinal muscle to bradykinin was recorded simply to correlate the contractile response of the ileum, with the appearance of prostaglandin-like material in the effluent. Close examination of the recordings suggested that addition of indomethacin or aspirin to the perfusate of the ileum, abolished the contractile response to bradykinin during mucosal, but not serosal perfusion. The observation during mucosal perfusion supported the findings of Crocker and Willavoys (1976), who suggested that prostaglandins participated in the contraction of the longitudinal muscle of rat isolated ileum to bradykinin. However, since these workers were studying the effect of bradykinin on a section of terminal ileum maintained in an organ bath, they were probably studying a predominantly serosal action of bradykinin. Therefore, to clarify the role of prostaglandins in the contractile response to bradykinin, it was decided to make a comparative investigation of bradykinin during mucosal and serosal perfusion of the rat isolated terminal ileum. Subsequently, the study was extended to the rat duodenum, a tissue which exhibits a relaxation to bradykinin (Horton, 1959). Additional preliminary studies were performed on the cat ileum, a tissue with specific bradykinin receptors (Barabe et al., 1975,1977), to determine if there was any

species variation in the nature of the response to bradykinin.

A. The Role of Cyclo-Oxygenase Products in the Contractile Response of the Rat Perfused Ileum to Bradykinin

The possible involvement of prostaglandins, or other cyclo-oxygenase products in the contractile action of the perfused ileum to bradykinin, was initially investigated by addition of the cyclo-oxygenase inhibitors indomethacin or aspirin (Vane, I97I) to the perfusate. The work was then extended to the use of tissues from animals pretreated with indomethacin. Finally, experiments were performed to try and reverse the effect of indomethacin by addition of prostaglandins to the perfusion solution.

(i) The Effect of Indomethacin or Aspirin on the Contractile Response of the Perfused Ileum to Bradykinin or Potassium Chloride

(a) Mucosal Perfused Ileum

The log dose-response curve of the longitudinal muscle to bradykinin, perfused over the mucosal surface, was depressed (Fig.I6) in the presence of either indomethacin (28μM) or aspirin (6I0μM). Thus, the maximal response of the longitudinal muscle to bradykinin was reduced to 20 ± 5% by 28μM indomethacin and to 47 ± 6% by 6I0μM aspirin (both n=9, $p < 0.00I$).

The log dose-response curve of the longitudinal muscle to potassium chloride (Fig.I7) was unaltered by indomethacin (28μM) and the maximal response was I00 ± 8% (n=I0, $p > 0.05$) compared to control.

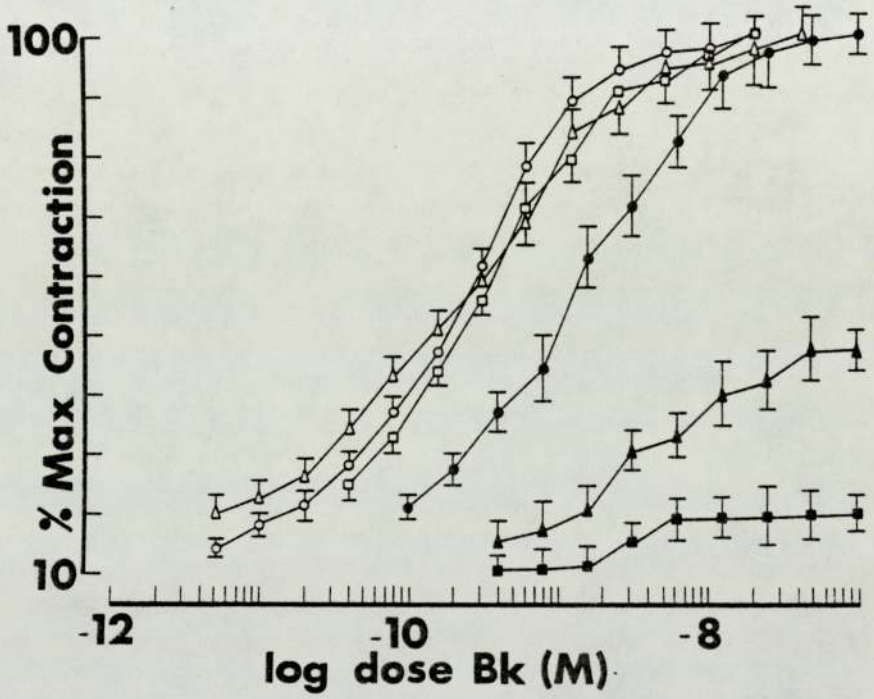

FIG.I6 Log dose-response relationships of longitudinal muscle of rat ileum to bradykinin (Bk). ○ represent serosally perfused ileum with 28μM indomethacin (□) or 6I0μM aspirin (△) in the perfusate. ● represent mucosally perfused ileum with 28μM indomethacin (■) or 6I0μM aspirin (▲) in the perfusate. Each point represents the mean ± s.e.m. (n=5-9).

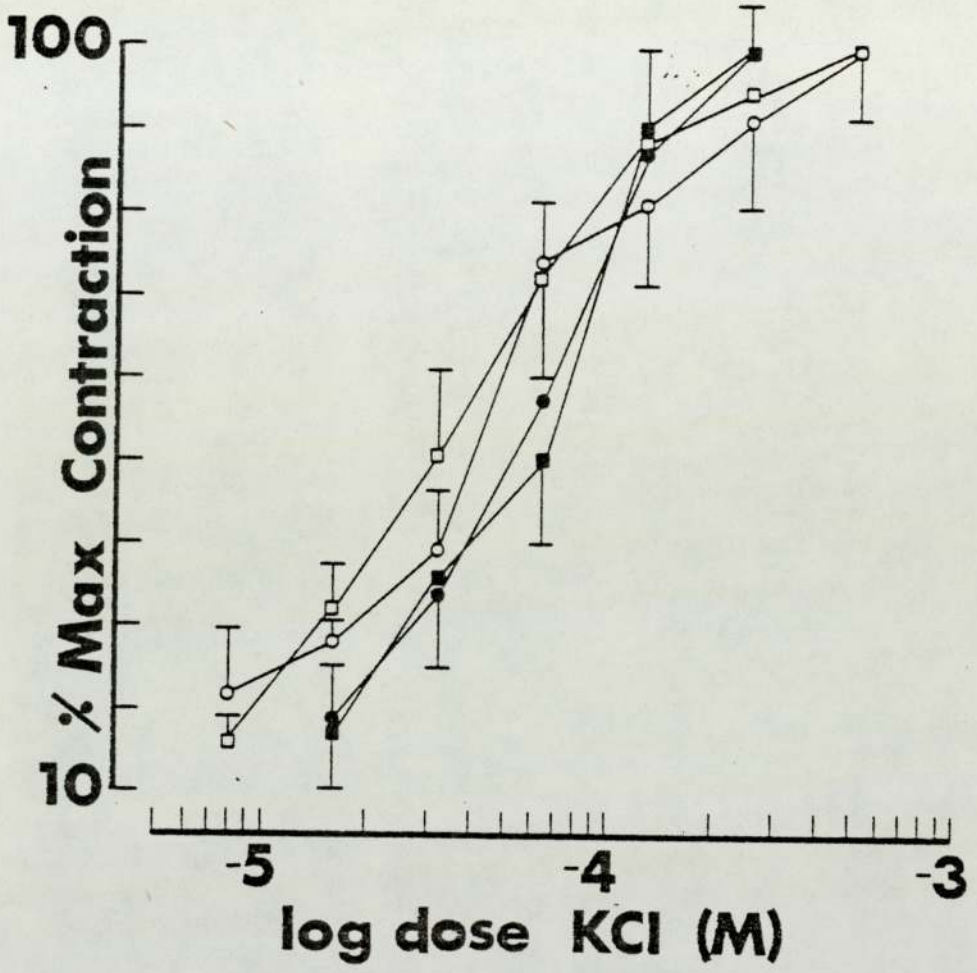

FIG.I7 Log dose-response relationships of longitudinal muscle of rat ileum to potassium chloride (KCl). ○ represent serosally perfused ileum with 28μM indomethacin (□) in the perfusate. ● represent mucosally perfused ileum with 28μM indomethacin (■) in the perfusate. Each point represents the mean ± s.e.m. (n=I0-I5).

(b) Serosal Perfused Ileum

The serosal surface was more sensitive to bradykinin than the mucosal surface (Fig.I6), the mean EC_{50} being 1.5×10^{-10}M and 8.1×10^{-10}M respectively. The maximal contraction to serosally applied bradykinin (5.0 ± 0.3g), n=6, was greater than seen during mucosal perfusion (2.4 ± 0.2g), n=9, $p < 0.01$.

The log dose-response curve to bradykinin perfused over the serosal surface was unaffected by the presence of either indomethacin (28μM) or aspirin (610μM) and the maximum responses were 100 ± 4% and 100 ± 6% (both n=6, $p > 0.05$) respectively of control.

Similarly, responses to potassium chloride were not significantly effected (Fig.I7) by indomethacin (28μM), the maximal response being 99 ± 10% (n=10, $p > 0.05$) compared to control.

These results suggested that the action of bradykinin during mucosal, but not serosal perfusion of the ileum, was dependent upon a functional cyclo-oxygenase system. These experiments had been designed as a preliminary study and had therefore used comparatively high concentrations of the cyclo-oxygenase inhibitors to ensure inhibition of the enzyme. To verify the results of the present study, it was necessary to use lower concentrations of the inhibitors to eliminate/ reduce possible non-specific actions of the drugs. In the following experiments 2.8μM indomethacin was added to the perfusion solution. Additionaly, some experiments were also performed on tissues from animals pretreated, subcutaneously, with indomethacin. This was to determine whether there was a diffusion barrier to indomethacin on the serosal surface,

since if a barrier existed pretreatment would probably have no effect on responses to bradykinin during mucosal perfusion.

(ii) The Effect of Indomethacin and Pretreatment of Rats with Indomethacin, on the Response of the Perfused Ileum to Bradykinin

Rats were pretreated with indomethacin 8mg.Kg subcutaneously, 2hr prior to killing the animal (8mg.Kg/s.c./2hr) and removal of the terminal ileum.

(a) Mucosal Perfused Ileum

The log dose-response curve of the longitudinal muscle to bradykinin, perfused over the mucosal surface, was depressed in the presence of indomethacin (2.8μM) and in tissues removed from animals pretreated with 8mg.Kg/s.c./2hr indomethacin (Fig.I8). Thus, the maximal response of the longitudinal muscle to bradykinin was reduced to 55 ± 4% after pretreatment with indomethacin (8mg.Kg/s.c./2hr) and and to 46 ± 4% by 2.8μM indomethacin (both n=5, $p < 0.001$).

(b) Serosal Perfused Ileum

The log dose-response curve of the longitudinal muscle to bradykinin, perfused over the serosal surface of the ileum, was unaffected by pretreatment of the rats with indomethacin (8mg.Kg/s.c./2hr). The maximal contraction during serosal perfusion was 98 ± 7% (n=5, $p > 0.05$) of the control response (Fig.I9).

These results provided further support for an interrelationship between bradykinin and cyclo-oxygenase products during mucosal perfusion of the rat isolated terminal ileum. Furthermore, if the response to bradykinin during mucosal perfusion was dependent upon prostaglandin synthesis, then

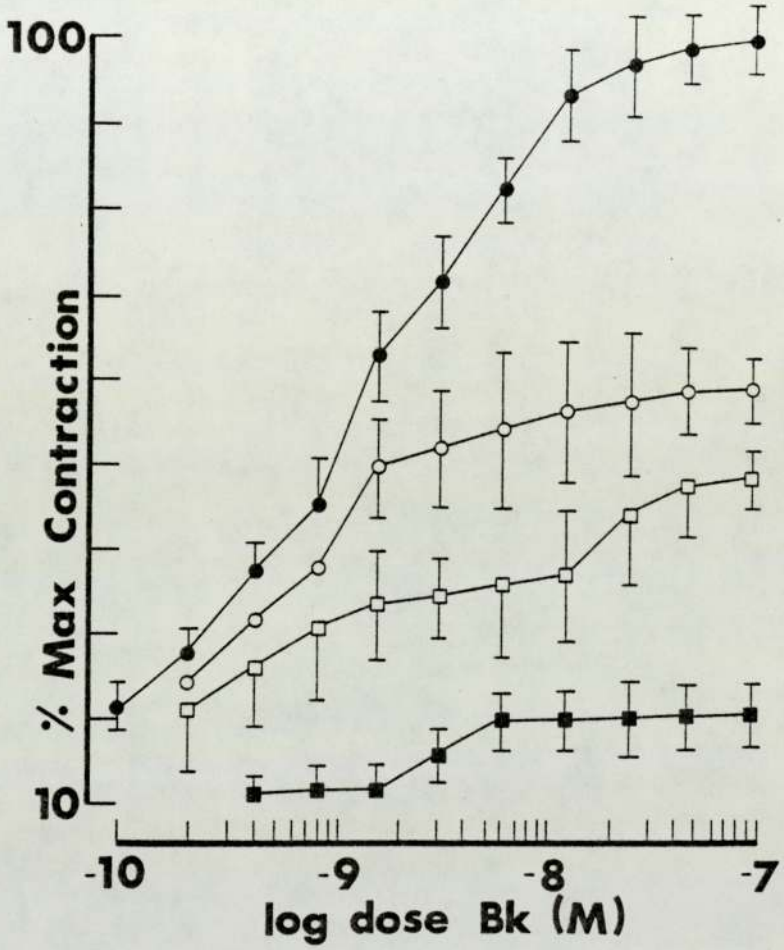

FIG.I8 Log dose-response relationships of the longitudinal muscle of the mucosally perfused rat ileum to bradykinin (Bk). ● represents mucosally perfused ileum with 2.8μM (□) or 28μM (■) indomethacin in the perfusate. ○ represents the response of the ileum from rats pretreated with indomethacin 8mg.Kg/s.c./2hr. Each point represents the mean ± s.e.m. (n=5-9).

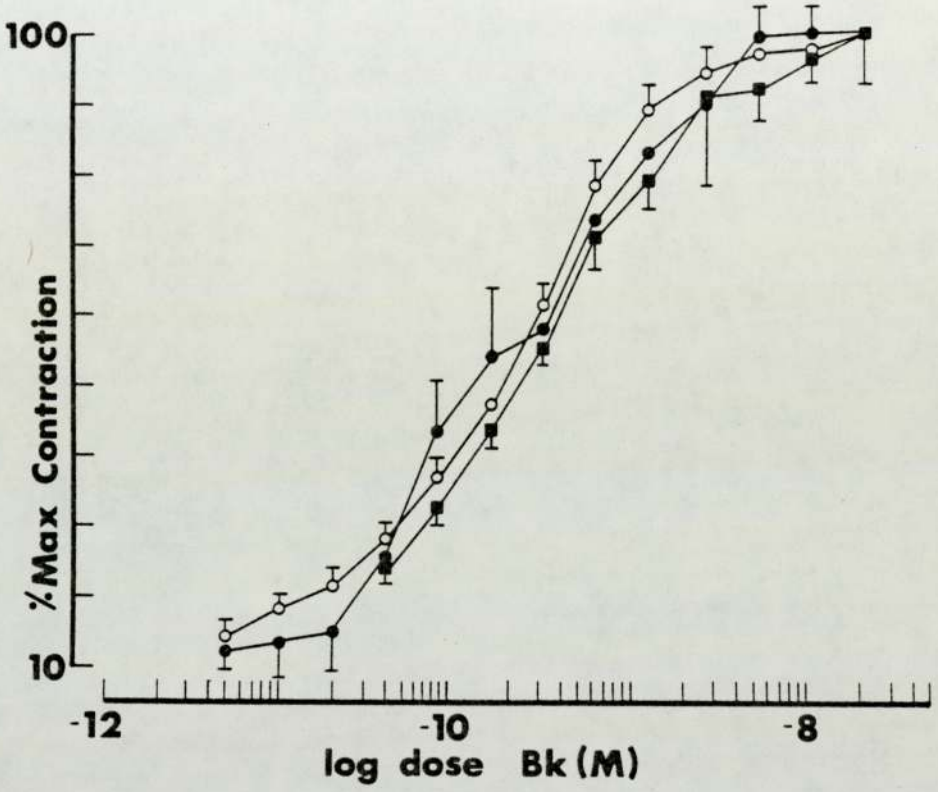

FIG.I9 Log dose-response relationships of longitudinal muscle of the serosally perfused rat ileum to bradykinin (Bk). O represents serosally perfused ileum with 2.8μM (■) indomethacin in the perfusate. ● represents the response of the ileum from rats pretreated with indomethacin 8mg.Kg/s.c./2hr. Each point represents the mean ± s.e.m. (n=5-6).

addition of exogenous prostaglandins might overcome this inhibition. Therefore, it was decided to investigate the effect of PGE_2 and $PGF_{2\alpha}$ on the response of the mucosal perfused ileum to bradykinin.

(iii) The Effect of Indomethacin, PGE_2 or $PGF_{2\alpha}$ on the Response of the Mucosal Perfused Rat Ileum to Bradykinin

The highest concentration of PGE_2 added to the perfusate, which had no effect on the resting tension of the mucosal perfused rat ileum was 1.4×10^{-12}M. The equivalent concentration of $PGF_{2\alpha}$ was 2.1×10^{-12}M. Addition of PGE_2 (1.4×10^{-12}M) together with indomethacin (2.8μM) to the mucosal perfusate, displaced the log dose-response curve for bradykinin to the left of that obtained in the presence of indomethacin (2.8μM) alone (Fig.20). In the presence of both indomethacin (2.8μM) and PGE_2 (1.4×10^{-12}M), the maximal contraction to bradykinin was 61 ± 6% (n=7) which was significantly larger ($p < 0.05$) than the maximal response obtained in the presence of indomethacin (2.8μM) alone, 44 ± 5% (n=10).

Addition of $PGF_{2\alpha}$ (2.1×10^{-12}M) together with indomethacin (2.8μM) to the mucosal perfusate, produced no displacement of the log dose-response curve to bradykinin, compared to that obtained in the presence of indomethacin (2.8μM) alone (Fig.21). The maximal contraction to bradykinin, in the presence of indomethacin (2.8μM) and $PGF_{2\alpha}$ (2.1×10^{-12}M) was 42 ± 6% (n=7). This was not significantly different from the maximal response obtained in the presence of indomethacin (2.8μM) alone, 44 ± 5% (n=10, $p > 0.05$).

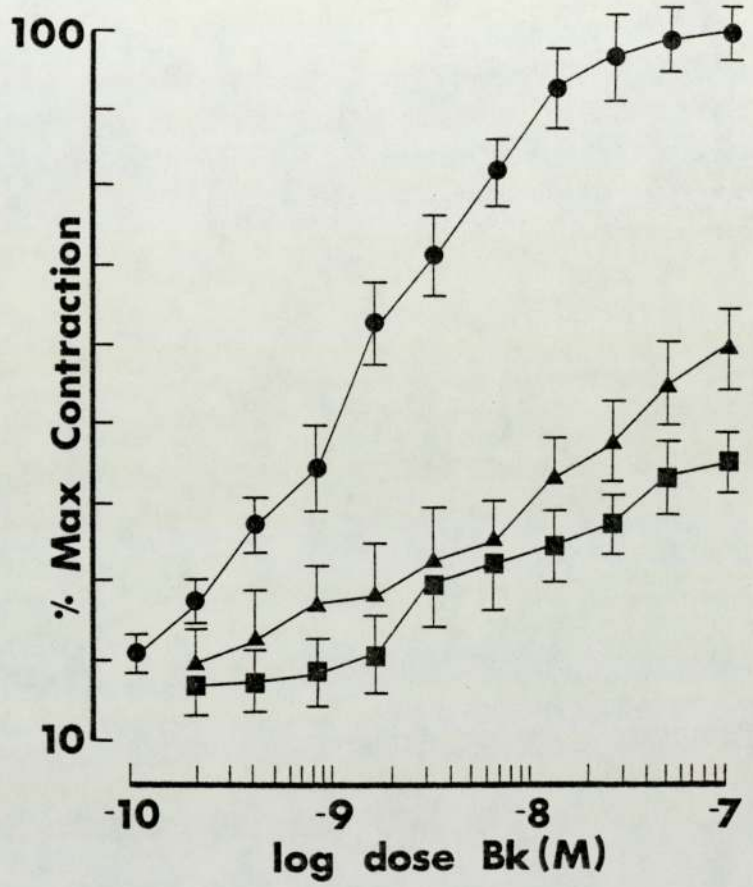

FIG.20 Log dose-response relationships of the longitudinal muscle of the mucosal perfused rat ileum to bradykinin (Bk). ● represents mucosally perfused ileum with 2.8μM (■) indomethacin, or 2.8μM indomethacin plus I.4 x 10^{-12}M PGE_2 (▲) in the perfusate. Each point represents the mean ± s.e.m. (n=7-10).

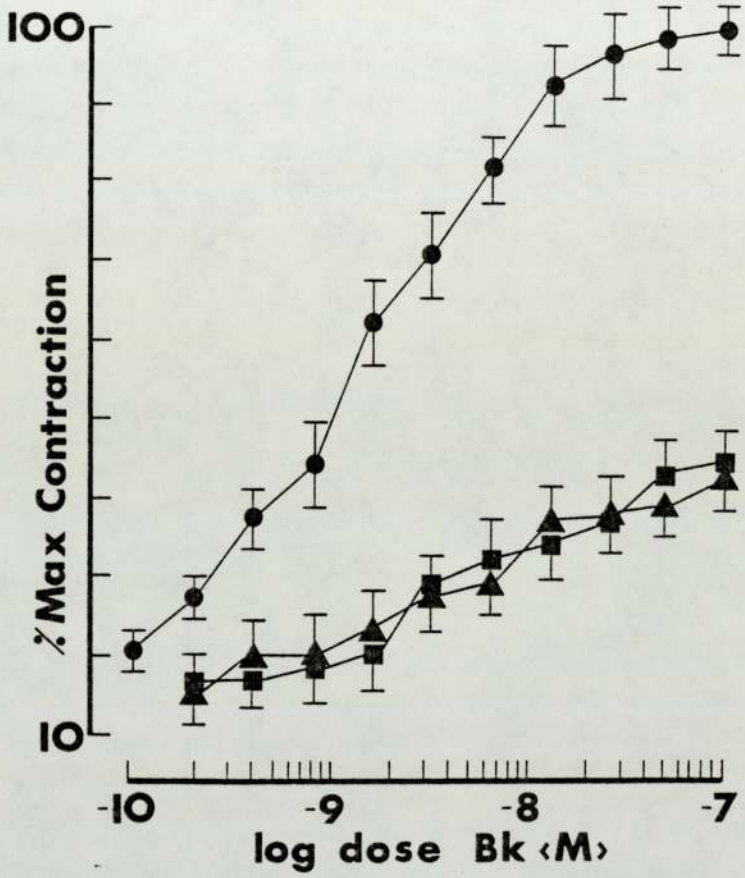

FIG.2I Log dose-response relationships of the longitudinal muscle of the mucosally perfused rat ileum to bradykinin (Bk). ● represents mucosally perfused ileum with 2.8μM (■) indomethacin, or 2.8μM indomethacin plus 2.1×10^{-12}M $PGF_{2\alpha}$ (▲) in the perfusate. Each point represents the mean ± s.e.m. (n=7-10).

These results demonstrated that during mucosal perfusion of the ileum with indomethacin, responses to bradykinin were only partially reversed by PGE_2 and appeared unaffected by $PGF_{2\alpha}$. This could have been due to the poor penetration of the prostaglandins to the active site. Alternatively, it might have been because PGE_2 and $PGF_{2\alpha}$ were not the main cyclo-oxygenase products involved in the response to bradykinin.

B. The Role of Cyclo-Oxygenase Products in the Relaxation of the Rat Isolated Perfused Duodenum to Bradykinin

The previous results suggested that products of the cyclo-oxygenase pathway might be involved in the contractile action of bradykinin during mucosal, but not serosal perfusion of the rat ileum. The study was extended to investigate whether a similar interrelationship might exist in the rat duodenum, a tissue which exhibits a relaxation to bradykinin (Horton, I959).

(i) The Effect of Indomethacin and Pretreatment of Rats with Indomethacin, on the Response of the Perfused Duodenum to Bradykinin

During perfusion of bradykinin over either surface of the duodenum there was a concentration range which gave an all-or-none response. This range was between 10^{-13}M to 0.8 x 10^{-12}M bradykinin during mucosal perfusion and 5 x 10^{-14}M to 0.4 x 10^{-12}M bradykinin during serosal perfusion. However, graded and reproducible relaxations could be obtained between 0.8 to 6.4 x 10^{-12}M during mucosal perfusion and between 0.4

to 3.2×10^{-12}M bradykinin during serosal perfusion (Fig.22). Isoprenaline, a β-adrenergic receptor stimulant, was used as the control agent in experiments on the rat duodenum.

(a) Mucosal Perfused Duodenum

The addition of indomethacin (2.8μM or 28μM) to the mucosal perfusate, depressed the log dose-response relationship to bradykinin (Fig.23) and reduced the maximal relaxation to 58 ± 6% and 47 ± 5% (both n=6, $p < 0.001$) respectively, of the control. The log dose-response relationship to bradykinin was also depressed in tissues removed from animals pretreated with indomethacin (8mg.Kg/s.c./2hr). The maximal relaxation was reduced to 62 ± 16% (n=7, $p < 0.05$) of the control.

(b) Serosal Perfused Duodenum

The relaxations of the duodenum to bradykinin perfused over the serosal surface were unaffected by the addition of indomethacin (2.8μM or 28μM) to the perfusate (Fig.24), or in tissues from animals pretreated with indomethacin (8mg.Kg/s.c./2hr).

The addition of indomethacin (2.8μM or 28μM) to the perfusate of either surface of the duodenum had no effect upon relaxations to isoprenaline. The relaxations induced by isoprenaline, perfused over either surface of the duodenum, were also unaffected by pretreatment of animals with indomethacin (8mg.Kg/s.c./2hr).

These results demonstrated that the involvement of cyclo-oxygenase products, in the relaxation of the duodenum, was specific to bradykinin. Furthermore, the results have demonstrated an involvement of the cyclo-oxygenase pathway in both the contractile response of the ileum and the relaxation of the duodenum to bradykinin. This suggested

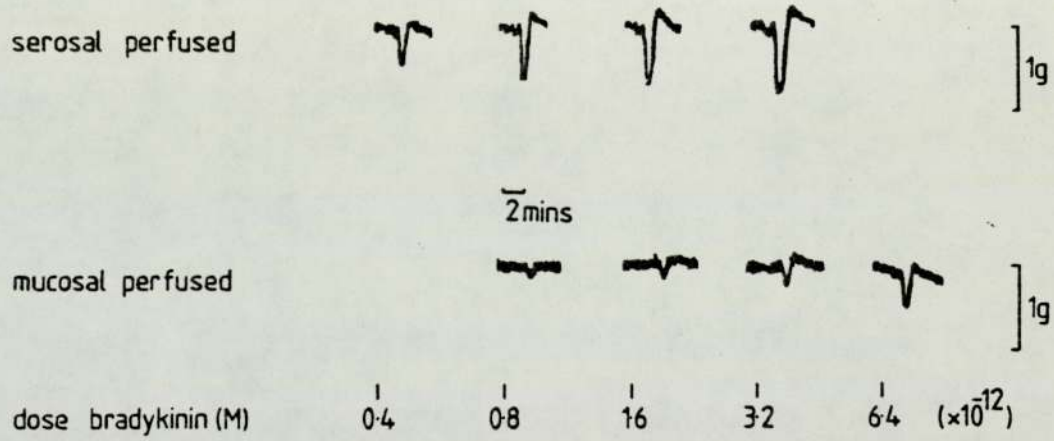

FIG.22 Traces showing the graded relaxations of the serosal or mucosal perfused rat duodenum to bradykinin. Vertical bars indicate scale in g.tension. Resting tension of the duodenum was adjusted to Ig, 4min prior to each injection of bradykinin. Dose cycle I2min.

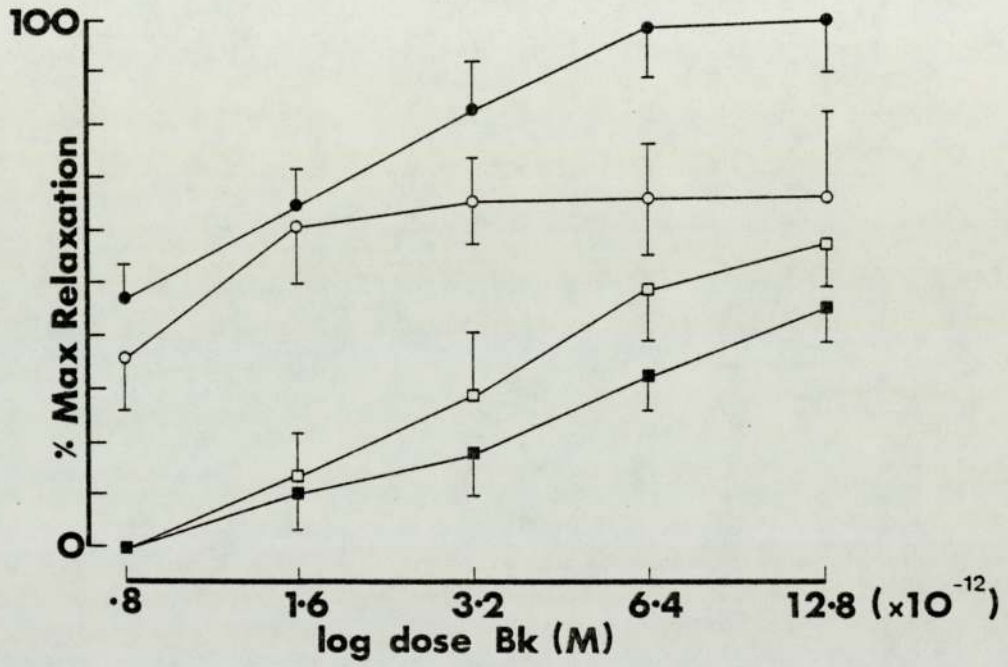

FIG.23 Log dose-response relationships of the longitudinal muscle of mucosal perfused duodenum to bradykinin (Bk). ● represents mucosally perfused duodenum with 2.8μM (□) or 28μM (■) indomethacin in the perfusate. ○ represents the response of the duodenum from rats pretreated with indomethacin 8mg.Kg/s.c./2hr. Each point represents the mean ± s.e.m. (n=6-9).

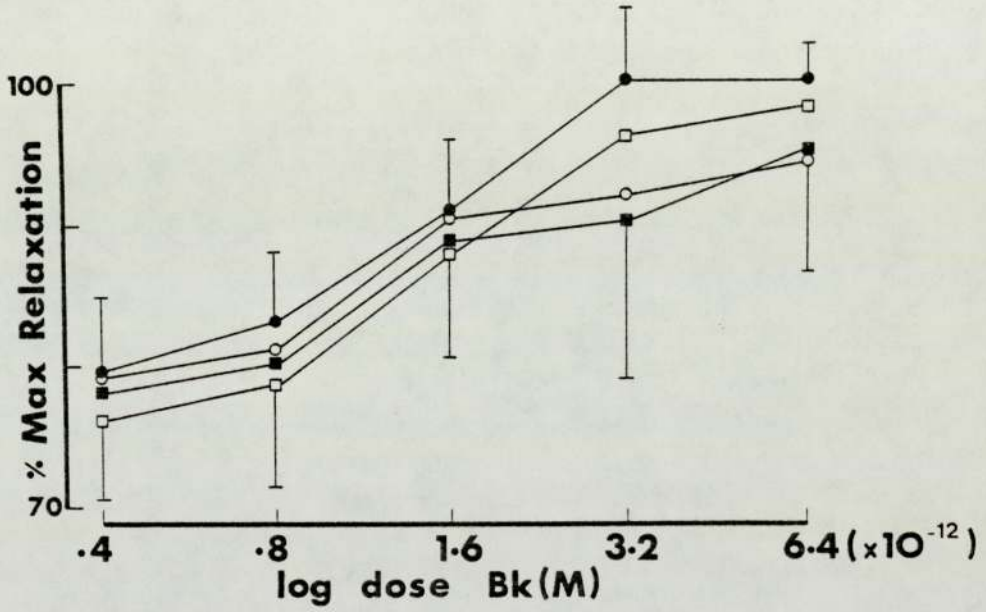

FIG.24 Log dose-response relationships of the longitudinal muscle of serosal perfused rat duodenum to bradykinin (Bk). ● represents serosally perfused duodenum with 2.8μM (□) or 28μM (■) indomethacin in the perfusate. ○ represents the response of the duodenum from rats pretreated with indomethacin 8mg.Kg/s.c./2hr. Each point represents the mean ± s.e.m. (n=5-7).

that cyclo-oxygenase products were common biochemical mediators in the response of the rat isolated perfused small intestine to bradykinin. It was of interest to investigate if such an involvement of the cyclo-oxygenase pathway with bradykinin, was species specific. Therefore, the study was extended to the cat ileum, a tissue with specific bradykinin receptors (Barabe et al., 1975,1977).

C. The Role of Cyclo-Oxygenase Products in the Contractile Response of the Cat Isolated Perfused Ileum to Bradykinin

Preliminary studies were performed to investigate the possible involvement of products of the cyclo-oxygenase pathway, in the contractile response of the cat isolated perfused ileum. The involvement of the cyclo-oxygenase pathway was investigated by the addition of indomethacin (2.8μM) to the perfusate of the ileum.

The serosal surface of the cat ileum was more sensitive to bradykinin than the mucosal surface, the mean EC_{50} being 2.8×10^{-9}M and 9.2×10^{-9}M respectively. The log dose-response relationship of the longitudinal muscle to bradykinin, perfused over either surface of the ileum, was unaffected by the presence of 2.8μM indomethacin (Fig.25). The maximal contraction in the presence of 2.8μM was 101 ± 14% of the mucosal control, 100 ± 9% (both n=4, $p > 0.05$). Similarly, during serosal perfusion, the maximal response to bradykinin in the presence of indomethacin (2.8μM) was 98 ± 15% of the serosal control, 100 ± 12% (both n=4, $p>0.05$).

These preliminary studies therefore suggested that the action of bradykinin was not dependent upon a functional

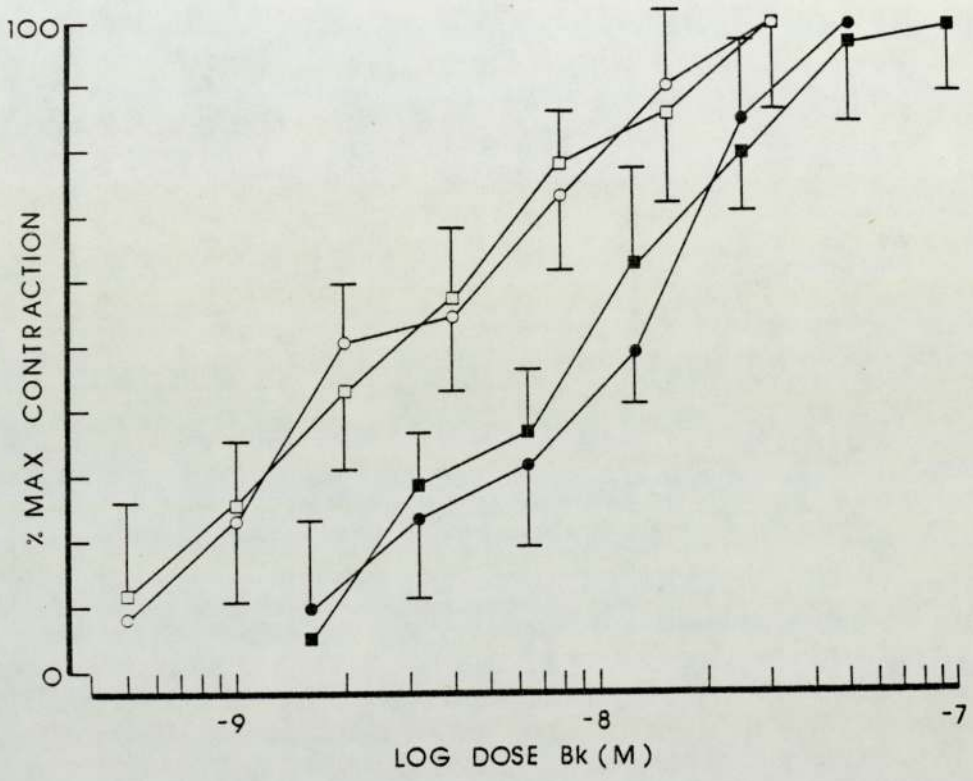

FIG.25 Log dose-response relationships of longitudinal muscle of cat ileum to bradykinin (Bk). ○ represent serosally perfused ileum with 2.8μM indomethacin (□) in the perfusate. ● represent mucosally perfused ileum with 2.8μM indomethacin (■) in the perfusate. Each point represents the mean ± s.e.m. (n=4).

cyclo-oxygenase system in the cat ileum.

SECTION 3 THE ACTION OF INDOMETHACIN ON THE CONTRACTILE RESPONSE OF THE RAT ILEUM TO BRADYKININ

The demonstration that aspirin-like drugs inhibited prostaglandin synthetase (Vane,I97I), provided a simple and reliable method for investigating the involvement of prostaglandins in a variety of situations. However, the specificity of these cyclo-oxygenase inhibitors, has been questioned (Smith,I975,I978; Northover,I977). In the experiments reported in this thesis, aspirin and indomethacin were used in concentrations previously shown to inhibit the enzyme cyclo-oxygenase. However, effects on other enzymes cannot be excluded (see Flower,I974) and furthermore, the ability of aspirin and indomethacin to induce mucosal damage has been known for a considerable time (Gross and Greenberg,I948; Lovgren and Allander,I964). Therefore, experiments were performed to exclude an action of indomethacin through non-specific enzyme inhibition or through induction of mucosal damage.

A. Time-Course for Inhibition of Bradykinin Responses by Indomethacin in the Mucosal Perfused Ileum

The measurement of prostaglandin release from isolated tissues has been used as an indication of cyclo-oxygenase activity. In the rabbit isolated spleen a marked inhibition of prostaglandin release by non-steroidal anti-inflammatory drugs was quickly obtained, although there was a delay of 45min to 3hr before maximal inhibition (Gryglewski and Vane, I972). In other studies, contractions induced by arachidonic

acid have been used to indicate cyclo-oxygenase activity. In the rabbit isolated jejunum, contractions induced by arachidonic acid were blocked 30min after indomethacin administration, although in some tissues responses were not blocked for Ihr to 2hr (Ferreira et al.,I976).

In this study, the time taken for indomethacin to inhibit contractions to bradykinin was investigated during mucosal perfusion of the rat ileum. The results of this study, were to be compared with the time taken by indomethacin to inhibit the basal release of prostaglandin-like material from the rat isolated perfused ileum (see p.72), and with the known time course for inhibition of cyclo-oxygenase in other isolated tissues.

Addition of indomethacin (2.8μM) to the mucosal perfusate, reduced the response of the ileum to bradykinin (8.I x $IO^{-IO}M$) to 82 ± 5% (n=5, $p > 0.05$) after IOmin, 64 ± 6% (n=5, $p < 0.0I$) after 20min, 54 ± 6% (n=5, $p < 0.00I$) after 40min, 52 ± 4% (n=5, $p < 0.00I$) after 80min and 46 ± 7% (n=5, $p < 0.00I$) after I80min, of the control contraction obtained in the absence of indomethacin (Fig.26).

Addition of indomethacin (28μM) to the mucosal perfusate reduced the contractions to bradykinin (8.I x $IO^{-IO}M$) to 76 ± 6% (n=5, $p > 0.05$) after IOmin, 53 ± 8% (n=5, $p < 0.0I$) after 20min, 52 ± 6% (n=5, $p < 0.00I$) after 40min, 35 ± 4% (n=5, $p < 0.00I$) after 80min and to 9 ± 6% (n=5, $p < 0.00I$) after I80min, of the control contraction obtained in the absence of indomethacin.

These results were consistent with the reported time course for inhibition of cyclo-oxygenase in isolated intestinal tissue. However, the continual decay of responses

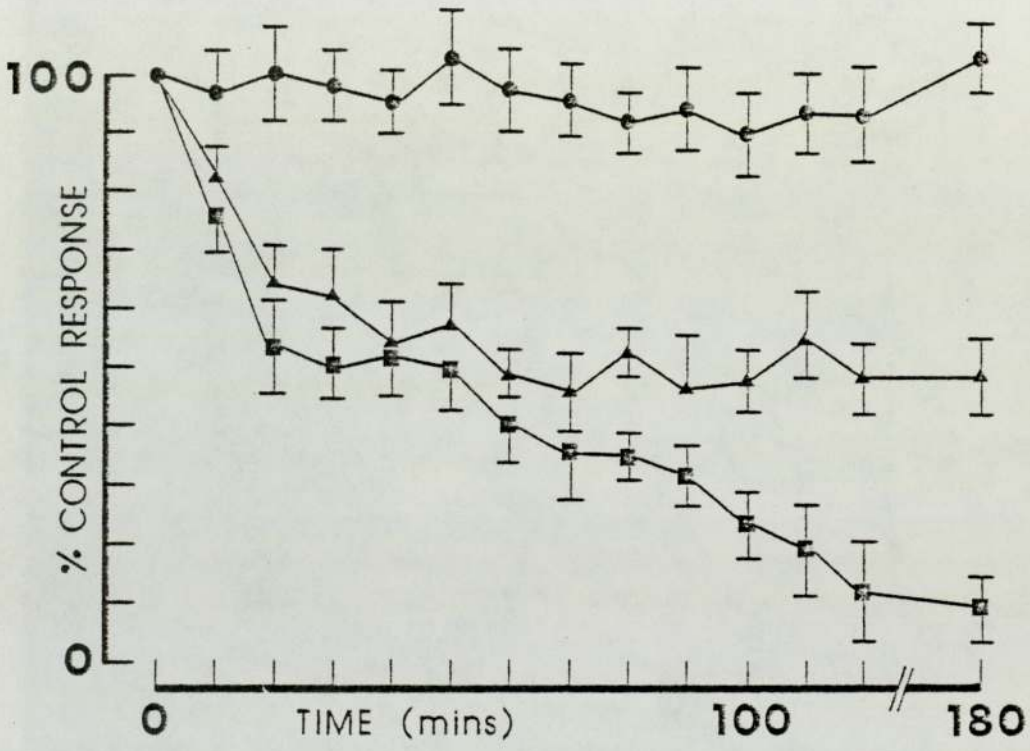

FIG.26 The time-course for inhibition of bradykinin responses by indomethacin in the mucosal perfused rat ileum. ● represents response of the mucosal perfused ileum to bradykinin ($8.I \times I0^{-I0}M$) perfused with Krebs solution alone, or in the presence of 2.8μM indomethacin (▲), or 28μM indomethacin (■). Each point represents the mean ± s.e.m. (n=5).

to bradykinin, seen in the presence of indomethacin (28μM), suggested a non-specific mechanism may also be involved.

B. The Effect of Indomethacin on the Mucosa of the Perfused Ileum

When indomethacin (28μM) was perfused over the mucosal surface of the rat ileum, a viscous material was deposited in the effluent of the ileum. This viscous material was collected by filtration of the effluent. The weight of the filtrate after perfusion with indomethacin was expressed as the percentage weight increase (% mucosal loss) compared to an adjacent section of ileum perfused with Krebs solution alone.

The addition of indomethacin 3.5μM and 7μM to the ileal perfusate had no significant effect upon the mucosal loss, 0.I ± I.0% (n=5, $p > 0.05$) and I.I ± I.5% (n=9, $p > 0.05$) respectively. However, the addition of indomethacin I4μM and 28μM produced a mucosal loss of 6.I ± I.8% (n=9, $p < 0.0I$) and I0.9 ± 2.5% (n=6, $p < 0.0I$) respectively (Fig.27). Addition of PGE_2 (I.4 x $I0^{-I2}$M) to the perfusate together with 3.5μM, 7μM, I4μM and 28μM indomethacin, produced values for the mucosal loss of -0.3 ± 0.6%, 0.2 ± I.0%, -0.I ± I.0% and 0.5 ± 0.9% respectively (all n=5, $p > 0.05$).

Thus, the results presented suggest indomethacin I4μM and 28μM induced a mucosal loss which was prevented by PGE_2, whilst concentrations less than 7μM indomethacin did not cause a mucosal loss. Furthermore, this mucosal loss appeared to be related to mucosal damage since preliminary histological evidence revealed that perfusion with

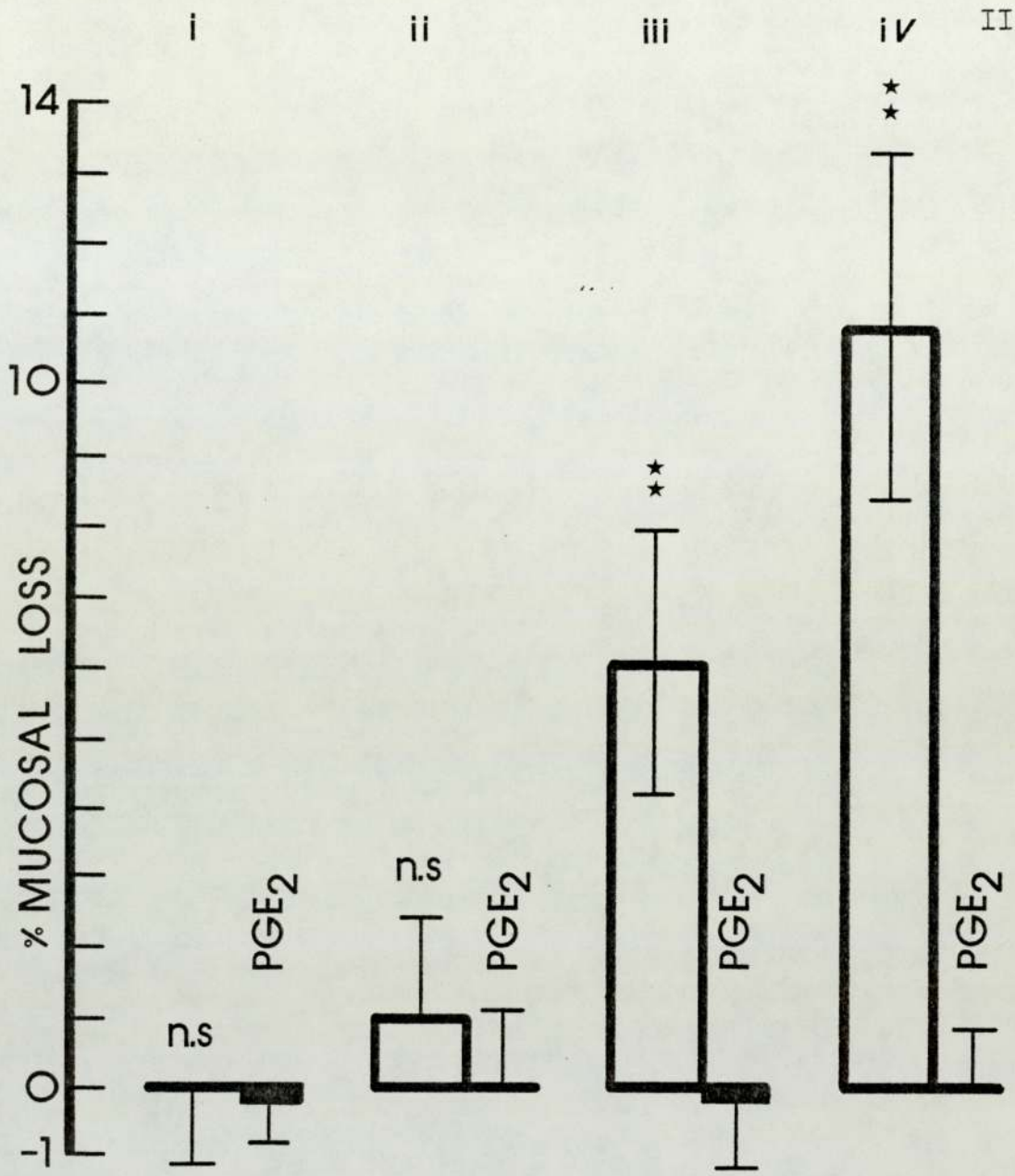

FIG.27 The effect of indomethacin on the mucosal loss of the rat isolated perfused ileum. Mucosal loss in the presence of indomethacin 3.5μM (i); 7μM (ii); I4μM (iii); and 28μM (iv) alone, or together with $I.4xI0^{-I2}$M PGE_2 ($+PGE_2$). n.s, not significant; **: $p < 0.0I$ compared to an adjacent section of ileum from the same rat perfused with Krebs alone. Vertical lines represent s.e.m. (n=5-9).

indomethacin (28μM) caused marked mucosal damage compared to indomethacin (2.8μM), or Krebs solution alone. Moreover, the simultaneous perfusion of PGE_2 and indomethacin (28μM) prevented this mucosal damage.

C. The Effect of Pretreatment of Rats with Indomethacin on the Release of PGE_2-like Material from the Perfused Ileum

Collier (I974) demonstrated that mechanical compression of sections of rat isolated intestinal tract released prostaglandins, which could be quantified by bioassay on the superfused rat fundus strip and rat colon. In this study, a modification of this procedure was used. It was found that when the tension of a perfused section of ileum was increased, there was a release of prostaglandin-like material which was detectable by direct bioassay on the rat fundus strip. This release was quantified by comparison with authentic PGE_2, and utilized to determine the extent of inhibition of prostaglandin synthesis in the ileum removed from rats pretreated with indomethacin.

(i) Mucosal Perfused Ileum

An increase in the tension of the mucosal perfused ileum from Ig to 6g, resulted in the release of 9 ± Ing PGE_2-like material/g.tissue (n=7), which was defined as the I00% mucosal control release. In tissues from rats pretreated with indomethacin 4,8,I6 and 32 mg.Kg/s.c./2hr prior to killing, the output of PGE_2-like material (Fig.28) was reduced to 92 ± 9% (n=4, $p > 0.05$), 45 ± 8% (n=7, $p < 0.0I$), I4 ± 8% (n=3, $p < 0.0I$) and I2 ± II% (n=2, $p < 0.0I$) respectively of this control release. Pretreatment of rats with

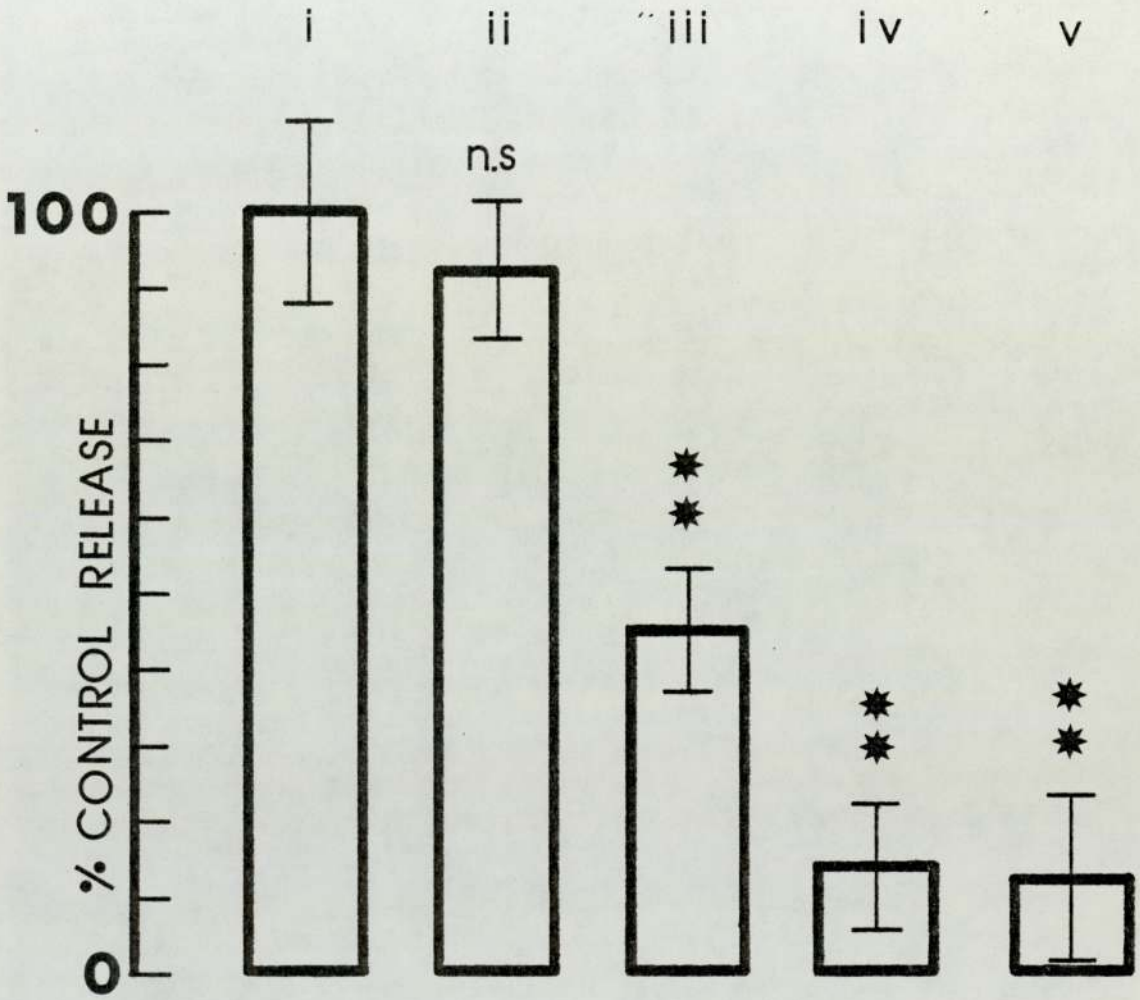

FIG.28 Amount of PGE_2-like material released from the mucosal perfused rat ileum, following an increase in the tissue tension from I-6g, expressed as a percentage of the control release (i). Effect of pretreatment with indomethacin (ii) 4mg.Kg; (iii) 8mg.Kg; (iv) I6mg.Kg; and (v) 32mg.Kg/s.c./2hr. n.s, not significant; ** :$p < 0.0I$ compared with control (i). Vertical lines represent s.e.m. (n=2-7).

indomethacin I6 and 32mg.Kg/s.c./2hr caused pronounced vascularization of the intestine with evidence of mucosal damage upon histological examination.

(ii) Serosal Perfused Ileum

An increase in the tension of the serosal perfused ileum from Ig to 6g, resulted in the release of 5 ± 0.5ng PGE_2-like material/g.tissue (n=7), which was defined as the I00% serosal control release. Thus, the control release from the serosal perfused ileum was significantly lower than the release from the mucosal perfused ileum ($p < 0.0I$). Tissues from rats pretreated with indomethacin 4,8,I6 and 32mg.Kg/ s.c./2hr reduced the release of PGE_2-like material (Fig.29) to 87 ± I2% (n=4, $p > 0.05$), 42 ± I0% (n=7, $p < 0.0I$), 8 ± 5% (n=3, $p < 0.00I$) and 6 ± 5% (n=2, $p < 0.00I$) respectively of the control.

Therefore, pretreatment with indomethacin 8mg.Kg/s.c./2hr appeared the most appropriate dosage regimen to use. There was a significant reduction in prostaglandin release, regardless of the surface of the ileum studied, and there was no evidence of mucosal damage on histological examination.

The results of the experiments performed in this section suggest that indomethacin (2.8μM), added to the perfusate, does not appear to have significant non-specific effects during mucosal perfusion. However, indomethacin (28μM) added to the perfusate induced significant mucosal damage, which might also have contributed to the continuous decline in the response of the mucosal perfused ileum to bradykinin. Thus, it appears valid to use only indomethacin (2.8μM) added to the perfusate, or tissues from animals pretreated with

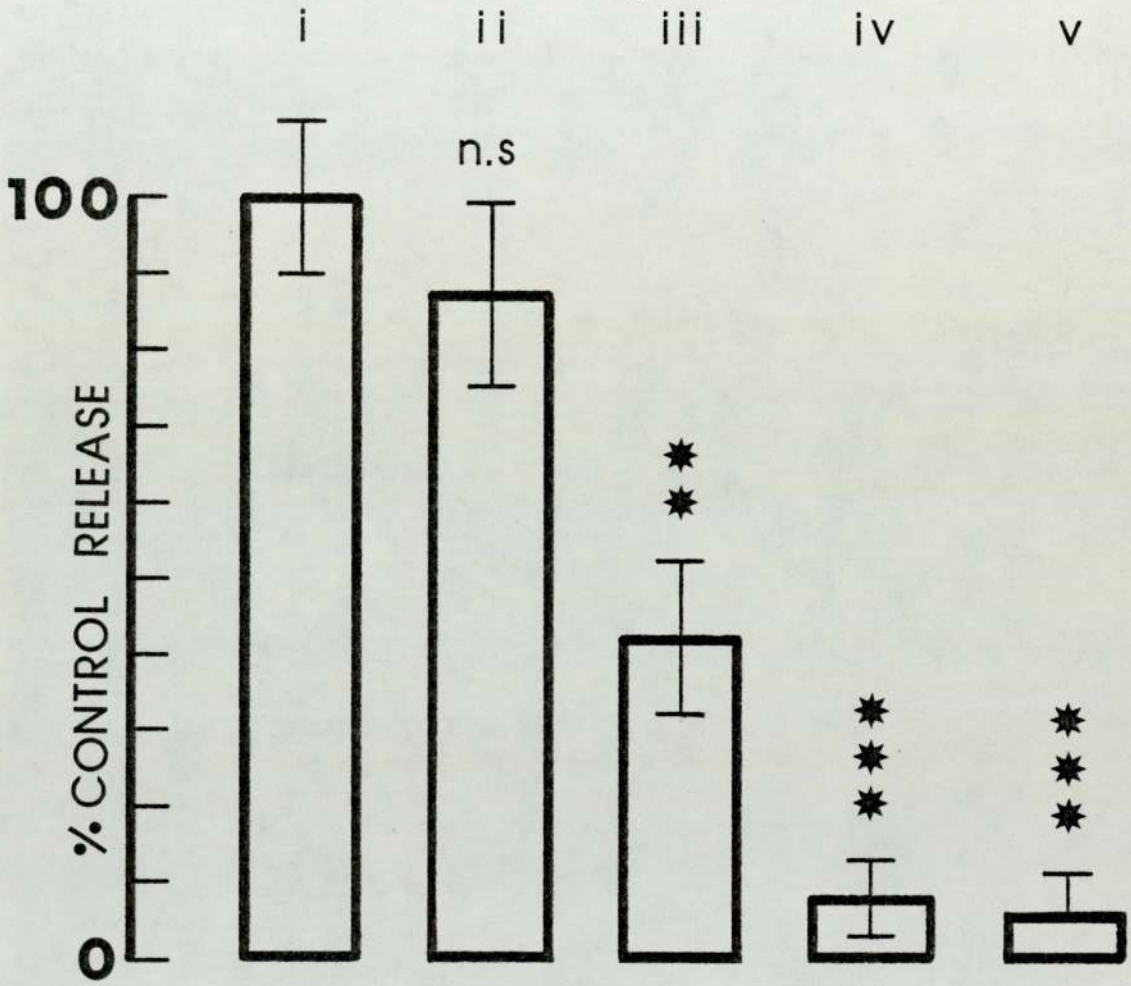

FIG.29 Amount of PGE_2-like material released from the serosal perfused rat ileum, following an increase in the tissue tension from I-6g, expressed as a percentage of the control release (i). Effect of pretreatment with indomethacin, (ii) 4mg.Kg; (iii) 8mg.Kg; (iv) I6mg.Kg; and (v) 32mg.Kg/s.c./2hr. n.s, not significant; ** :$p < 0.0I$; *** :$p < 0.00I$ compared with control (i). Vertical lines represent s.e.m. (n=2-7).

indomethacin (8mg.Kg/s.c./2hr), whilst studying the effect of bradykinin during mucosal perfusion.

SECTION 4 EXPERIMENTS TO INVESTIGATE A POSSIBLE INVOLVEMENT OF CYCLIC NUCLEOTIDES OR A NEURONAL MECHANISM IN THE RESPONSE OF RAT INTESTINE TO BRADYKININ

The experiments performed on the rat isolated perfused small intestine, demonstrated that products of the cyclo-oxygenase pathway were probably common biochemical mediators in the response of the mucosal perfused ileum to bradykinin. This involvement of cyclo-oxygenase products in the response to bradykinin during mucosal perfusion, occurred whether the tissue relaxed or contracted. Therefore, to explain the opposite responses, an additional effector mechanism must be implicated. Possible contenders for such an additional mechanism include an involvement of cyclic nucleotides or a neuronal mechanism.

A. The Role of Cyclic Nucleotides and Cyclo-Oxygenase Products in the Response of the Rat Perfused Small Intestine to Bradykinin

The possible involvement of cyclic AMP was investigated, since prostaglandins have been shown to stimulate the formation of cyclic nucleotides in several systems and conversely, cyclic AMP has been shown to stimulate prostaglandin biosynthesis (see Samuelsson et al., I978b). Furthermore, bradykinin-induced accumulation of cyclic AMP has been found to be related to prostaglandin synthesis in guinea-pig lung slices (Stoner et al., I973) and synovial fibroblasts (Fahey et al., I977). It has been demonstrated that the relaxation of the rat duodenum to bradykinin is potentiated

by activators of adenylate cyclase or inhibitors of cyclic AMP-phosphodiesterase (Paegelow et al., I977). Therefore, it was decided initially to investigate a possible interrelationship between bradykinin, prostaglandins and cyclic AMP in the rat isolated perfused duodenum and then extend the study to the rat ileum. The role of cyclic nucleotides was investigated with theophylline and imidazole, an inhibitor and stimulator respectively, of cyclic AMP-phosphodiesterase (Butcher and Sutherland, I962).

(i) The Effect of Theophylline and Indomethacin on the Relaxation of the Perfused Duodenum to Bradykinin

The relaxation of the rat duodenum to 0.8 x $IO^{-I2}M$ and 0.4 x $IO^{-I2}M$ bradykinin, the threshold concentrations for graded relaxations during mucosal and serosal perfusion respectively, represented the I00% control responses on their respective surface.

(a) Mucosal Perfused Duodenum

The addition of theophylline (5.6 x $IO^{-5}M$) to the mucosal perfusate increased the relaxation of the duodenum to bradykinin (0.8 x $IO^{-I2}M$), to I34 $\pm$ I6% (n=8, $p < 0.0I$) of the control value. Addition of indomethacin (2.8μM) together with theophylline (5.6 x $IO^{-5}M$), reduced the relaxation produced by bradykinin (0.8 x $IO^{-I2}M$) to I6 $\pm$ I4% (n=8, $p < 0.00I$), of the control value. This relaxation to bradykinin (0.8 x $IO^{-I2}M$) in the presence of indomethacin (2.8μM) and theophylline (5.6 x $IO^{-5}M$) was significantly less ($p < 0.00I$) than the relaxation observed in the presence of theophylline (5.6 x $IO^{-5}M$) alone. The relaxation of the mucosal perfused duodenum to bradykinin (0.8 x $IO^{-I2}M$) in the

presence of indomethacin (2.8μM) alone, was 4 ± 4% (n=5, $p < 0.001$) of the control value (Fig.30), and was not significantly different ($p > 0.05$) from the relaxation observed in the presence of indomethacin (2.8μM) and theophylline (5.6×10^{-5}M).

(b) Serosal Perfused Duodenum

In the presence of theophylline (5.6×10^{-5}M), the relaxation of the duodenum to bradykinin (0.4×10^{-12}M) during serosal perfusion, was increased to 119 ± 7% (n=8, $p < 0.05$) of the control value (Fig.31). The relaxation of the duodenum to bradykinin (0.4×10^{-12}M) in the presence of theophylline (5.6×10^{-5}M) and indomethacin (2.8μM), was 118 ± 9% (n=8, $p > 0.05$) of the control value and was not significantly different from that observed in the presence of theophylline (5.6×10^{-5}M) alone. In the presence of indomethacin (2.8μM) alone, the relaxation of the serosal perfused duodenum to bradykinin (0.4×10^{-12}M) was 95 ± 13% (n=5, $p > 0.05$) of the control value.

The relaxation of the duodenum to bradykinin (0.4×10^{-12}M) in the presence of indomethacin (2.8μM) alone, was not significantly different ($p > 0.05$) from the response obtained in the presence of indomethacin (2.8μM) and theophylline (5.6×10^{-5}M) or theophylline (5.6×10^{-5}M) alone.

(ii) The Effect of Imidazole and Indomethacin on the Relaxation of the Perfused Duodenum to Bradykinin

(a) Mucosal Perfused Duodenum

The effect of imidazole (2×10^{-4}M) on the response of the mucosal perfused duodenum to bradykinin (0.8×10^{-12}M)

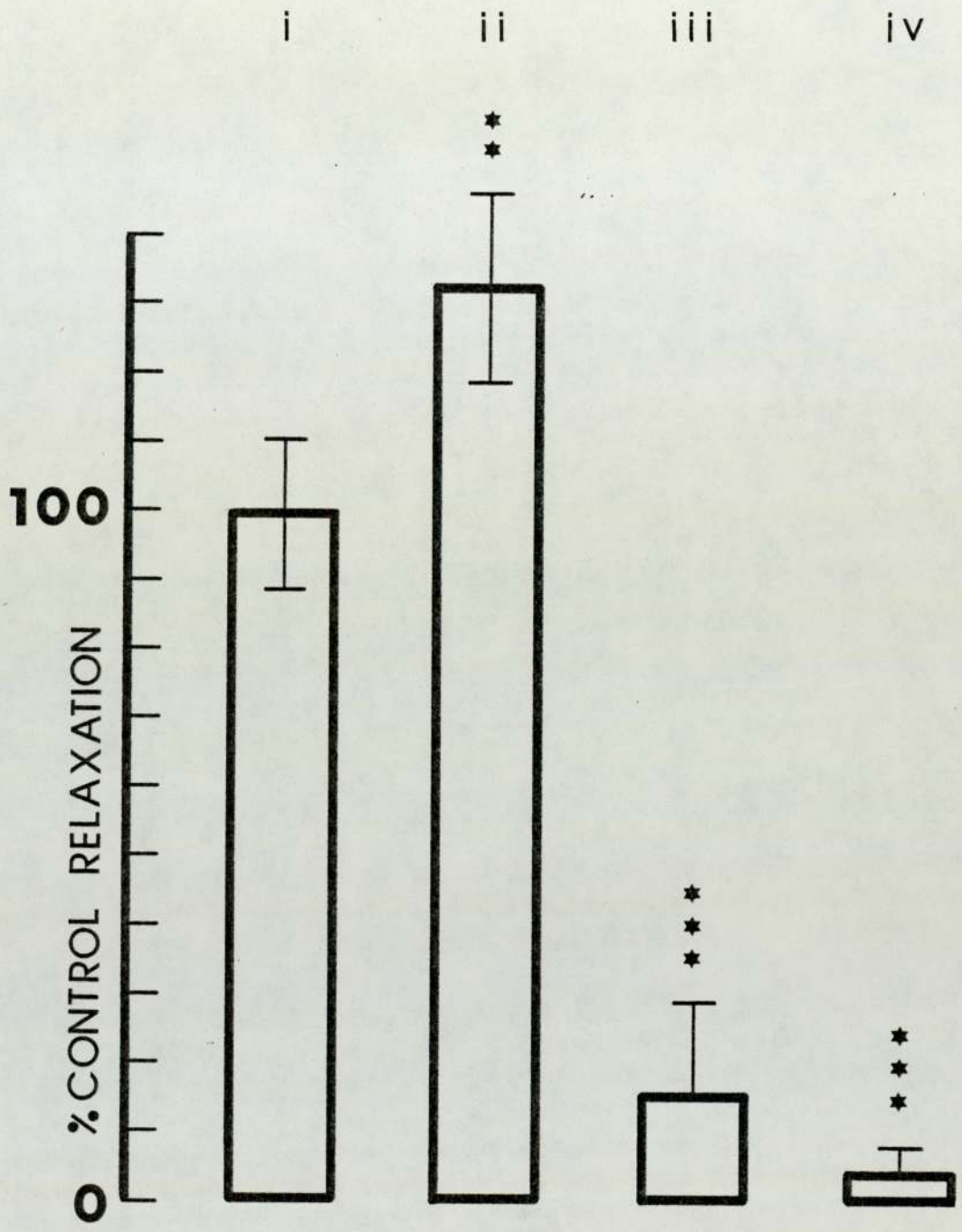

FIG.30 Response of mucosal perfused rat duodenum to 0.8 x 10^{-12}M bradykinin (i), and in the presence of theophylline (5.6 x 10^{-5}M) (ii); 5.6 x 10^{-5}M theophylline and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant; ** :$p < 0.01$; *** :$p < 0.001$ compared with control (i). Vertical lines represent s.e.m. (n=5-8).

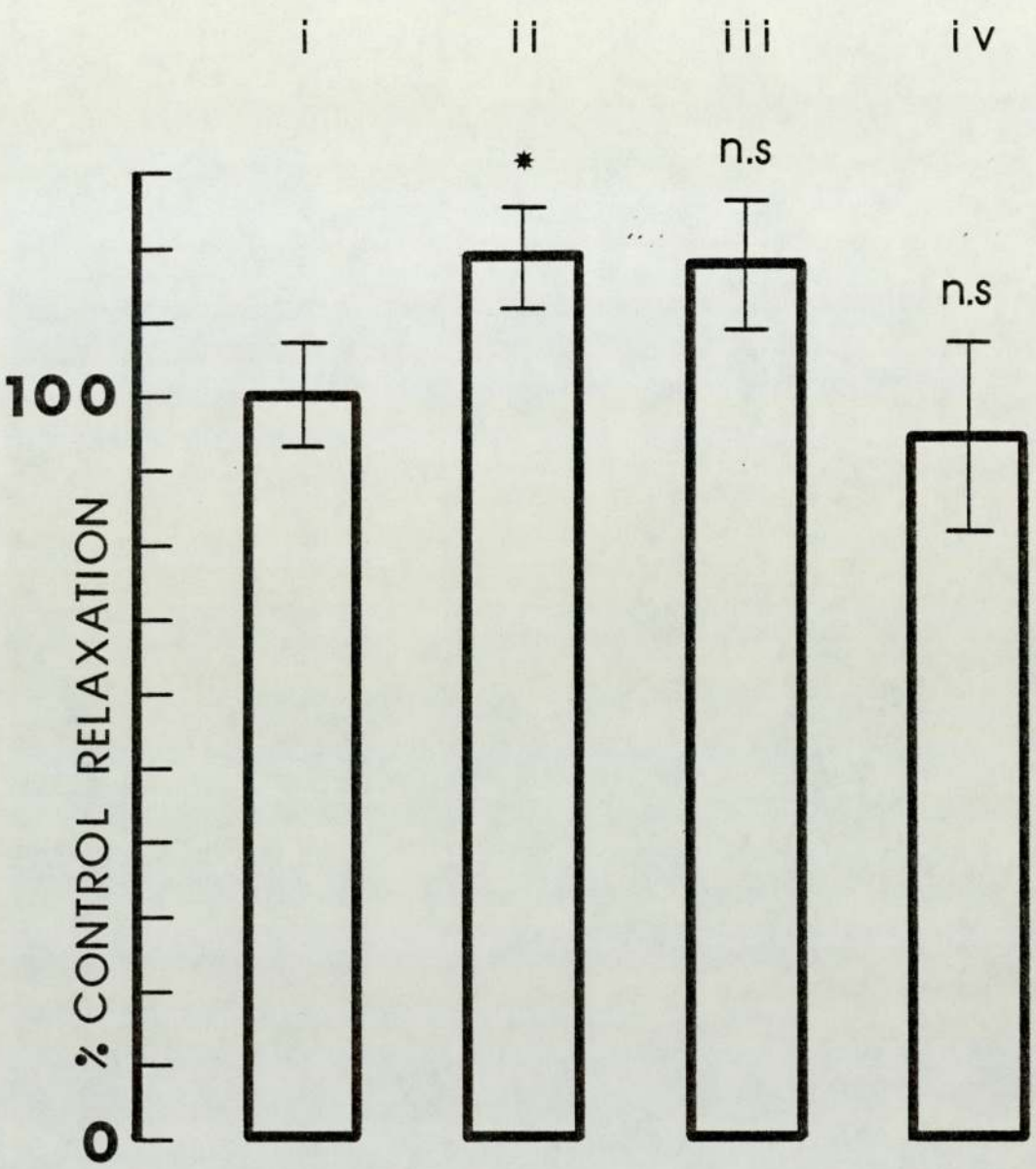

FIG.3I Response of serosal perfused rat duodenum to 0.4 x 10^{-12}M bradykinin (i); and in the presence of 5.6 x 10^{-5}M theophylline (ii); 5.6 x 10^{-5}M theophylline and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant; * :$p < 0.05$ compared with control (i). Vertical lines represent s.e.m. (n=5-8).

and to bradykinin (0.8×10^{-12}M) in the presence of indomethacin (2.8μM), is shown in Fig.32. The addition of imidazole (2×10^{-4}M) to the mucosal perfusate of the duodenum reduced the relaxation to bradykinin (0.8×10^{-12}M), to 58 ± 7% (n=8, $p < 0.01$) of the control value. The relaxation of the duodenum to bradykinin (0.8×10^{-12}M) in the presence of imidazole (2×10^{-4}M) and indomethacin (2.8μM) was 8 ± 6% (n=8, $p < 0.001$) of the control value. This relaxation to bradykinin (0.8×10^{-12}M) in the presence of imidazole (2×10^{-4}M) and indomethacin (2.8μM), was significantly less ($p < 0.001$) than that obtained in the presence of imidazole (2×10^{-4}M) alone. The relaxation of the duodenum to bradykinin (0.8×10^{-12}M) in the presence of indomethacin (2.8μM) alone, was 4 ± 4% (n=5, $p < 0.001$) of the control value and was not significantly different ($p > 0.05$) from the relaxation to bradykinin (0.8×10^{-12}M) in the presence of indomethacin (2.8μM) and imidazole (2×10^{-4}M).

(b) Serosal Perfused Duodenum

The effect of imidazole (2×10^{-4}M) on the response of the serosal perfused duodenum to bradykinin (0.4×10^{-12}M) and to bradykinin (0.4×10^{-12}M) in the presence of indomethacin (2.8μM), is shown in Fig.33. In the presence of imidazole (2×10^{-4}M) the relaxation of the duodenum to bradykinin (0.4×10^{-12}M), was reduced to 48 ± 10% (n=8, $p < 0.01$) of the control value. The response of the duodenum to bradykinin (0.4×10^{-12}M) in the presence of imidazole (2×10^{-4}M) and indomethacin (2.8μM) was 54 ± 9% (n=8, $p < 0.01$) of the control, which was not significantly different from that seen in the presence of imidazole (2×10^{-4}M) alone. In the presence of indomethacin alone, the response to bradykinin

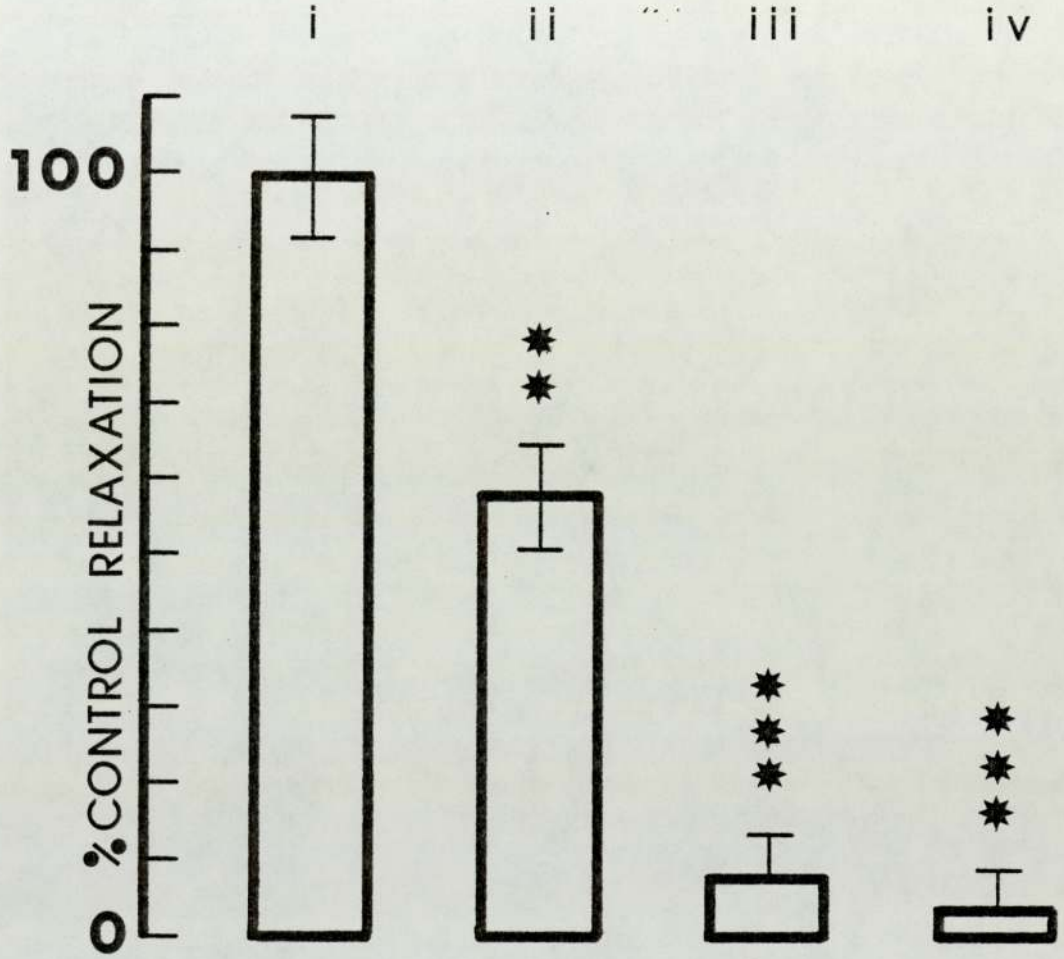

FIG.32 Response of mucosal perfused rat duodenum to 0.8×10^{-12}M bradykinin (i), and in the presence of 2×10^{-4}M imidazole (ii); 2×10^{-4}M imidazole and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant; ** :$p < 0.01$; *** :$p < 0.001$ compared with control (i). Vertical lines represent s.e.m. (n=5-9).

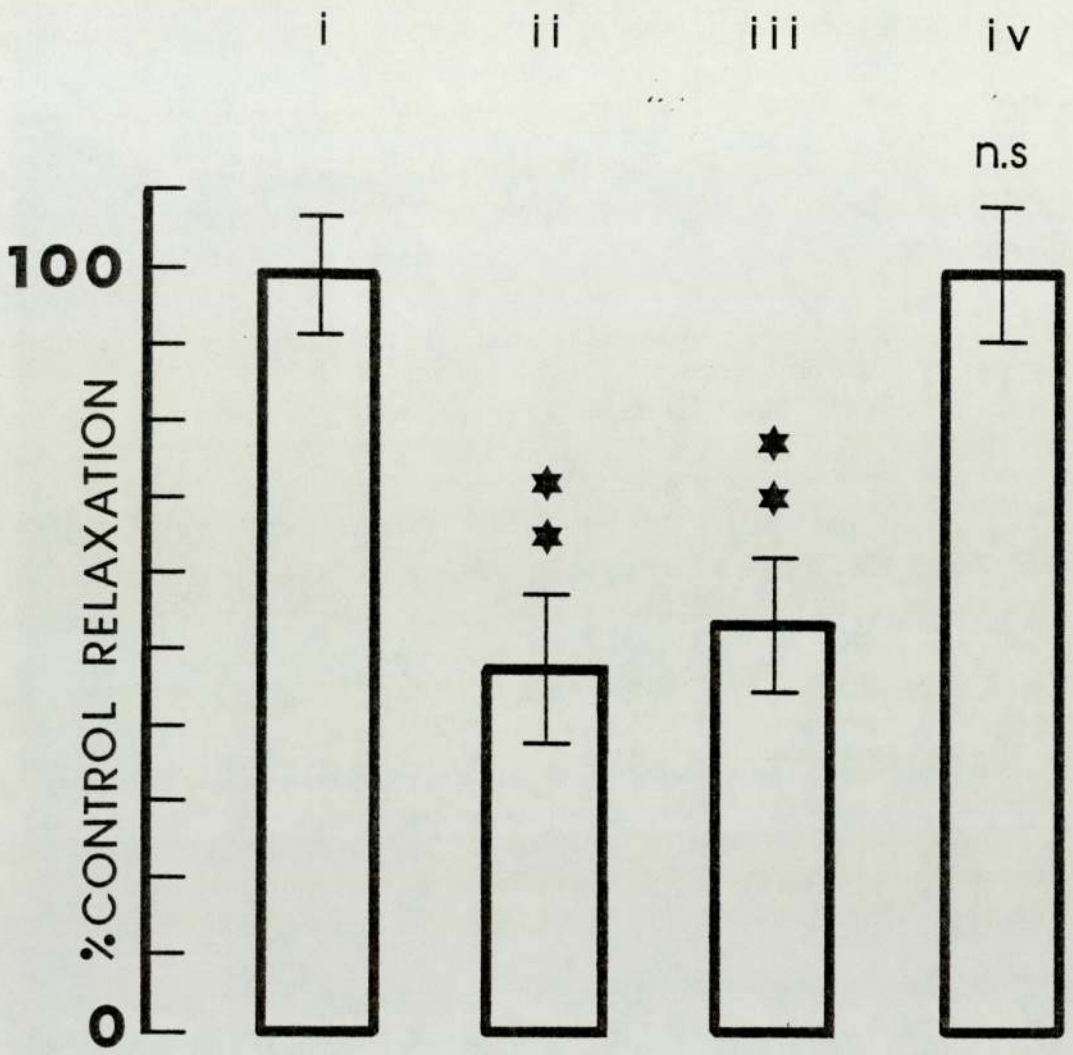

FIG.33 Response of serosal perfused rat duodenum to 0.4×10^{-12}M bradykinin (i), and in the presence of 2×10^{-4}M imidazole (ii); 2×10^{-4}M imidazole and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant; ** :$p < 0.01$; compared with control. (i). Vertical lines represent s.e.m. (n=8-9).

(0.4×10^{-12}M) was I00 ± 9% (n=9) of the control value. The relaxation of the serosal perfused duodenum to bradykinin (0.4×10^{-12}M) in the presence of indomethacin (2.8μM) and imidazole (2×10^{-4}M) was significantly less ($p < 0.01$) than the response in the presence of indomethacin (2.8μM) alone.

Thus, the results demonstrated that the phosphodiesterase inhibitor theophylline increased the bradykinin-induced relaxation of the mucosal or serosal perfused rat duodenum. Alternatively, stimulation of phosphodiesterase activity with imidazole, reduced the bradykinin-induced relaxation of the mucosal or serosal perfused duodenum. Therefore, the results suggested there was an interrelationship between the adenylate cyclase system and the action of bradykinin in the rat duodenum, which supports the findings of Paegelow et al. (1977). However, addition of indomethacin to the perfusate inhibited the effect of theophylline during mucosal, but not serosal perfusion of the rat duodenum. Similarly, indomethacin appeared to override the effect of imidazole during mucosal, but not serosal perfusion. This suggested there was an interrelationship between prostaglandins and cyclic nucleotides in the mucosal perfused rat duodenum.

(iii) The Effect of Theophylline and Indomethacin on the Contractile Response of the Perfused Ileum to Bradykinin or Potassium Chloride

The contraction of the rat isolated ileum to 8.1×10^{-10}M bradykinin during mucosal perfusion and 1.5×10^{-10}M bradykinin during serosal perfusion, represented the 100% control responses on their respective surfaces. The corresponding concentration for potassium chloride perfused over

either surface of the ileum was 4 x 10^{-5}M. All concentrations used corresponded to their EC_{50} values on the respective ileal surface.

(a) Mucosal Perfused Ileum

The effect of theophylline (5.6 x 10^{-5}M) on contractions of the mucosal perfused ileum to bradykinin (8.1 x 10^{-10}M) and to bradykinin (8.1 x 10^{-10}M) in the presence of indomethacin (2.8μM), is shown in Fig.34. The response of the mucosal perfused ileum to bradykinin (8.1 x 10^{-10}M) in the presence of theophylline (5.6 x 10^{-5}M) was 93 ± 13% (n=5, $p > 0.05$) of the control value. The contraction of the ileum to bradykinin (8.1 x 10^{-10}M) in the presence of theophylline (5.6 x 10^{-5}M) and indomethacin (2.8μM) was 33 ± 12% (n=5, $p < 0.01$) of the control value and was significantly less ($p < 0.01$) than the response observed in the presence of theophylline (5.6 x 10^{-5}M) alone. The contraction of the ileum to bradykinin (8.1 x 10^{-10}M) in the presence of indomethacin (2.8μM) alone was 36 ± 8% (n=5, $p < 0.01$) of the control value, and was not significantly different from that obtained in the presence of theophylline (5.6 x 10^{-5}M) and indomethacin (2.8μM).

The contractile response to potassium chloride (4 x 10^{-5}M) perfused over the mucosal surface of the ileum, in the presence of theophylline (5.6 x 10^{-5}M), theophylline (5.6 x 10^{-5}M) and indomethacin (2.8μM), or indomethacin (2.8μM) alone (Fig.35) was 118 ± 16%, 101 ± 14% and 94 ± 13% respectively, (all n=5, $p > 0.05$) of the control response. There was no significant difference between any treatment group in their response to potassium chloride (4 x 10^{-5}M).

(b) Serosal Perfused Ileum

The contractile response to bradykinin (1.5 x 10^{-10}M)

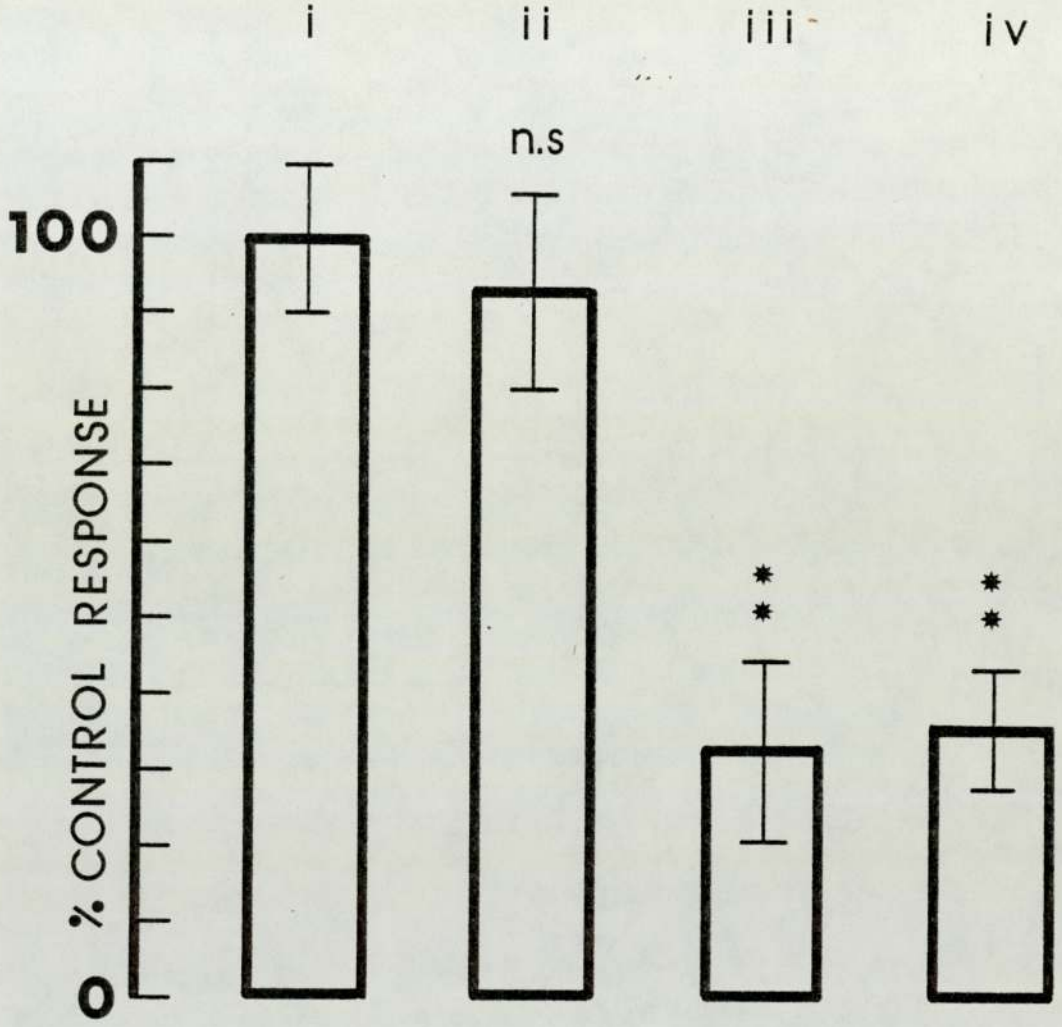

FIG.34 Response of mucosal perfused rat ileum to 8.1×10^{-10}M bradykinin (i); and in the presence of 5.6×10^{-5}M theophylline (ii); 5.6×10^{-5}M theophylline and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant; ** :$p < 0.01$ compared with control (i). Vertical lines represent s.e.m. (n=5).

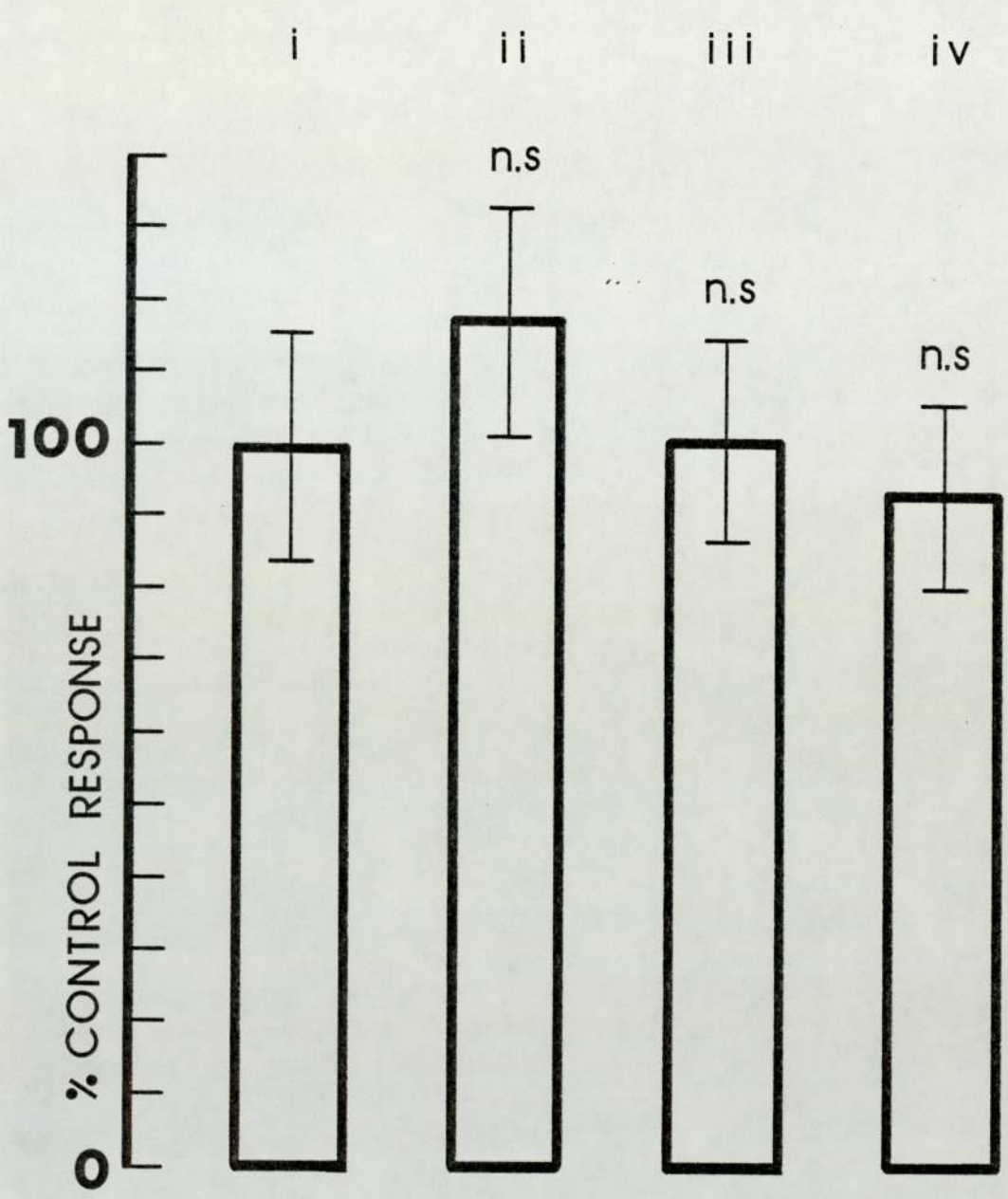

FIG.35 Response of mucosal perfused rat ileum to 4 x 10^{-5}M potassium chloride (i), and in the presence of 5.6 x 10^{-5}M theophylline (ii); 5.6 x 10^{-5}M theophylline and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=5).

perfused over the serosal surface of the ileum, in the presence of theophylline (5.6×10^{-5}M), theophylline (5.6×10^{-5}M) and indomethacin (2.8μM), or indomethacin (2.8μM) alone (Fig.36) was 95 ± 8%, 96 ± 6% and 98 ± 6% respectively, (all n=5, $p > 0.05$) of the control response. The corresponding responses to potassium chloride (4×10^{-5}M) were 108 ± 6%, 102 ± 11% and 95 ± 12% respectively, (all n=5, $p > 0.05$) of the control value (Fig.37). There was no significant difference between any treatment group in their response to bradykinin (1.5×10^{-10}M) or potassium chloride (4×10^{-5}M).

(iv) The Effect of Imidazole and Indomethacin on the Contractile Response of the Perfused Ileum to Bradykinin or Potassium Chloride

(a) Mucosal Perfused Ileum

The effect of imidazole (2×10^{-4}M) added to the mucosal perfusate, or added together with indomethacin (2.8μM), on the response of the rat ileum to bradykinin (8.1×10^{-10}M) is shown in Fig.38. The addition of imidazole (2×10^{-4}M) to the mucosal perfusate of the ileum increased the response to bradykinin (8.1×10^{-10}M) to 186 ± 12% (n=11, $p < 0.001$) of the control value. The response of the ileum to bradykinin (8.1×10^{-10}M) in the presence of imidazole (2×10^{-4}M) and indomethacin (2.8μM) was 56 ± 9% (n=10, $p < 0.001$) of the control value. This response to bradykinin (8.1×10^{-10}M) in the presence of indomethacin (2.8μM) and imidazole (2×10^{-4}M) was significantly smaller ($p < 0.001$) than that obtained in the presence of imidazole (2×10^{-4}M) alone. The response of the ileum to bradykinin (8.1×10^{-10}M) in the presence of indomethacin (2.8μM), was 37 ± 10% (n=5, $p < 0.001$) of the

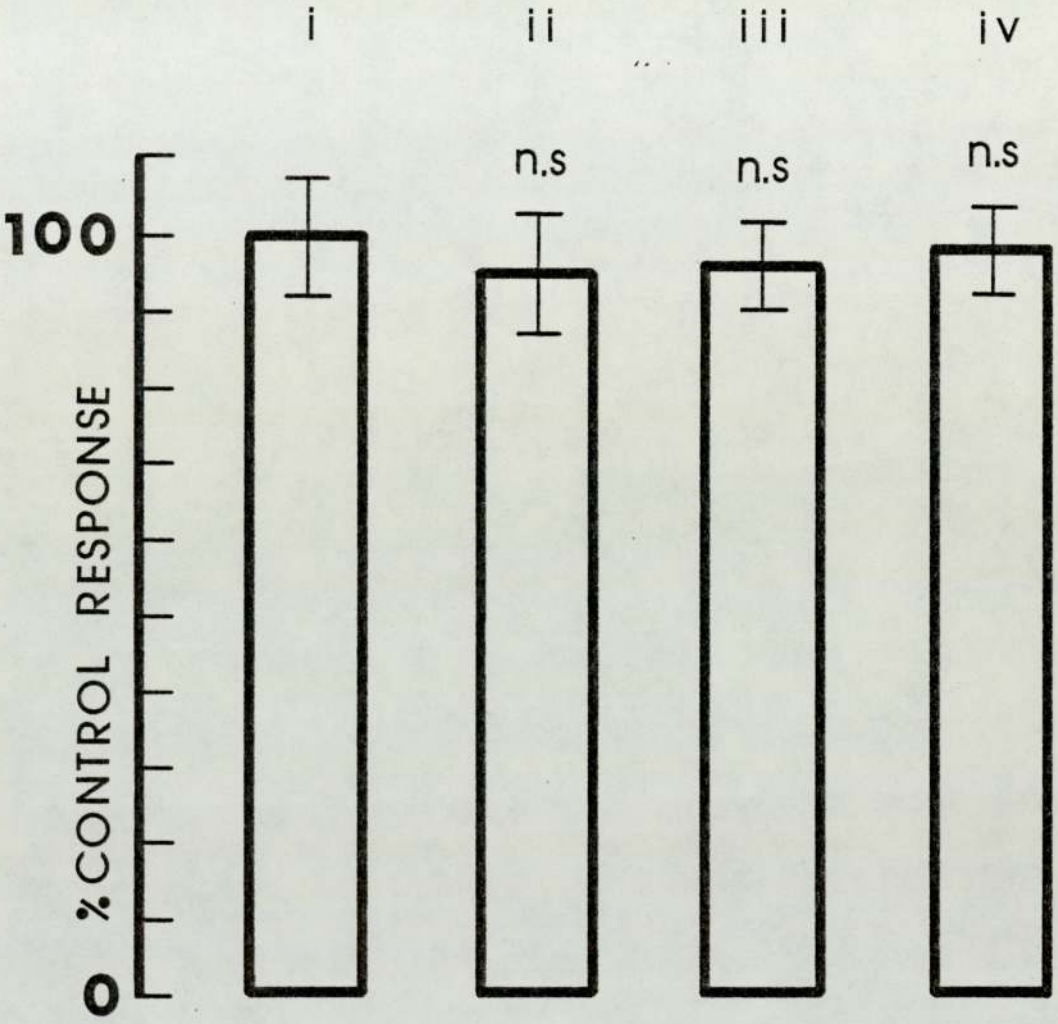

FIG.36 Response of serosal perfused rat ileum to 1.5×10^{-10}M bradykinin (i); and in the presence of 5.6×10^{-5}M theophylline (ii); 5.6×10^{-5}M theophylline and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=5).

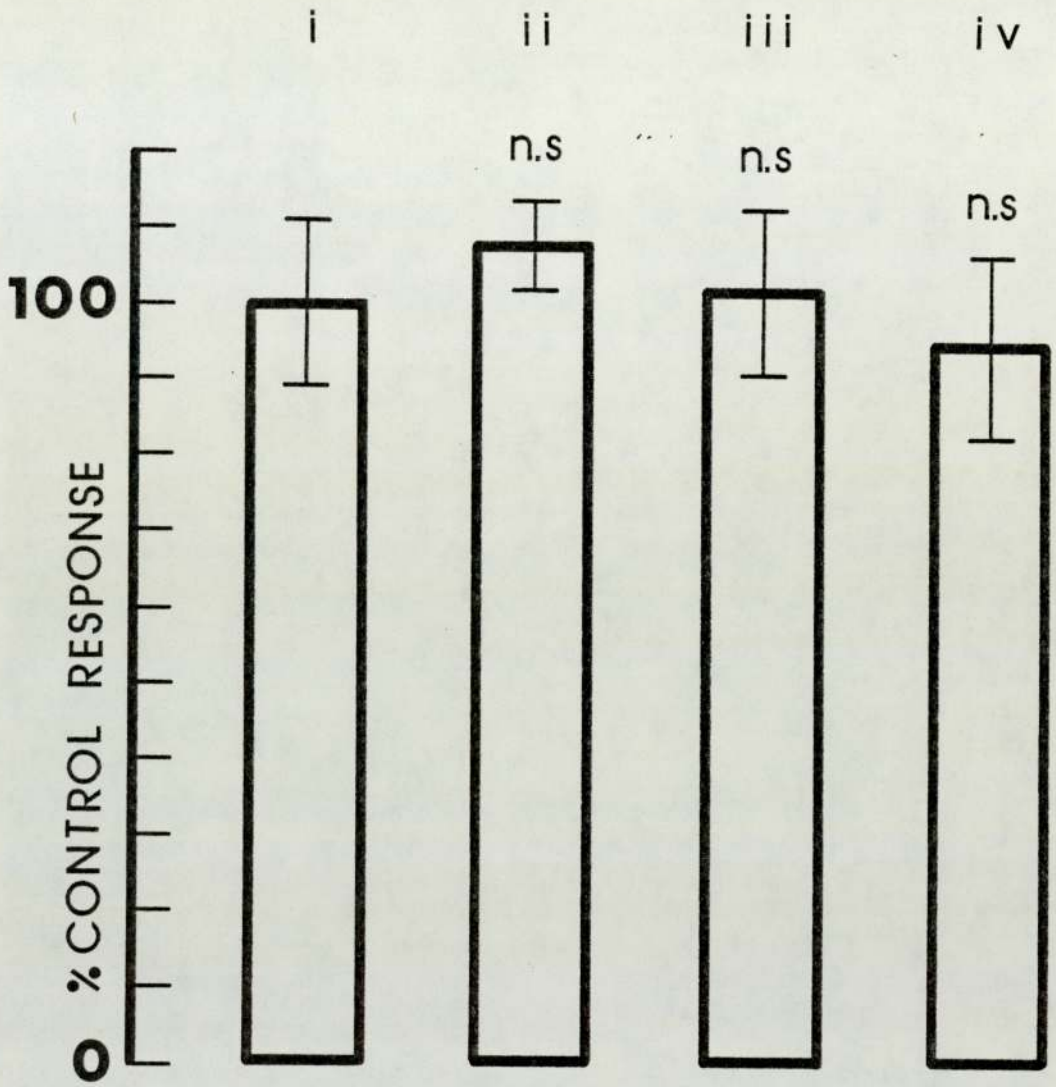

FIG.37 Response of serosal perfused rat ileum to 4 x 10^{-5}M potassium chloride (i); and in the presence of 5.6 x 10^{-5}M theophylline (ii); 5.6 x 10^{-5}M theophylline and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=5).

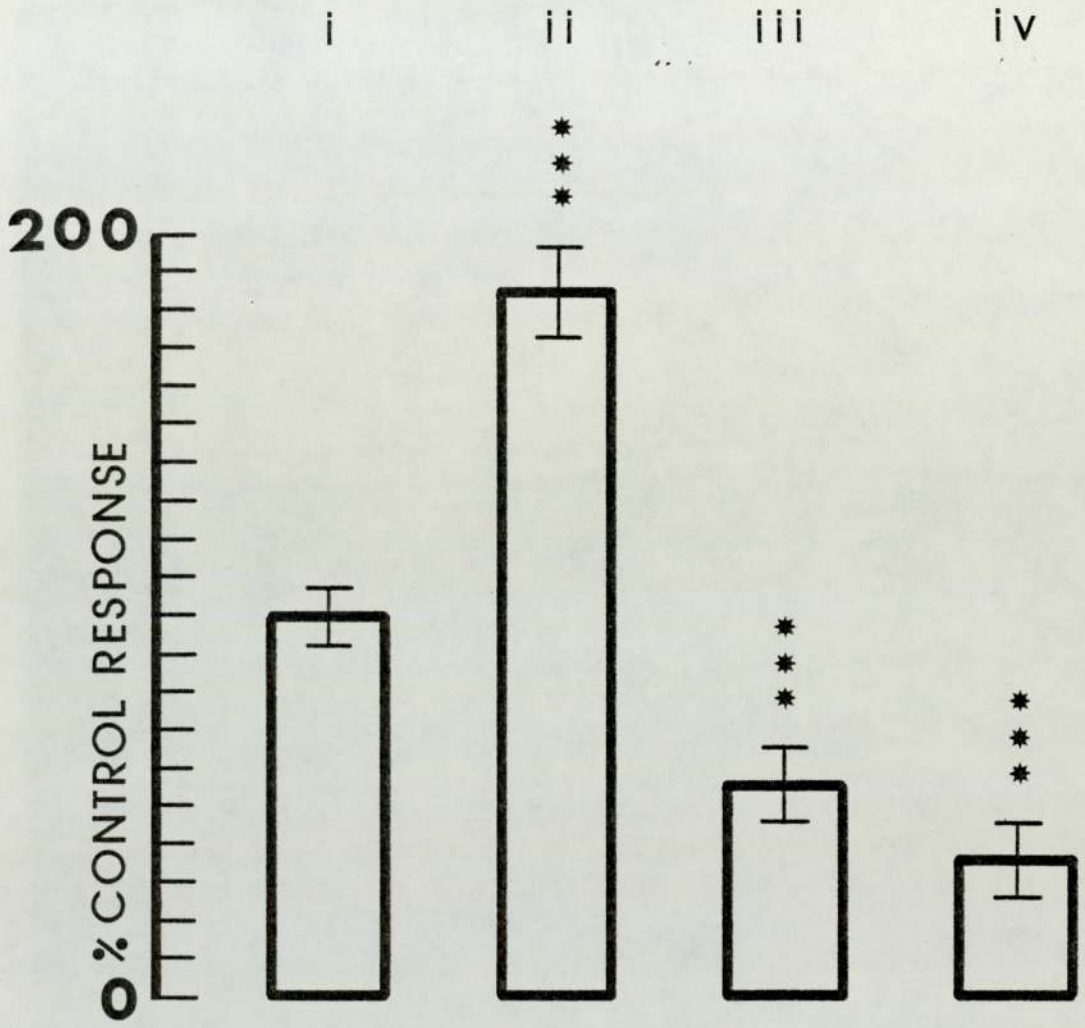

FIG.38 Response of mucosal perfused rat ileum to 8.1×10^{-10}M bradykinin (i); and in the presence of 2×10^{-4}M imidazole (ii); 2×10^{-4}M imidazole amd 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. *** :$p < 0.001$ compared with control (i). Vertical lines represent s.e.m. (n=5-11).

control value, which was not significantly different ($p > 0.05$) from the response to bradykinin ($8.I \times IO^{-IO}M$) in the presence of indomethacin (2.8μM) and imidazole ($2 \times IO^{-4}M$).

The contractile response to potassium chloride ($4 \times IO^{-5}M$) perfused over the mucosal surface of the ileum, in the presence of imidazole ($2 \times IO^{-4}M$), imidazole ($2 \times IO^{-4}M$) and indomethacin (2.8μM), or indomethacin (2.8μM) alone, was I09 ± I3%, 98 ± I2% and 95 ± 9% respectively (all n=5, $p > 0.05$) of the control value (Fig.39). There was no significant difference in the response to potassium chloride ($4 \times IO^{-5}M$) between any treatment group.

(b) Serosal Perfused Ileum

The contractile response to bradykinin ($I.5 \times IO^{-IO}M$) perfused over the serosal surface of the ileum, in the presence of imidazole ($2 \times IO^{-4}M$), imidazole ($2 \times IO^{-4}M$) and indomethacin (2.8μM), or indomethacin (2.8μM) alone (Fig.40), was I20 ± I2%, II7 ± I3% and I02 ± 9% respectively, (all n=IO, $p > 0.05$) of the control response. The corresponding responses to potassium chloride ($4 \times IO^{-5}M$) were II6 ± I3%, II4 ± I0% and I04 ± 9% respectively, (all n=5, $p > 0.05$) of the control value (Fig.4I). There was no significant difference between any treatment group in their response to bradykinin ($I.5 \times IO^{-IO}M$) or potassium chloride ($4 \times IO^{-5}M$).

These results demonstrated that theophylline had no significant effect upon the response to bradykinin or potassium chloride, during either mucosal or serosal perfusion of the rat ileum. However, the addition of imidazole to the mucosal perfusate significantly increased the response to bradykinin, but had no effect upon the response to potassium

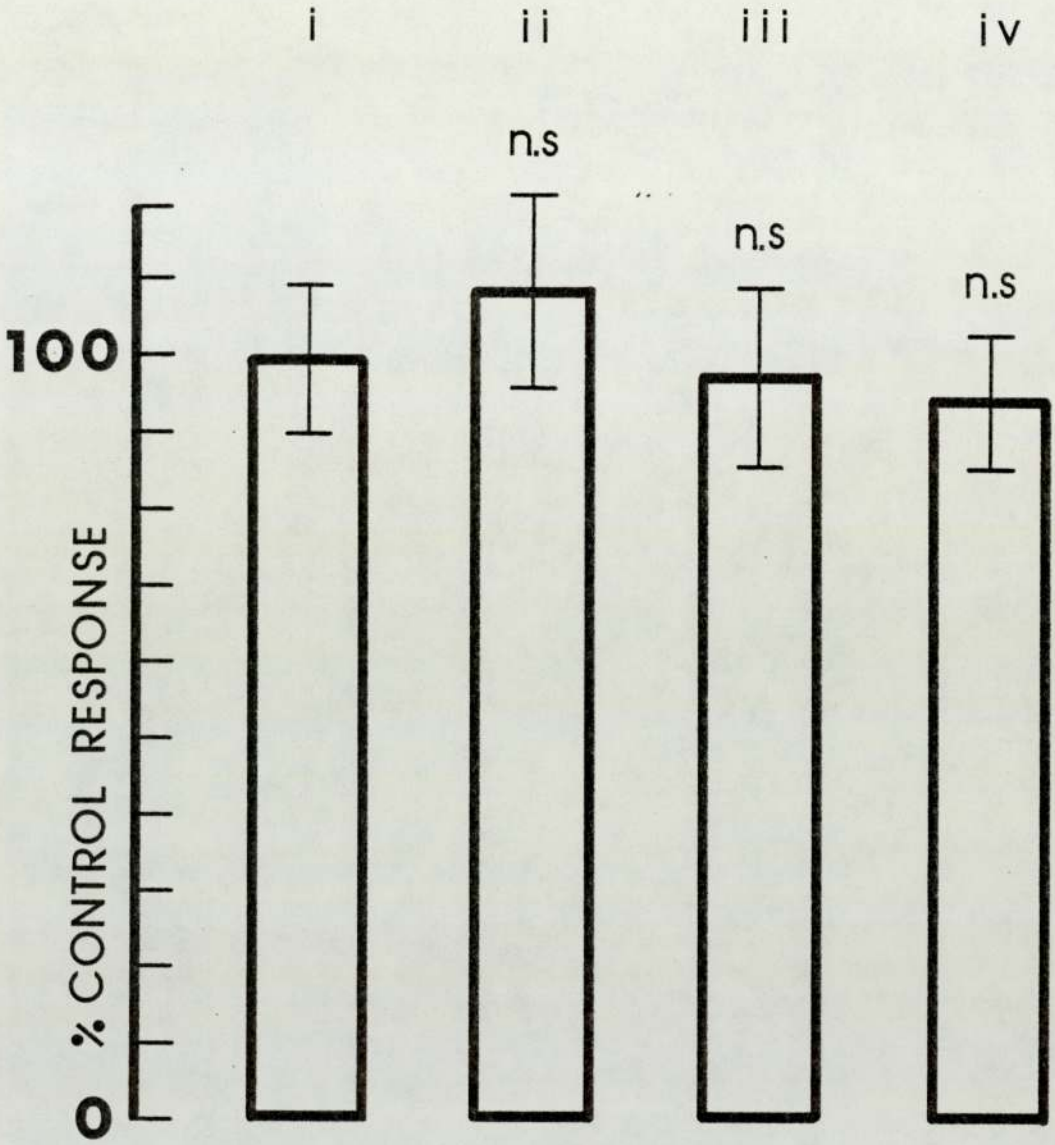

FIG.39 Response of mucosal perfused rat ileum to 4×10^{-5}M potassium chloride (i); and in the presence of 2×10^{-4}M imidazole (ii); 2×10^{-4}M imidazole and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv)alone. n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=5).

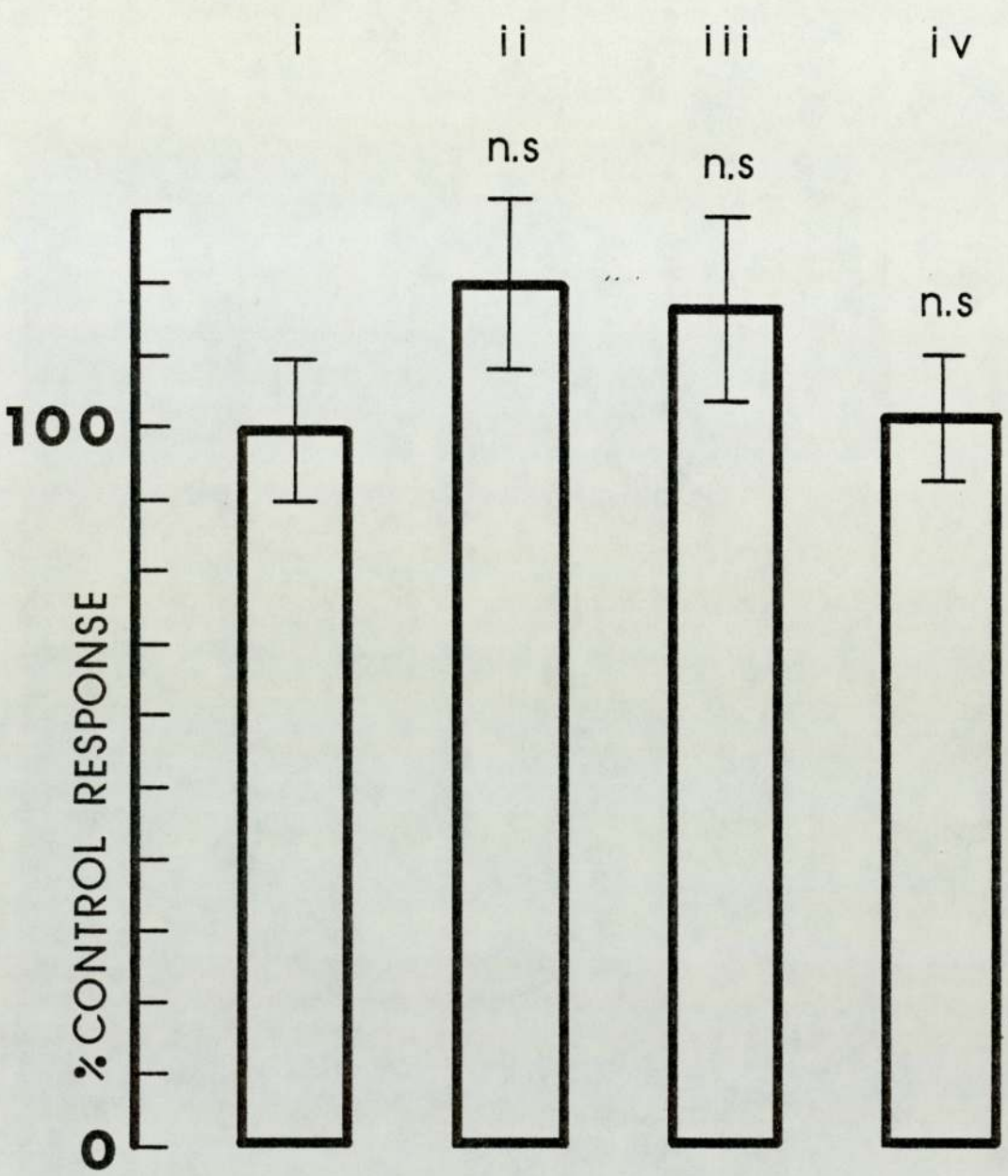

FIG.40 Response of serosal perfused rat ileum to I.5 x 10^{-10}M bradykinin (i); and in the presence of 2 x 10^{-4}M imidazole (ii); 2 x 10^{-4}M imidazole and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=10).

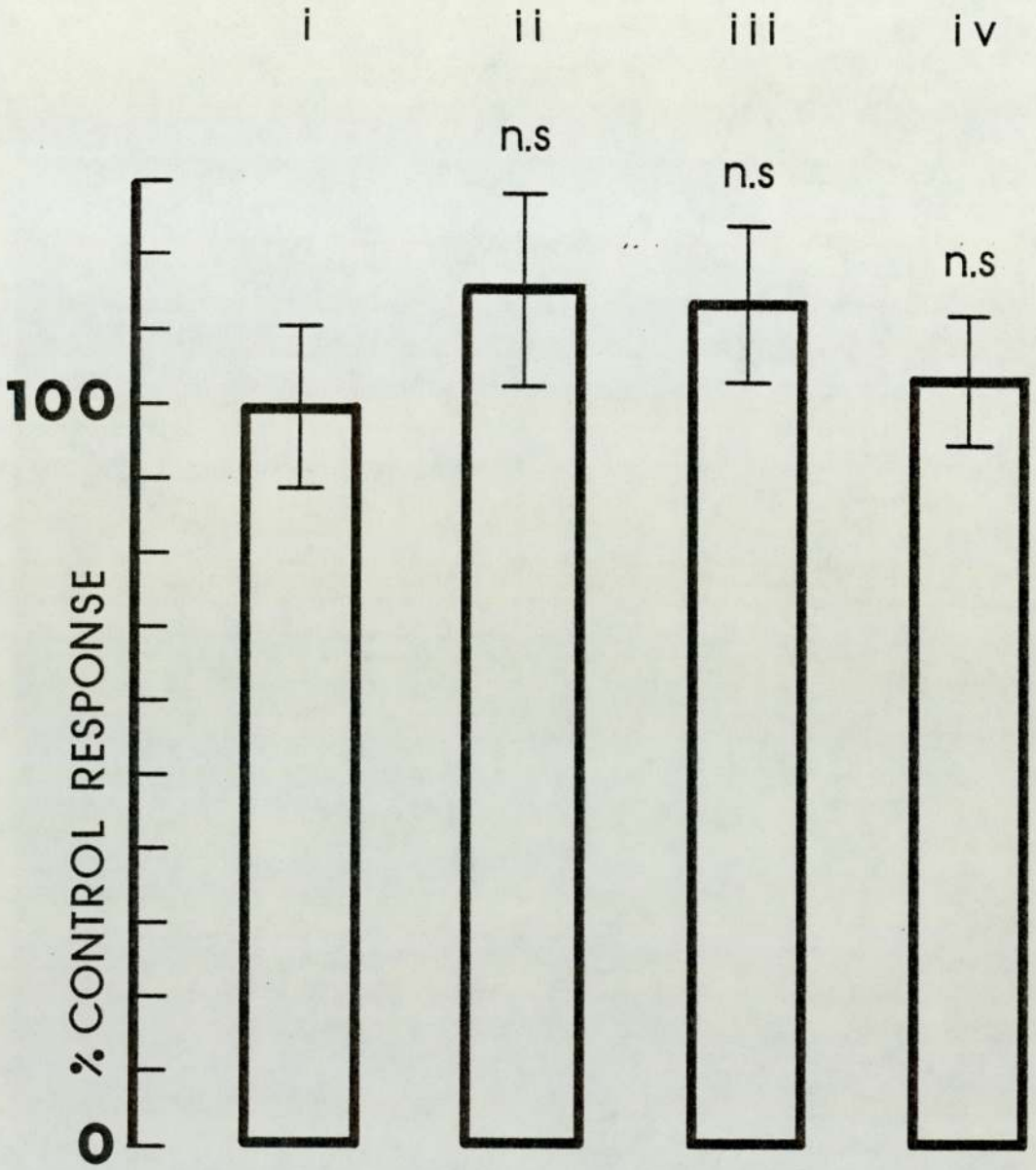

FIG.4I Response of serosal perfused rat ileum to 4 x 10^{-5}M potassium chloride (i); and in the presence of 2 x 10^{-4}M imidazole (ii); 2 x 10^{-4}M and 2.8μM indomethacin (iii); and 2.8μM indomethacin (iv) alone. n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=5).

chloride, which suggested the effect of imidazole was specific to bradykinin. The addition of indomethacin abolished this increased response to bradykinin seen in the presence of imidazole alone, thus implicating an involvement of cyclo-oxygenase products. Furthermore, during serosal perfusion, imidazole had no significant effect upon the response to bradykinin or potassium chloride.

Therefore, it appears that during mucosal perfusion of both the rat duodenum and ileum, the action of bradykinin probably involves both products of the cyclo-oxygenase pathway and cyclic nucleotides. The reason for the apparent resistance of the mucosal perfused ileum to the effect of theophylline remains unclear.

B. The Role of Nerves and Cyclo-Oxygenase Products in the Response of the Rat Perfused Ileum to Bradykinin

The contractile action of bradykinin on intestinal smooth muscle has been postulated to be direct and not to involve a neuronal component (Khairallah and Page, I96I; Day and Vane, I963; Gershon, I967; Barabe et al., I975), although an indirect cholinergic mechanism has been suggested (Wiegershausen et al., I964). However,these results presumably represented the action of bradykinin on the serosal surface. The postulated involvement of prostaglandins with bradykinin during mucosal perfusion, as developed in this thesis, may modify this picture, particularly since prostaglandins have recently been suggested to act physiologically as modulators of cholinergic transmission in the intestinal tract (Kadlec et al., I978). This study was designed to

investigate the possibility of a neuronal involvement in the contractile response of the rat isolated perfused ileum to bradykinin. The study used tetrodotoxin as a specific inhibitor of sodium-dependent action potentials in autonomic (and other) nerves (Kuriyama et al., 1966; Gershon, 1967; Evans, 1972).

(i) The Effect of Tetrodotoxin and Indomethacin on the Response of the Perfused Ileum to Bradykinin or Potassium Chloride

The contraction of the rat isolated ileum to 8.1 x 10^{-10}M bradykinin during mucosal perfusion and 1.5 x 10^{-10}M bradykinin during serosal perfusion, represented the 100% control responses on their respective surfaces. The corresponding concentration for potassium chloride perfused over either surface of the ileum was 4 x 10^{-5}M. All concentrations used corresponded to their EC_{50} values on the respective ileal surface.

(a) Mucosal Perfused Ileum

The resting tension of the mucosal or serosal perfused ileum was unaffected by the presence of tetrodotoxin (6.3μM). The addition of tetrodotoxin (6.3μM), tetrodotoxin (6.3μM) and indomethacin (2.8μM), or indomethacin (2.8μM) alone to the mucosal perfusate, reduced the response to 8.1 x 10^{-10}M bradykinin (Fig.42), to 46 ± 6% (n=10, $p < 0.01$), 48 ± 8% (n=7, $p < 0.01$) and 44 ± 10% (n=5, $p < 0.01$) of the control value. There was no significant difference in the response to bradykinin (8.1 x 10^{-10}M) between any treatment group.

The contractile response to potassium chloride (4 x 10^{-5}M) perfused over the mucosal surface of the ileum, in

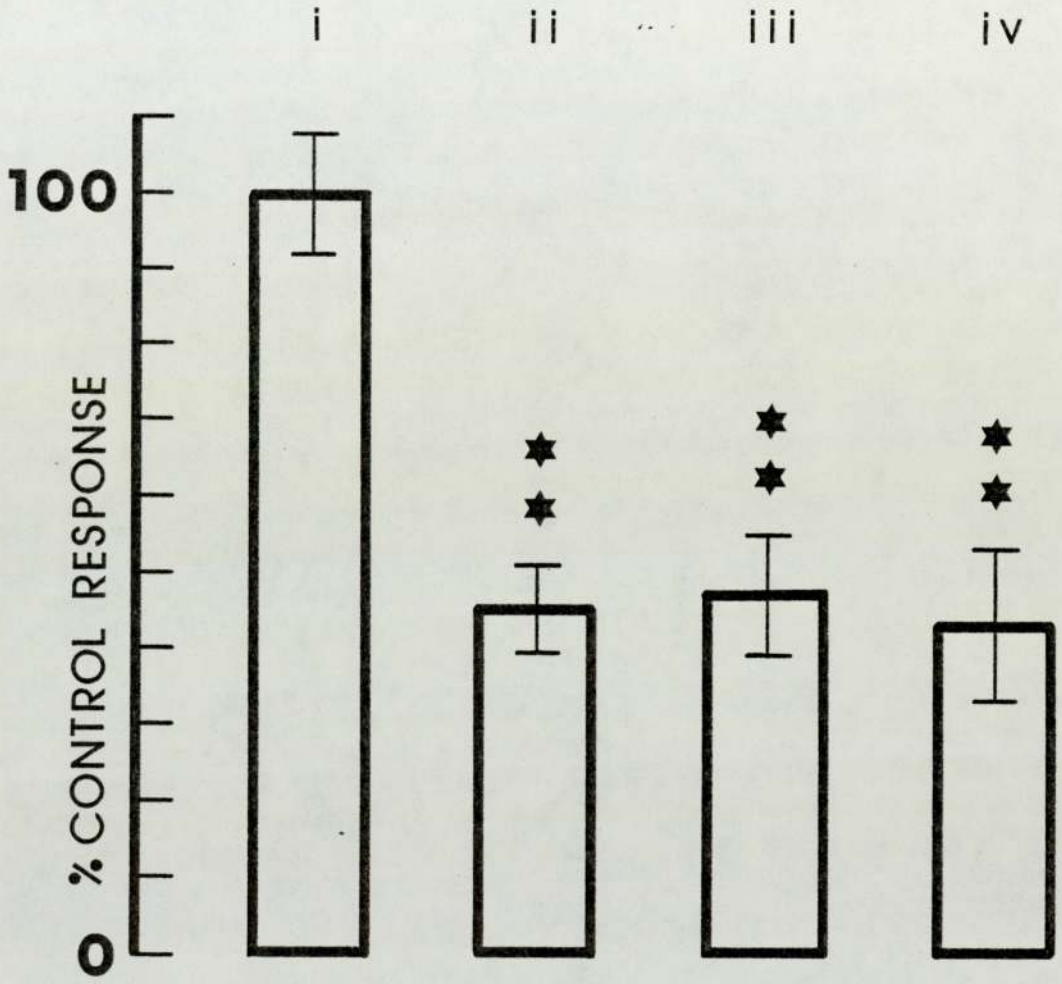

FIG.42 Response of the mucosal perfused rat ileum to 8.1×10^{-10}M bradykinin (i); and in the presence of 6.3μM tetrodotoxin (ii); 6.3μM tetrodotoxin and 2.8μM indomethacin (iii); and 2.8μM indomethacin alone (iv). **: $p < 0.01$ compared with control (i). Vertical lines represent s.e.m. (n=5-10).

the presence of tetrodotoxin (6.3μM), tetrodotoxin (6.3μM) and indomethacin (2.8μM), or indomethacin (2.8μM) alone (Fig.43), was 90 ± I2%, 96 ± 9% and IOI ± 7% respectively, (all n=5, $p > 0.05$) of the control response.

(b) Serosal Perfused Ileum

The contractile response to bradykinin (I.5 x $IO^{-IO}M$) perfused over the serosal surface of the ileum, in the presence of tetrodotoxin (6.3μM), tetrodotoxin (6.3μM) and indomethacin (2.8μM), or indomethacin (2.8μM) alone (Fig.44), was 92 ± I3%, 94 ± II% and IOI ± 8% respectively, (all n=5, $p > 0.05$) of the control response. The corresponding responses to potassium chloride (4 x $IO^{-5}M$) were 87 ± IO%, 93 ± I2% and 97 ± 7% respectively, (all n=5, $p > 0.05$) of the control response (Fig.45). There was no significant difference between any treatment group in their response to bradykinin (I.5 x $IO^{-IO}M$) or potassium chloride (4 x $IO^{-5}M$).

These results suggested the involvement of a neuronal mechanism in the response of the mucosal perfused ileum to bradykinin, but not to potassium chloride. Furthermore, the addition of either tetrodotoxin or indomethacin to the mucosal perfusate reduced the response to bradykinin by similar amounts, whilst the simultaneous addition of indomethacin and tetrodotoxin produced an inhibition no larger than either individual component. This suggested that both inhibitors were probably acting on the same mechanism.

(ii) The Effect of Various Antagonists on the Response of the Mucosal Perfused Ileum to Bradykinin

The results from the previous study suggested that the action of bradykinin during mucosal perfusion of the rat

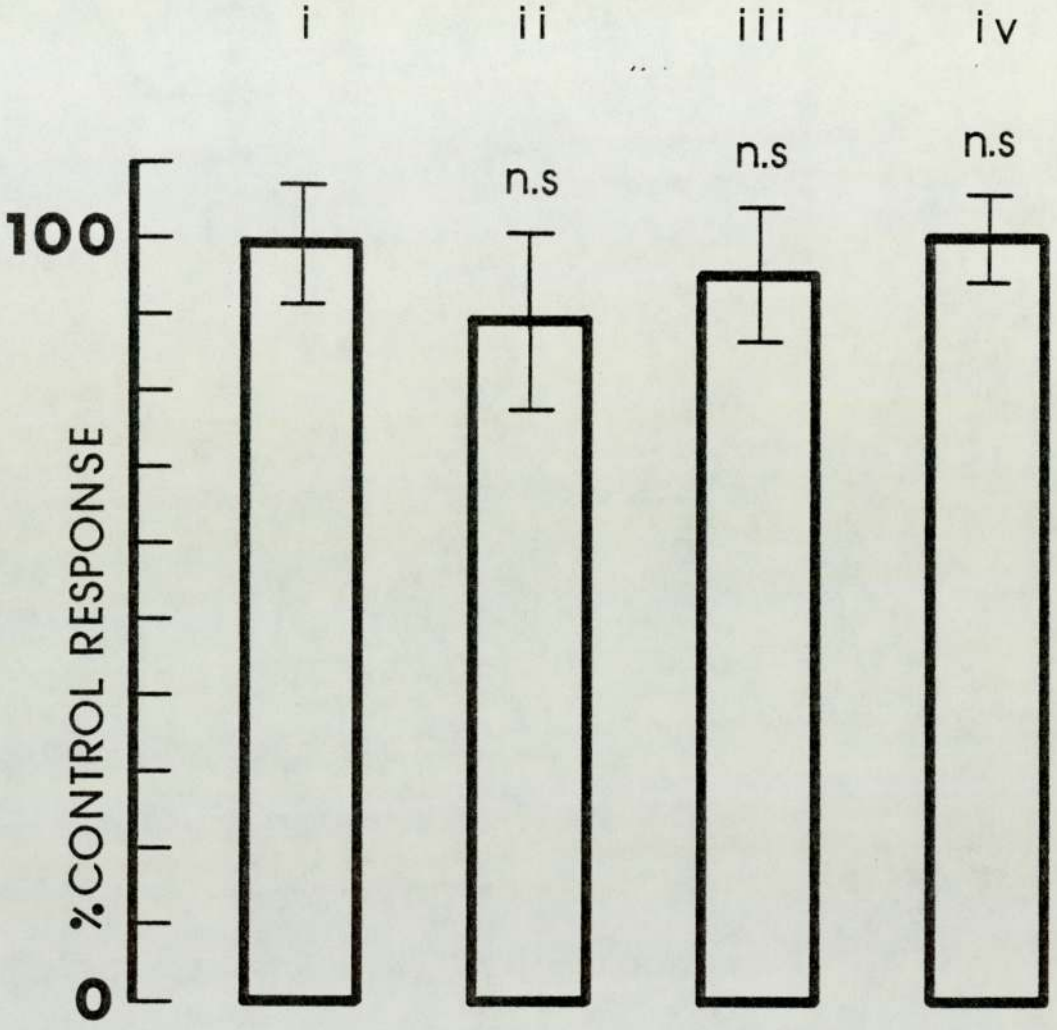

FIG.43 Response of the mucosal perfused rat ileum to 4 x 10^{-5}M potassium chloride (i); and in the presence of 6.3μM tetrodotoxin (ii); 6.3μM tetrodotoxin and 2.8μM indomethacin (iii); and 2.8μM indomethacin alone (iv). n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=5).

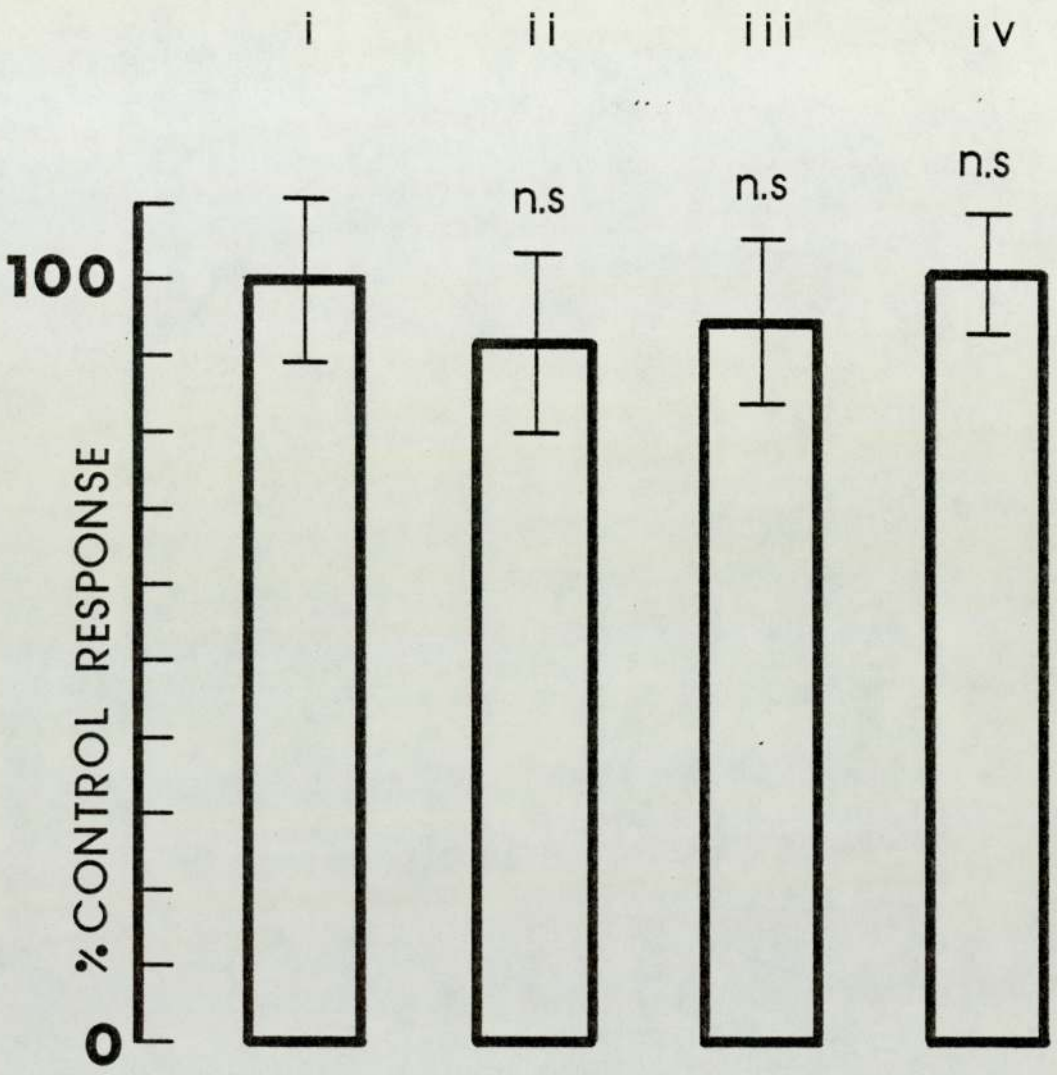

FIG.44 Response of the serosal perfused rat ileum to 1.5×10^{-10}M bradykinin (i); and in the presence of 6.3μM tetrodotoxin (ii); 6.3μM tetrodotoxin and 2.8μM indomethacin (iii); and 2.8μM indomethacin alone (iv). n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=5).

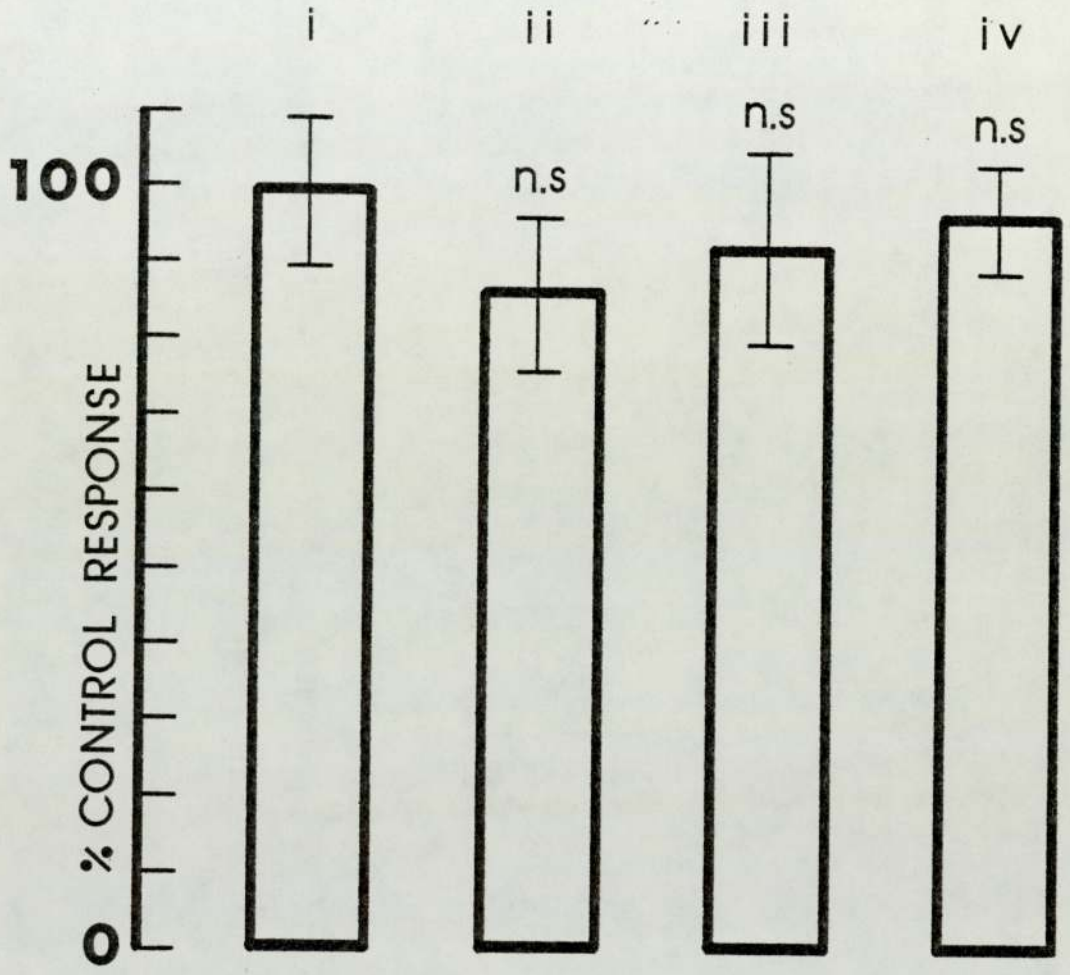

FIG.45 Response of the serosal perfused rat ileum to 4 x 10^{-5}M potassium chloride (i); and in the presence of 6.3μM tetrodotoxin (ii); 6.3μM tetrodotoxin and 2.8μM indomethacin (iii); and 2.8μM indomethacin alone (iv). n.s, not significant compared with control (i). Vertical lines represent s.e.m. (n=5).

isolated ileum could have involved a neuronally mediated component. The object of this investigation was to identify the type of nervous mechanism involved and the possible site of action of bradykinin i.e. pre-ganglionic, post-ganglionic.

The response of the mucosal perfused ileum to bradykinin (8.I x $IO^{-IO}M$), the concentration giving a 50% maximal response, was studied in the absence and presence of several antagonists (Table 3). The concentrations of the antagonists used were sufficient to significantly reduce ($p < 0.0I$) the response of their respective agonist. In those cases where the mucosal perfused ileum did not respond to the agonist, the highest concentration of antagonist was used which had no effect upon the response to potassium chloride (4 x $IO^{-5}M$). Thus, the action of pempidine, mepyramine, phentolamine and propranolol against their respective agonists (nicotine, histamine, catecholamines) was not tested because these agonists were inactive on the mucosal perfused ileum.

The addition of hyoscine (4.6 x $IO^{-6}M$), methysergide (5 x $IO^{-5}M$) or mepyramine (5 x $IO^{-6}M$) to the mucosal perfusate, had no significant effect upon the response to bradykinin (8.I x $IO^{-IO}M$). The contractile responses to bradykinin (8.I x $IO^{-IO}M$) were also unaffected by the presence of pempidine (3 x $IO^{-5}M$) and morphine ($IO^{-4}M$) in the perfusate, which suggested that bradykinin did not stimulate intramural ganglia or release acetylcholine from cholinergic nerve endings.

Although catecholamines relax the rat ileum, phentolamine (8 x $IO^{-6}M$) and propranolol (I.7 x $IO^{-5}M$) were used to eliminate any possible interference of endogenous catecholamines in the contractile response to bradykinin (8.I x $IO^{-IO}M$).

ANTAGONISTS (M)	AGONISTS (M)	% MAX RESPONSE: In absence of antagonist	In presence of antagonist	n	P
Hyoscine (4.6×10^{-6}M)	Bk 8.1×10^{-10}M	51.5 ± 7.8	65.0 ± 12.7	8	N.S.
	Ach 2.7×10^{-7}M	50.0 ± 7.6	11.9 ± 7.1	7	$p<0.01$
Morphine (10^{-4}M)	Bk 8.1×10^{-10}M	45.2 ± 9.0	42.8 ± 9.5	11	N.S.
Pempidine (3×10^{-5}M)	Bk 8.1×10^{-10}M	47.8 ± 3.7	52.2 ± 8.0	9	N.S.
Methysergide (5×10^{-5}M)	Bk 8.1×10^{-10}M	54.2 ±11.5	58.7 ± 11.9	8	N.S.
	5HT 2×10^{-4}M	51.0 ± 7.0	8.3 ± 8.3	7	$p<0.01$
Phentolamine (8×10^{-6}M)	Bk 8.1×10^{-10}M	44.2 ± 2.6	43.5± 3.8	6	N.S.
Propranolol (1.7×10^{-5}M)	Bk 8.1×10^{-10}M	49.7 ± 6.2	46.3 ± 7.6	9	N.S.
Mepyramine (5×10^{-6}M)	Bk 8.1×10^{-10}M	52.5 ± 3.1	56.7 ± 5.6	9	N.S.

TABLE 3 Specificity of the contractile response to bradykinin (Bk) in the isolated mucosal perfused rat ileum. (Ach) acetylcholine; (5HT) 5-hydroxytryptamine; (N.S.) not significant.

The addition of either antagonist to the mucosal perfusate had no significant effect upon the tone of the ileum, nor on the contractile response to bradykinin (8.1×10^{-10}M).

Therefore, the results suggested that the neuronal mechanism postulated to participate, together with cyclo-oxygenase products, in the response of the mucosal perfused ileum to bradykinin, is of a non-cholinergic, non-adrenergic nature.

D I S C U S S I O N

GENERAL INTRODUCTION

Bradykinin is a large positively charged molecule and it is therefore probable that it does not readily enter cells. Moreover, it is a small peptide, for which specific receptors have been shown to exist in several smooth muscles (e.g. Barabe et al., I977). The action of bradykinin on intestinal smooth muscle has been shown to be direct or indirect (see pp.3I-33). It has been postulated that prostaglandins might be involved in the contractile response of intestinal smooth muscle to bradykinin (Crocker and Willavoys, I976). Prostaglandins possess a wide range of biological activities, they function as modulators and mediators of the actions of many hormones, including bradykinin, and they contribute to basal or resting function of some tissues. These actions by which prostaglandins may participate in the regulation of the functional state of an organ are most easily established for those organs, such as the kidney, in which prostaglandin release is relatively high (Lonigro et al., I973). The gastrointestinal tract has a low biosynthetic capacity for prostaglandins compared to the kidney (Christ and van Dorp, I972), and this probably contributes to the poor understanding of the role of prostaglandins in the action of bradykinin on this system. However, there is evidence for an interrelationship between bradykinin and prostaglandins in the rat intestinal tract. In addition to the report of Crocker and Willavoys (I976) mentioned above, it has recently been demonstrated that bradykinin increases the formation of PGE_2 and $PGF_{2\alpha}$ in

microsomal fractions of rat duodenum (Liebmann and Arold, I978). It has also been shown that the increase in potential difference across the rat jejunum and colon during exposure to bradykinin is mediated by prostaglandins (Hardcastle et al., I978). Thus, the objective of the present study was to determine whether prostaglandins were involved in the contractile action of bradykinin on intestinal smooth muscle, and, if so, to investigate the nature of this interaction.

SECTION I MEASUREMENT OF PROSTAGLANDIN RELEASE FROM THE RAT ILEUM

It has been shown that bradykinin stimulates the release of prostaglandins from several organs (see p.35). The initial objective of the present study was to investigate the effect of bradykinin upon prostaglandin release from the rat isolated ileum. The prostaglandins released from the ileum into the surrounding bathing fluid were extracted and quantified by radioimmunoassay and bioassay. The study was subsequently extended to measure, by direct immediate bioassay, the release of prostaglandins from the perfused ileum.

A. Radioimmunoassay and Comparative Bioassay of the Prostaglandins Released from the Rat Ileum

(i) Measurement by Radioimmunoassay of the Prostaglandins Released from the Rat Ileum

The release of prostaglandin-like material from the rat isolated ileum was measured by radioimmunoassay, which is both a sensitive and specific assay method for prostaglandins, with the further advantage of a large sample capacity. In this way it was hoped to measure the release of individual prostaglandins, and to detect relative changes in the release of the prostaglandins.

PGE_2 and $PGF_{2\alpha}$ were measured by radioimmunoassay since preliminary studies, which involved extraction, thin-layer chromatography and bioassay, had demonstrated that they were the main prostaglandins released from the rat isolated

ileum. These findings were consistent with those of Collier (1974), who suggested PGE_2 was the main prostaglandin produced by compression of the rat gastrointestinal tract, and Bennett (1976a) who reported that prostaglandins of the E and F series were the main types found in the mammalian gastrointestinal tract. However, Knapp et al. (1978) has subsequently described the release of PGE_2, $PGF_{2\alpha}$ and PGD_2, measured by gas chromatography-mass spectrometry, from whole cell preparations of rat jejunum and colon. Since the release of PGD_2 from the rat isolated ileum was not measured in this study, its possible contribution to the results obtained is unknown.

In addition to the release of prostaglandins PGE_2, $PGF_{2\alpha}$ and PGD_2, other products of the cyclo-oxygenase pathway might also have been released. These include the more recently discovered endoperoxide products, prostacyclin, 6-keto $PGF_{I\alpha}$ and thromboxanes, all of which have been isolated from the gastrointestinal tract (see Vapaatalo and Parantainen, 1978). The full implications of these relatively new cyclo-oxygenase products, and their isolation from the rat intestinal tract, only became apparent after the completion of this particular study. It is unlikely that the unstable endoperoxides PGG_2 and PGH_2, thromboxane A_2 or prostacyclin would be present in the extracts. However, the extraction, chromatographic and immunological profiles of the stable degradation products thromboxane B_2 and 6-keto $PGF_{I\alpha}$, formed from thromboxane A_2 and prostacyclin respectively, are unknown. Recently, Cottee et al. (1977) reported that in the conventional systems of thin-layer chromatography, 6-keto $PGF_{I\alpha}$ was not separated from PGE_2. Thus, the possible contribution of 6-keto $PGF_{I\alpha}$,

and thromboxane B_2 to the results reported for the radioimmunoassay are also unknown.

(a) Establishment of the Radioimmunoassay

Prostaglandins have a molecular weight of around 350, and thus they have to be coupled to larger molecules to become antigenic. The antisera used in this study were prepared by coupling the carboxyl group of the prostaglandin molecule to the amino group of bovine serum albumin. This conjugation results in masking of the carboxyl group, and the Δ^5 double bond becomes close to the coupling site. Thus, the antibodies so produced seem to have a better recognition for those structures most distant from the coupling site such as the cyclopentane ring, the Δ^{13} double bond and the structure at the ω-end (see Granstrom and Kindahl, I978). Since there are a large number of prostaglandins which only differ from one another by small structural changes, it was essential to check the specificity of the antiserum produced. Preliminary studies also had to be performed to determine whether it was necessary to extract samples prior to radioimmunoassay. Several prostaglandin radioimmunoassays have been reported which have used untreated and unextracted plasma, urine, and other biological fluids (see Salmon and Karim, I976 and Kindahl, I978). It is, however, sometimes necessary to carry out extractions or other separatory procedures before the radioimmunoassay, in order to concentrate the prostaglandin, to separate it from certain of its metabolites or from other structurally related prostaglandins.

The PGE_2 and $PGF_{2\alpha}$ antisera used in the present study were supplied, together with data on their specificity, by Dr Levine. The cross reactivity of the antisera provided was

verified for $PGF_{2\alpha}$, PGE_2 and PGA_2. The antiserum raised against PGE_2 appeared to be unspecific and cross reacted to a high degree with PGA_2 and PGB_2. The reason for the low specificity of the antibody could have been that the PGE_2 was dehydrated whilst being coupled to bovine serum albumin during the production of the antiserum (Levine et al., 1971). Furthermore, the antisera raised against $PGF_{2\alpha}$ and PGE_2 had a high degree of cross reactivity with $PGF_{1\alpha}$ and PGE_1 respectively. This suggests the antisera did not recognise the Δ^5 double bond in prostaglandins of the 2 series.

Preliminary studies were performed on unextracted samples, and on extracted samples which had not been chromatographed, but the values obtained for prostaglandin release in both studies were unphysiologically high (mgPG/g.tissue/hr). This suggested that there was non-immunological inhibition which was probably caused by variations in ionic strength, protein concentration, presence of low molecular weight impurities in the sample etc. (see Kirkham and Hunter, 1971). The sample preparation procedure eventually used, involved extraction and subsequent thin-layer chromatography on silica gel plates developed in the AI solvent system of Green and Samuelsson (1964). This method gave a good separation between the different prostaglandin series, but it did not separate the individual members of each series. Preliminary studies were performed with silver nitrate impregnated silica gel plates, developed in the AII solvent system of Green and Samuelsson (1964). This method separates the individual members of each prostaglandin series that have a different number of double bonds in the molecule. However, it was found that when impregnated plates and the AII solvent

system were used, a high non-specific binding (> I5% of control binding) was obtained in the radioimmunoassay. Therefore, since neither the extraction and separation procedure, nor the antisera, distinguished between individual members of the PGE or PGF series, the results were presented as PGE-like and PGF-like.

The main disadvantage of performing extraction and chromatography, is the introduction of non-specific interfering factors into the assay. These factors contribute to the method blank and are probably due to impurities in the solvents, or in the nitrogen used to evaporate the solvents. In addition, the presence of dioctylpthalate, a plasticizer which is readily leached from standard laboratory plastic apparatus e.g. pipette tips, has been shown to interfere in radioimmunoassays (Granstrom, I979). This could also contribute to the method blank.

The inclusion of extraction and chromatography steps in the sample preparation, made it necessary to estimate the recovery of prostaglandins throughout the entire process, since variations were inevitable. It was found, using labelled prostaglandins, that over a third of the original prostaglandin content was lost during thin-layer chromatography, which was similar to the 20% to 40% loss reported by Willis (I970). In addition, the overall extraction efficiency compared favourably with other workers using the same procedure but extracting from blood (Joyner and Strand, I978).

(b) The Basal Release of Prostaglandins

To quantify the release of prostaglandins from the rat ileum, it was necessary to establish whether the tissue could metabolize any of the prostaglandins released. It was found

that there was no significant metabolism of exogenous labelled prostaglandin by the rat ileum during a 60min incubation with the tissue. This is consistent with the findings of Bennett et al. (I973) and Ferreira et al. (I976), who reported that neither human stomach homogenates nor isolated rabbit jejunum metabolized exogenous prostaglandins. However, the metabolism of PGE_1 by rat isolated jejunum (Parkinson and Schnieder, I969) and PGE_2 by guinea-pig isolated ileum (Bennett and Charlier, I977) has been demonstrated. A possible explanation for this variation in metabolism is that the enzyme prostaglandin I5-hydroxydehydrogenase, which catalyzes the initial and rate limiting step in the metabolic degradation of prostaglandins (Anggard and Samuelsson, I964, I966), has only a short half-life in isolated tissues (half-life in rat kidney and lung 45-75min, Blackwell et al., I975). Thus, the ability to metabolize prostaglandins probably not only depends upon the species and tissue studied, but also upon the time after removal of the tissue from the animal. In the present study, the metabolic degradation, by the rat ileum, of prostaglandins added to the bathing fluid was not assessed until 45min after the animal was killed. Therefore, the tissue may have lost its dehydrogenase activity before the test, which would account for the lack of metabolism of exogenous prostaglandins.

Several workers have reported the release of prostaglandins from various gastrointestinal preparations (see pp.I3-I4). It has also been reported that manipulation of tissue, such as handling the dog spleen in vivo (Ferreira and Vane, I967b), stroking the external surface of guinea-pig lungs in vitro (Piper and Vane, I97I), or distension of the

guinea-pig isolated ileum (Yagasaki et al., I974) release prostaglandins. In addition, Ferreira et al. (I976) suggested that prostaglandin generation, and consequent release from the rabbit isolated jejunum, was proportional to the amount of manipulation of, or damage to, the tissue. Thus, in the present study the release of PGE-like and PGF-like material was measured from 30min after the tissue was mounted in the organ bath. This period was adopted to minimize the effect of tissue manipulation upon the prostaglandin levels measured. After this stabilization period, the release of PGE-like and PGF-like material from the rat ileum, was found to be constant over the next two consecutive 60min incubation periods. Similar findings were reported by Yamaguchi et al. (I976) and Kadlec et al. (I978), who studied the release of PGE-like and PGF-like material from the guinea-pig taenia-coli and isolated ileum. However, in both these studies the output of prostaglandin-like material did not stabilize until 60min after mounting the respective tissue in the organ bath, although it then remained constant for 3hr to 5hr. In contrast Ferreira et al. (I976) did not observe a period of stable prostaglandin output from the rabbit isolated jejunum. They found the spontaneous release of PGE_2-like material decreased over the first 2hr and then steadily increased. Similarly, Knapp et al. (I978) demonstrated in rat jejunum and colon mince incubates, that in two consecutive 30min periods the output of PGE_2 decreased whilst the release of $PGF_{2\alpha}$ remained constant. The existence of a stable output of prostaglandin-like material as reported by Yamaguchi et al. (I976), Kadlec et al. (I978) and in the present study, compared to the variable output observed by Ferreira et al.

(I976) and Knapp et al. (I978), may represent a tissue/species variation. Moreover, it could reflect the basic stability of the preparation due to careful tissue manipulation.

In the present study it was demonstrated that more PGE-like than PGF-like material was released from the rat ileum. Edery and Shemesh (I978) also studied the release of prostaglandin-like material from the rat isolated ileum by radioimmunoassay. These workers found the release of PGF-like material was approximately twice that of PGE-like material when measured over a period of 2.5hr. However, the results of the present study are consistent with the findings of Liebmann and Arold (I978), who demonstrated that incubation of microsomal fractions of rat duodenum with arachidonic acid produced more PGE_2 than $PGF_{2\alpha}$. Additionally, Knapp et al. (I978) demonstrated, by mass spectrometry, that the release of $PGF_{2\alpha}$ was significantly less than the corresponding value for PGE_2 from rat jejunum and colon mince incubates, whilst Collier (I974) showed by bioassay, that PGE_2 was the main prostaglandin produced by compression of all regions of the rat gastrointestinal tract. Thus, the consensus of opinion suggests that PGE-like material is the main primary prostaglandin formed in the rat gastrointestinal tract, which is consistent with the findings of this study.

After a period of stable prostaglandin output had been established for the rat ileum, the effect of the cyclo-oxygenase inhibitor indomethacin (Vane I97I), was investigated on this release. This experiment was performed to verify that the radioimmunoassay was measuring prostaglandins, and was sensitive to changes in prostaglandin concentration. Incubation of the ileum with indomethacin reduced the release of

PGE-like material, but it had no significant effect upon the release of PGF-like material. Since indomethacin inhibits prostaglandin synthesis (Vane, I97I), a reduction in the release of both PGE-like and PGF-like material was expected. However, Sanders and Ross (I978), using radioimmunoassay, similarly demonstrated that the basal concentration of PGE in the cat ileum, in situ, was not reduced by intravenous indomethacin.

Potassium chloride, at a dose which gave a similar contraction height to bradykinin, was used as the control spasmogen in the present study. The non-receptor stimulant potassium chloride has been shown to cause a fast contraction due to calcium influx associated with membrane depolarization (Goodman and Weiss, I97I; van Breeman et al., I972; Marshall and Kroeger, I973). Incubation of the ileum with potassium chloride had no effect upon the release of PGE-like or PGF-like material from the ileum when compared to the basal release. This is consistent with the findings of Levy (I978b) who demonstrated that the contractile response of rat isolated ileum to potassium chloride does not involve prostaglandins. Thus, the results of the present study demonstrate that there is no detectable release of prostaglandin-like material associated with activation of the contractile mechanism.

In the presence of bradykinin there was an almost ten fold increase in the release of PGF-like material, whilst a reduction in the release of PGE-like material was observed. This suggests that whilst bradykinin increases the measured total release of prostaglandins from the ileum, it also appears to stimulate preferentially the formation of PGF-like material at the expense of PGE-like material.

(ii) Bioassay of Prostaglandins Released from the Rat Ileum

Bioassay was developed as one of the earliest quantitative methods for the measurement of prostaglandins (see Ferreira, I977 and Moncada et al., I978). Today, a series of new and more sophisticated chemical methods such as gas chromatography, radioimmunoassay, mass spectrometry, mass fragmentometry etc. are being developed and perfected. However, bioassay still has a role to play since it is a simple and sensitive technique for the detection of known and novel cyclo-oxygenase products.

Initially, bioassay was used to validate the results of the radioimmunoassay, by measurement of the prostaglandin content in the extracts used for the radioimmunoassay. The samples were bioassayed by the "dose bracket" method on the superfused rat fundus strip and colon. The contractions of the rat fundus strip and colon were recorded isometrically, although it has been reported that the rat fundus strip in particular does not perform well under such conditions (see Ferreira, I977). In the present study it was found that, perhaps at the expense of threshold sensitivity, under isometric conditions the rat fundus strip and colon gave reproducible responses and maintained a stable baseline throughout the experiment. The threshold concentration for PGE_2 on the superfused rat fundus strip was comparable to that of other workers (Willis, I969; Harada and Katori, I974; Bult and Bonta, I976), although the sensitivity of the rat colon to $PGF_{2\alpha}$ was approximately I0 to 20 fold less than that reported by Juan and Lembeck (I976) and Henman et al. (I978).

The bioassay of the extracts produced values for the

release of PGE-like material, which were not significantly different from those obtained by radioimmunoassay. Thus, incubation of the ileum with indomethacin reduced the release of PGE-like material, whilst in the presence of potassium chloride the release of PGE-like material was similar to the basal release, neither value being significantly different from the corresponding radioimmunoassay value. However, during incubation with bradykinin the reduction in the output of PGE-like material from the rat ileum was not significant. In contrast, a significant reduction was revealed by radioimmunoassay. The difference in this result obtained by the two methods was probably due to the presence of biological activity in the sample which was not immunologically PGE-like. However, it is unlikely that PGD_2, or the stable degradation products of prostacyclin and thromboxane A_2, 6-keto $PGF_{I\alpha}$ and thromboxane B_2 respectively, contributed to this biological activity despite their possible presence. Although PGD_2 contracts the rat fundus strip, the equipotent molar ratios for $PGF_{2\alpha}$ and PGD_2 have been shown to be 3.5 and 47 with respect to PGE_2 (=I) (Horton and Jones, I974). Omini et al. (I977) reported that 6-keto $PGF_{I\alpha}$ was inactive on the rat fundus strip and colon, whilst thromboxane B_2 is also considered to be biologically inactive (see Horton, I976 and ElAttar, I978).

Due to the poor sensitivity of the rat colon to $PGF_{2\alpha}$, the only level of PGF-like material detectable, was after incubation with bradykinin. The value for the release of PGF-like material obtained by bioassay was again not significantly different from the corresponding radioimmunoassay value. Thus, it appeared that bradykinin had increased

the release of PGF-like material from the rat ileum.

In conclusion, the results obtained with bioassay generally support the findings of the radioimmunoassay. It appears that bradykinin increases the release of PGF-like material but has no effect (bioassay) or reduces (radioimmunoassay) the release of PGE-like material from the rat ileum. Since potassium chloride has no effect upon the release of either PGF-like or PGE-like material, this implies that the action of bradykinin is not due to the contractile process. Furthermore, since PGD_2, 6-keto $PGF_{I\alpha}$ and thromboxane B_2 are relatively biologically inactive, and the bioassay and radioimmunoassay results are similar, this suggests there was no major interference by these cyclo-oxygenase products in the radioimmunoassay.

(iii) Interpretation of Bioassay and Radioimmunoassay Results

(a) The Action of Indomethacin on the Release of Prostaglandin-like Material

In the present study the inhibitory action of indomethacin on the release of prostaglandin-like material from the ileum as assessed both by radioimmunoassay and bioassay, was not as marked as initially expected. Moreover, no inhibitory effect of indomethacin was observed upon the release of PGF-like material measured by radioimmunoassay.

The apparent weak inhibitory action of indomethacin, could have been due to a slow or poor penetration of the drug into the tissue, resistance of the ileal cyclo-oxygenase to the drug, or a combination of these effects (see Flower, I974). The slow and/or poor penetration of the drug is

particularly marked in isolated tissues, since they no longer have an intact vascular supply to facilitate penetration. The action of indomethacin, on the release of prostaglandins from the rabbit isolated spleen, was shown to be rapid in onset but with a 45min to 3hr delay before maximal inhibition (Gryglewski and Vane, I972). Studies on the rabbit isolated jejunum showed a delay of 30min before inhibition of responses to arachidonic acid by indomethacin (Ferreira et al., I976). Furthermore,it has been demonstrated in the present study that in the presence of indomethacin it took 33min and 36min to inhibit the release of prostaglandin-like material from the mucosal or serosal perfused rat ileum respectively, as indicated by the tension of the rat fundus strip superfused with the effluent from the ileum. Therefore, it is possible that the prostaglandin levels measured in this study, in the presence of indomethacin, reflect the release of prostaglandins before penetration and inhibition of cyclo-oxygenase.

Recently, Sanders and Ross (I978), using radioimmunoassay, found that indomethacin did not reduce the PGE content of the non-stimulated cat intestine in situ, although it blocked the acetylcholine-induced stimulation of PGE. They suggested that the PGE content of the ileum is compartmentalized. One compartment readily synthesizes PGE in response to acetylcholine, and a second compartment exists that has a slower rate of disposition and therefore undergoes no rapid change when PGE synthesis is blocked by indomethacin. The results of the present study are consistent with a similar compartmentalization in the rat ileum. However, in studies which have used radioimmunoassay the apparent resistance to

indomethacin, particularly with regard to the release of PGF-like material in the present study, may be due to the measurement of non-prostaglandin compounds. This non-immunological inhibition which has been reported to be caused by agents such as dioctylpthalate (Granstrom, I979), would not be reduced by indomethacin. It is unlikely that non-immunological inhibition accounts for the results obtained in the present study, since the results of the bioassay also demonstrate a weak inhibitory action of indomethacin on the release of prostaglandin-like material from the rat ileum.

(b) The Action of Bradykinin on the Release of Prostaglandin-like Material

The results of both the radioimmunoassay and bioassay, demonstrate an increase in the total amount of prostaglandin-like material released from the rat ileum by bradykinin. There is substantial evidence demonstrating that bradykinin can release prostaglandins from several isolated tissues (see p.35). Vargaftig and Dao Hai (I972) suggested that bradykinin activated an acylhydrolase which caused the release of prostaglandin precursors. This action of bradykinin has been demonstrated to be relatively specific to the release of only those fatty acids which act as substrate for the cyclo-oxygenase enzyme (Hseuh et al., I977). Such an action of bradykinin on the rat ileum, would account for the overall increase in the release of prostaglandin-like material. However, it does not explain the ability of bradykinin to preferentially stimulate the release of PGF-like material from the ileum as measured in both assays, and to reduce (radioimmunoassay) or have no effect (bioassay) on the release of PGE-like material.

In addition to the stimulation of an acylhydrolase, bradykinin has been reported to stimulate the enzyme PGE 9-ketoreductase in bovine isolated veins (Wong et al., I977b). The distribution of this enzyme, which reduces PGE to PGF, was studied in rat organs by Leslie and Levine (I973). They found the highest activity of the enzyme in kidney, brain and liver, with negligible activity in smooth muscle and no detectable activity in homogenates of the rat ileum. However, these results do not conclusively demonstrate the absence of the enzyme from the rat intestinal tract, since only one such experiment was performed on the rat ileum. Furthermore, the activity of the enzyme was quantified by the amount of $PGF_{2\alpha}$ generated, measured with a $PGF_{2\alpha}$ antiserum, after incubation for 60min with PGE_2 and NADH. Thus, this does not preclude a low basal activity of PGE 9-ketoreductase in the rat ileum, which could be stimulated by bradykinin.

Therefore, the increase in prostaglandin release from the rat ileum is consistent with the known ability of bradykinin to activate an acylhydrolase, which stimulates the release of cyclo-oxygenase substrate and the consequent production of prostaglandins. The demonstrated ability of bradykinin to modulate the prostaglandin release profile, tentatively suggests an activation of the enzyme PGE 9-ketoreductase. However, the evidence for such a mechanism is circumstantial and further work must be performed to verify the hypothesis.

B. Direct Bioassay of the Prostaglandins Released from the Perfused Ileum

The results of the previous experiments demonstrated that bradykinin could stimulate the release of prostaglandins during a 60min incubation period. Thus, it was decided to investigate whether the contractile response of the ileum to bradykinin was correlated with the release of prostaglandin -like material into the effluent of the perfused ileum. The effluent from the mucosal or serosal perfused ileum was subject to immediate direct bioassay on the superfused rat fundus strip.

The use of the rat fundus strip for direct measurement of the release of prostaglandin-like material from the perfused ileum, offers several obvious advantages, which include sensitivity and experimental convenience, since the sample is tested without any chemical treatment. However, the assay tissue must be continuously superfused with a mixture of antagonists (Gilmore et al., I968) and indomethacin, to render the tissue more selective and sensitive to prostaglandins. In the present study, the presence of these antagonists was demonstrated to have no effect upon the response of the rat fundus strip to bradykinin or PGE_2, although a slight antagonism of responses to $PGF_{2\alpha}$ was observed. These findings are consistent with the observations of Henman et al. (I978).

In the present study the introduction of a section of rat terminal ileum, into the Krebs stream superfusing a rat fundus strip, increased the resting tension of the assay tissue to a similar extent regardless of the surface of the ileum perfused. The increased tension was abolished by the

addition of either indomethacin or aspirin to the perfusate of the ileum. This suggested that there was a continuous basal output of prostaglandin-like material from the perfused ileum. Furthermore, since the increased tension of the assay tissue was similar, regardless of the surface of the ileum perfused, it suggested the synthesis and consequent release of prostaglandin-like material was the same from both the mucosal and serosal surfaces. However, since no formal identification of the released prostaglandin-like material was performed, the possibility that each ileal surface produced different cyclo-oxygenase products, which had different activities on the assay tissue, cannot be excluded. An additional observation which has arisen from this study, is that although the basal release of prostaglandin-like material appears to be similar irrespective of the ileal surface studied, in those studies where the ileal tension was increased, there was more prostaglandin-like material (assayed as PGE_2-like material) released from the mucosal than the serosal surface. The implications of this observation are not clear. The mucosa of the gastrointestinal tract has been shown to produce more prostaglandin-like material than the muscle layers, although the converse appears to be true in certain situations (see pp.I3-I5). The results of the present study may reflect a greater functional reserve for the mucosal cyclo-oxygenase, particularly when activated by stretch.

Bradykinin, but not potassium chloride, perfused over either surface of the ileum contracted the rat fundus strip more than when the ileum was absent. Thus, it initially appeared that bradykinin released something from the ileum

which increased its contractile response on the rat fundus strip. Since this increased response to bradykinin was reduced after the addition of indomethacin or aspirin to the perfusate of either ileal surface, it suggested there was a release of a prostaglandin-like material from the ileum by bradykinin. The release of this prostaglandin-like material from the ileum appeared to be closely associated with the presence of bradykinin, since the response shape of the rat fundus strip to bradykinin, was the same whether bradykinin was injected in the presence or absence of the ileum. To verify that the increased response of the rat fundus strip to bradykinin was due to the release of a prostaglandin-like material, the effect of the prostaglandin receptor antagonist, SC I9220, was investigated on the rat fundus strip.

SC I9220, a derivative of a series of dibenzoxazepine compounds synthesized by Coyne and Cusic (I968), was first shown to antagonize the response to prostaglandins by Sanner (I969). Bennett and Posner (I97I) demonstrated that SC I9220 blocked the response of the rat fundus strip to PGE_2 and $PGF_{2\alpha}$. In the present study, SC I9220 was shown to inhibit the increased response of the rat fundus strip to bradykinin, after perfusion over either surface of the ileum. Thus, the results provide further evidence of a release of prostaglandin-like material from the perfused ileum.

(i) Studies to Quantify on the Rat Fundus Strip the Release of Prostaglandin-like Material

The previous results with both inhibitors of cyclo-oxygenase and prostaglandin receptor antagonists, suggested there was a release of prostaglandin-like material from the

perfused ileum. In this investigation it was hoped to measure, by direct bioassay, the prostaglandin-like material released from the ileum by bradykinin. The results were to be compared with those previously obtained after incubation of the ileum with bradykinin.

Juan and Lembeck (I976) described a method for the measurement, by direct bioassay on the rat fundus strip, of the prostaglandin-like material released from the isolated perfused rabbit ear by bradykinin. However, unlike the isolated perfused ileum, the isolated ear does not have a continuous release of prostaglandin-like material. Therefore, it was necessary to assess the effect of this continuous basal release of prostaglandin-like material on the response of the rat fundus strip to bradykinin. The increased contraction to bradykinin on the rat fundus strip may have been due to a potentiation by this basal release, to an increased release of prostaglandin-like material by bradykinin, or to a combination of both effects.

To investigate the effect of a continuous basal release of prostaglandin-like material, the ileum was removed from the system. PGE_2 or $PGF_{2\alpha}$, in concentrations which it was thought might simulate the release of endogenous prostaglandins from the ileum, were added to the perfusion solution, and the contractions of the rat fundus strip were determined to bradykinin or potassium chloride. It was found that only continuous superfusion with PGE_2 (0.7×10^{-12}M and 1.4×10^{-12}M) increased the contractions of the rat fundus strip to bradykinin. However, it was observed that $PGF_{2\alpha}$ could augment the increase in the bradykinin contraction caused by PGE_2. There was no apparent relationship between the

increase in the contraction of the rat fundus strip to bradykinin and the change in the baseline tension due to the prostaglandin. However, the marked increase in baseline tension produced by PGE_2 (2.8×10^{-12} M) might have accounted for the failure to observe an increase in the contraction to bradykinin at this concentration.

In the presence of the prostaglandins studied, the contractions of the rat fundus strip to bradykinin were shown to be dependent upon the prostaglandin used, its concentration and with the further possibility of modification by a combination of prostaglandins. It appeared that the responses to bradykinin were potentiated by prostaglandins. Potentiation of responses to spasmogens by prostaglandins, particularly those of the E series, was described by Hall and Pickles (1963). In the present study this property of prostaglandins was not observed during contractions to potassium chloride, demonstrating it is also dependent upon the spasmogen used.

It appeared that the results reflected a complex interaction between prostaglandins, and possibly other cyclo-oxygenase products, with bradykinin on the rat fundus strip. Thus, it did not seem possible to quantify on the rat fundus strip, the release of prostaglandins in response to bradykinin from a tissue which showed a continuous basal release of prostaglandin-like material.

In conclusion, the use of direct bioassay demonstrated a continuous basal release of prostaglandin-like material from the perfused ileum. In contrast to the experiments involving incubation with bradykinin, the injection of bradykinin into the perfused ileum did not appear to stimulate

the release of prostaglandin-like material. Although the concentration-response relationship of the rat fundus strip to bradykinin after injection over either ileal surface, was displaced to the left of that obtained in the absence of the ileum, this could have been due to a potentiation by the basal release of prostaglandin-like material. If bradykinin had caused a concentration related increase in the output of prostaglandin-like material from the ileum, it would be expected that the gradient of the concentration-response relationship for bradykinin on the rat fundus strip would have been steeper after injection through the ileum, than in its absence. This does not exclude the possibility that all concentrations of bradykinin studied induced maximal stimulation of prostaglandin output, or that the increased release of prostaglandin-like material after injection of bradykinin was too small to be detected by bioassay, particularly if the prostaglandins were not released as a single bolus. Moreover, if bradykinin only increased the release of prostaglandins of the F series, which are considered to be less active than prostaglandins of the E series on the rat fundus strip (see Ferreira, I977), this would probably be masked by the basal release of prostaglandin-like material and have been undetected by the assay. In addition, it is likely that the short contact time with bradykinin, compared to the incubation experiments, also contributed to the failure to observe an increase in the release of prostaglandin-like material from the perfused ileum.

SECTION 2 THE ROLE OF CYCLO-OXYGENASE PRODUCTS IN THE RESPONSE OF THE LONGITUDINAL MUSCLE OF RAT SMALL INTESTINE TO BRADYKININ

The experiments described in the previous section were concerned with the measurement of the prostaglandin-like material released during perfusion of the rat isolated terminal ileum. It was also observed that contractions of the ileum to bradykinin were reduced after the addition of cyclo-oxygenase inhibitors to the mucosal perfusate. Therefore, in view of this observation, and the fact that Crocker and Willavoys (I976) suggested prostaglandins participate in the contraction of the rat ileum to bradykinin, it appeared relevant to investigate the role of cyclo-oxygenase products in the response of the rat isolated perfused small intestine to bradykinin.

A. The Role of Cyclo-Oxygenase Products in the Contractile Response of the Rat Terminal Ileum to Bradykinin

The contractile response of the ileal longitudinal muscle was more sensitive to bradykinin during serosal perfusion than during mucosal perfusion. The addition of either indomethacin or aspirin to the perfusate of the ileum had an effect upon the response to bradykinin which was dependent upon the surface studied. During serosal perfusion, the contraction of the ileal longitudinal muscle to bradykinin was unaffected by the cyclo-oxygenase inhibitors indomethacin and aspirin. However, during mucosal perfusion, the cyclo-oxygenase inhibitors markedly reduced the

contractions to bradykinin. The contractions to potassium chloride were similar on both surfaces and were unaffected by indomethacin or aspirin.

These results suggested that the action of bradykinin during mucosal perfusion was dependent upon a functional cyclo-oxygenase pathway. The concentration of the cyclo-oxygenase inhibitors used, were those which had previously been shown by Crocker and Willavoys (I976) to be effective in reducing the contractile response of the longitudinal muscle of rat ileum to bradykinin. However, since these concentrations of indomethacin (28μM) and aspirin (6I0μM) were comparatively high, it was not possible to exclude effects additional to inhibition of cyclo-oxygenase (see Flower, I974). Several of these possibilities were investigated to ensure that non-specific effects were not significantly contributing to the results obtained.

(i) The Action of Indomethacin on the Contractile Response to Bradykinin

Experiments were performed to exclude an action of indomethacin on non-specific enzyme inhibition or on mucosal damage. In addition, the possible existence of a serosal barrier to the penetration of indomethacin was investigated. Although the experiments performed were of a very simple nature, they at least gave some indication of the possible mechanism of action.

(a) Time-Course for Inhibition of Bradykinin-Induced Contractions by Indomethacin

It has been shown that the introduction of a section of isolated perfused rat ileum into the superfusion stream

of the rat fundus strip, increased the resting tension of the rat fundus strip due to the release of a prostaglandin -like material. This increased resting tension was abolished after 33min and 36min by the addition of indomethacin(28μM) to the mucosal or serosal perfusate respectively. However, with some tissues there appeared to be a delay of up to 65min before the assay tissue returned to its original tension, and this was independent of the surface studied. The return of the rat fundus strip to baseline tension was used to indicate inhibition of the ileal cyclo-oxygenase system. This relies heavily on the hypothesis proposed by Piper and Vane (I97I), that cells do not store prostaglandins and that release is in most cases equivalent to de novo synthesis.

The time-course for the inhibition of the release of prostaglandin-like material from the rat ileum is consistent with the finding of Ferreira et al. (I976). They used the inhibitory action of indomethacin on arachidonic acid-induced contractions of rabbit isolated jejunum as an index of cyclo-oxygenase activity, and found contractions were blocked 30min after indomethacin administration. Although in some tissues they reported that responses to arachidonic acid were not blocked for I-2hr. In addition, the results of the present study are also supported by the findings of Gryglewski and Vane (I972), who demonstrated that indomethacin produced a maximal inhibition of prostaglandin release from the rabbit isolated spleen within 45min to 3hr. Both groups reported an initial rapid inhibitory action of indomethacin.

The addition of indomethacin (28μM) to the mucosal perfusate, reduced bradykinin-induced contractions rapidly at first and then gradually over the remaining 3hr study

period. The addition of indomethacin (2.8μM) to the mucosal perfusate also rapidly reduced responses to bradykinin, although after 40min to 60min the contractions stabilized at a level which was maintained over the remaining period studied. The time-course for the reduction of bradykinin contractions to a stable level, with indomethacin (2.8μM), is consistent with inhibition of cyclo-oxygenase as indicated by the time to inhibit the release of prostaglandin-like material from the rat ileum. These results are also consistent with the findings of Gryglewski and Vane (I972) and Ferreira et al. (I976). However, the continual depression of contractions to bradykinin in the presence of indomethacin (28μM), despite the demonstrated inhibition of prostaglandin-like material release after 33min, implicates an effect in addition to inhibition of cyclo-oxygenase. Such an additional effect could include inhibition of phosphodiesterase (Flores and Sharp, I972), or calcium antagonism (see Northover, I977).

(b) Studies on the Possible Induction of Mucosal Damage by Indomethacin

The potent gastrointestinal irritant properties of indomethacin were first reported in humans by Lovgren and Allander (I964). In the rat, Kent et al. (I969) were first to report that the administration of indomethacin, intragastrically or intramuscularly, produced multiple ulcerative lesions of the small intestine which involved both the jejunum and ileum. In I97I, Vane having previously demonstrated that indomethacin inhibited prostaglandin synthesis, suggested the gastrointestinal intolerance associated with indomethacin treatment in humans was due to a deficiency of endogenous prostaglandins. This hypothesis was tested in

rats by Robert (I975) who showed that several natural and synthetic prostaglandins inhibited the intestinal ulceration caused by indomethacin in vivo. This inhibition was found to be dose dependent, and PGA and PGE compounds were the most effective. This ability of prostaglandins to protect the cells of the intestinal epithelium from agents which cause necrosis was termed cytoprotection by Robert (I974, I975, I976). Prostaglandins have been extracted from both the stomach and intestine where they are endogenously synthesized (see pp.I3-I5) and have been postulated to have a physiological cytoprotective role (see Robert, I977).

In the present study it appeared possible that indomethacin-induced mucosal damage might account for the reduced response to bradykinin during mucosal perfusion. This was supported by the observation that perfusion of the ileal mucosal surface with indomethacin (28μM), resulted in the deposition of a viscous material in the effluent of the ileum. This "mucosal loss" induced by indomethacin was found to be related to the concentration of indomethacin, at concentrations greater than 7μM. Preliminary histological studies showed that perfusion with indomethacin (28μM) caused pronounced disruption of the mucosal epithelial cells, whereas indomethacin (2.8μM) did not appear to have such disruptive properties. Perfusion of the mucosal surface with indomethacin (28μM) and PGE_2, prevented the mucosal loss and damage caused by indomethacin alone. This prevention of indomethacin-induced mucosal loss and damage by PGE_2 is consistent with the known cytoprotective action of prostaglandins in vivo (Robert, I974, I975, I976). The results of the present in vitro study are also consistent with the findings

of Chaudhury and Jacobson (I978), who demonstrated that indomethacin damaged the isolated canine gastric mucosa and that prostaglandins offered protection against this damage.

Therefore, the results presented suggest that indomethacin (28μM) induced mucosal damage which was probably related to the continual decline in contractions to bradykinin. There was no significant mucosal damage induced by indomethacin (2.8μM), and the time course for reduction of bradykinin contractions was consistent with inhibition of cyclo-oxygenase. It therefore appeared that a concentration of 2.8μM indomethacin was more suitable for use in further studies.

(c) Pretreatment of Rats with Indomethacin

It was realized that the addition of indomethacin to the perfusate of the ileum had two disadvantages, in addition to the induction of mucosal damage. Firstly, during perfusion of the ileum, indomethacin was in direct contact with the ileal surface studied. Thus, the physical presence of the drug might have interfered with the response to bradykinin during mucosal, but not serosal perfusion. Secondly, the serosal surface might have been resistant to the action of indomethacin, due to the existence of a barrier to the penetration of indomethacin. It was felt that the use of tissues from rats pretreated subcutaneously with indomethacin might help to clarify the situation.

The initial problem was the determination of a dose of indomethacin which reduced cyclo-oxygenase activity, but which did not cause mucosal damage. Whittle (I978a) reported that indomethacin (5-30mg/Kg s.c.) caused the formation of rat gastric mucosal erosions within Ihr to 6hr, the incidence

and severity of which was dependent upon the concentration and duration of administration. In the present study, rats were pretreated with indomethacin (8mg/Kg s.c.) 2hr prior to killing the animal. Following an increase in tension there was significantly less PGE_2-like material released from either ileal surface of preparations removed from these pretreated animals, and this is consistent with inhibition of cyclo -oxygenase activity. Furthermore, there were no signs of mucosal damage in the ileum from these pretreated rats. The tissues from rats pretreated with higher doses of indomethacin showed a greater reduction in the release of PGE_2-like material from both surfaces. However, at these higher doses of indomethacin there was pronounced vascularization of the ileum, which was indicative of intestinal irritation. This observed reduction of cyclo-oxygenase activity, which is not associated with signs of mucosal damage, is consistent with the recent findings of Whittle (1979). He demonstrated that the synthesis of prostacyclin in the small intestine from rats 3hr after indomethacin (10mg/Kg s.c.) was reduced by 85%, whereas the formation of intestinal ulcers by indomethacin (10mg/Kg s.c.) was only macroscopically apparent after 36hr to 48hr.

In conclusion, these studies on the action of indomethacin have suggested that the addition of indomethacin (28μM) to the mucosal perfusate of the ileum induced mucosal damage. In contrast, the addition of indomethacin (2.8μM) to the mucosal perfusate, or the ileum from rats 2hr after indomethacin (8mg/Kg s.c.), appeared to reduce cyclo-oxygenase activity without inducing mucosal damage or having non -specific actions. Thus, the results of these studies gave

an indication of the concentrations of indomethacin which are probably fairly selective to inhibition of cyclo-oxygenase acivity in the rat ileum.

B. Further Studies on the Role of Cyclo-Oxygenase Products in Contractions of the Rat Ileum to Bradykinin

It was demonstrated in the previous sub-section that the concentration of indomethacin used in the original study of Crocker and Willavoys (I976), might have involved non-specific actions. Therefore, it was decided to perform further studies with the apparently more selective concentration of 2.8μM indomethacin, and in addition to use tissues from rats pretreated with indomethacin.

The addition of indomethacin (2.8μM) to the ileal perfusate had no effect upon contractions of the longitudinal muscle of the serosally perfused ileum to bradykinin. However, during mucosal perfusion indomethacin (2.8μM) markedly reduced contractions to bradykinin. These results were consistent with those obtained during mucosal and serosal perfusion of the ileum with 28μM indomethacin. The ileum from rats 2hr after indomethacin (8mg/Kg s.c.) also showed a reduction in contractions to bradykinin during mucosal perfusion, whilst contractions during serosal perfusion were unaffected. These findings provided further support for an involvement of cyclo-oxygenase products in the contractile response to bradykinin during mucosal perfusion.

In further experiments PGE_2 or $PGF_{2\alpha}$ was added, together with indomethacin (2.8μM), to the mucosal perfusate

of the rat ileum. In the presence of $PGF_{2\alpha}$ and indomethacin, contractions to bradykinin were similar to those obtained in the presence of indomethacin alone. The addition of PGE_2 together with indomethacin, increased the contractions of the ileum to bradykinin, compared to those obtained in the presence of indomethacin alone. However, this increase was only significant at the higher concentrations of bradykinin studied. Thus, despite the apparent involvement of prostaglandins in the response to bradykinin, the effect of indomethacin was not readily reversed by the addition of PGE_2, and was unaffected by $PGF_{2\alpha}$. These results are consistent with the observations of McGiff et al. (I976), who suggested that exogenous prostaglandins cannot mimic the release of endogenous prostaglandins in terms of specific localization of activity, sequence of structures affected, or concentrations that may be achieved at or near their site of release. In addition to these points, it must also be considered that prostaglandins may not be the major products of the cyclo-oxygenase pathway involved in the action studied. The small increase in response of the mucosal perfused ileum to bradykinin seen in the presence of PGE_2, might have been related to the beneficial cytoprotective properties of the prostaglandin.

Therefore, the results of the studies on the rat isolated perfused ileum suggest a dual action for bradykinin on longitudinal muscle contractions. The results following mucosal perfusion support a role for products of the cyclo-oxygenase pathway in the contraction to bradykinin, and are consistent with the previous findings of Crocker and Willavoys (I976). However, the failure of the cyclo-oxygenase

inhibitors to reduce contractions to bradykinin following serosal perfusion is consistent with the findings of Barabe et al. (I977), who proposed that bradykinin has a direct action on longitudinal muscle strips from cat ileum, and segments of rat uterus. A similar dual action for bradykinin has been reported in the rat isolated uterus (Whalley, I978), where it was suggested that bradykinin has a direct action on the myometrium and an indirect action on the endometrium via release of prostaglandin(s).

Preliminary studies were performed on the cat isolated ileum, a tissue in which bradykinin has a direct action on specific B_2 receptors (Barabe et al., I977). In these studies, indomethacin was found to have no effect upon the contraction of the ileal longitudinal muscle to bradykinin during either mucosal or serosal perfusion. These findings are consistent with those of Barabe et al. (I975, I977). Thus, it appears there may be a species variation in the response of ileal longitudinal muscle to bradykinin. However, further experiments are required to confirm this, particularly on sections of ileum which have not been stored before use. The sections of cat ileum used in this study and in those of Barabe et al. (I975, I977), were stored for I2hr to 24hr before use as recommended by Erspamer and Erspamer (I962).

C. The Role of Cyclo-Oxygenase Products in the Relaxation of the Longitudinal Muscle of Rat Isolated Duodenum

The previous results suggest that products of the cyclo-oxygenase pathway are involved in the contractile action of bradykinin during mucosal but not serosal perfusion

of the rat ileum. It was of interest to investigate whether a similar interrelationship existed in the rat duodenum, a tissue which exhibits a relaxation to bradykinin (Horton, I959), and in which bradykinin has been shown to stimulate the production of PGE_2 and $PGF_{2\alpha}$ in microsomal fractions (Liebmann and Arold, I978).

The relaxation response of the longitudinal muscle of rat isolated duodenum was more sensitive to bradykinin during serosal perfusion than during mucosal perfusion. The addition of indomethacin to the perfusate reduced bradykinin-induced relaxations during mucosal but not serosal perfusion. Similarly, in the duodenum from animals pretreated with indomethacin, bradykinin-induced relaxations were reduced during mucosal but not serosal perfusion.

These results further suggest an involvement of cyclo-oxygenase products in the response of the rat small intestine to bradykinin only during mucosal perfusion. It appears that the involvement of cyclo-oxygenase products is not confined to the contractile action of bradykinin, since it also occurred during the relaxation of the duodenum to bradykinin. The mechanism by which cyclo-oxygenase products mediate the contraction of the rat ileum and the relaxation of the rat duodenum to bradykinin, during mucosal perfusion, is unclear. Whilst most prostaglandins contract the longitudinal muscle of isolated intestinal tissue (see pp.I5-I6), PGE_I has been shown to relax the rat duodenum (Khairallah et al., I967). Therefore, if bradykinin stimulates the synthesis of different prostaglandins during mucosal perfusion in the ileum, compared to those in the duodenum, then the type of prostaglandin synthesized may be the factor which determines

the nature of the response. Alternatively, it may be the more labile products of the cyclo-oxygenase pathway which mediate the response to bradykinin. Again, bradykinin may stimulate the synthesis of different products in the ileum, compared to the duodenum. However, it is unlikely that the actual response to bradykinin during mucosal perfusion is caused by the cyclo-oxygenase product formed. The concentration of the cyclo-oxygenase products attained, as indicated by the measurement of prostaglandin-like material released from the ileum, would appear to be too small to cause a contraction or relaxation of the magnitude recorded. This of course does not take into account those cyclo-oxygenase products which could be formed in the ileum but for which release was not measured, or the fact that the intracellular concentration of endogenous prostaglandin(s) required to elicit a smooth muscle response, is much smaller than the concentration of exogenous prostaglandin to elicit an equivalent response. The more probable role for the cyclo-oxygenase products is the regulation of an additional effector mechanism. The most likely candidates for such an additional mechanism, which were subsequently investigated, appeared to be the cyclic nucleotides or a neuronal mechanism.

SECTION 3 STUDIES ON THE INVOLVEMENT OF CYCLIC NUCLEOTIDES OR A NEURONAL MECHANISM IN THE RESPONSE OF THE RAT INTESTINE TO BRADYKININ

A. The Interrelationship between Cyclic Nucleotides and Cyclo-Oxygenase Products in the Response to Bradykinin

Cyclic AMP has been shown to play a role in both smooth muscle contraction and relaxation. Relaxation of smooth muscle, in particular the relaxation induced by β-adrenergic agents, has been associated with increased intracellular levels of cyclic AMP. However, other mechanisms, in addition to cyclic AMP, are probably involved in the regulation of contractility and it appears that there are no differences in the response patterns and importance of these various regulatory pathways in different smooth muscles (see Diamond, I978). In the present study, the role of cyclic AMP was investigated by the use of theophylline and imidazole, which are respectively, an inhibitor and stimulator of cyclic AMP-phosphodiesterase (Butcher and Sutherland, I962).

The phosphodiesterase inhibitor theophylline was found to increase the bradykinin-induced relaxation of the rat duodenum, during both mucosal and serosal perfusion. This is consistent with the findings of Paegelow et al. (I977) and implies that the potentiated relaxation is associated with an increase of cyclic AMP levels. However, the results of the present study do not permit any positive conclusions regarding the nature of the relationship between cyclic nucleotides and bradykinin. The bradykinin-induced relaxation of the rat duodenum has been suggested to involve liberation

of catecholamines (Turker et al., I964; Montgomery, I968). Furthermore, it has been reported that the relaxation of smooth muscle by catecholamines is associated with increased cyclic AMP levels (Sutherland and Robison, I966; Anderson, I972; Bar, I974). However, the current opinion appears to be that the bradykinin-induced relaxation of the rat duodenum is not due to stimulation of β-adrenergic receptors (Ohashi et al., I967; Antonio, I968; Hall and Bonta, I973; Ufkes and van der Meer, I975; Paegelow et al., I977). Thus, whilst there is no evidence for a direct relationship between bradykinin and cyclic nucleotides in the rat duodenum, at least the possibility that cyclic nucleotides are involved as a consequence of catecholamine release can probably be eliminated.

The addition of indomethacin, together with theophylline, abolished the increased relaxation of the mucosal perfused duodenum to bradykinin observed in the presence of theophylline alone. The bradykinin-induced relaxation in the presence of theophylline and indomethacin was now reduced to the same extent as that observed in the presence of indomethacin alone. This suggests that a functional cyclo-oxygenase system is required for the action of theophylline upon the response to bradykinin during mucosal perfusion. In the presence of theophylline and indomethacin, the bradykinin-induced relaxation of the serosal perfused duodenum was also no longer significantly potentiated. However, the absence of a significant increase in these experiments was possibly due to the large standard error for these results, rather than an inhibition of the effect of theophylline by indomethacin.

Stimulation of phosphodiesterase activity by imidazole resulted in a reduction of the bradykinin-induced relaxation during both mucosal and serosal perfusion. This is again consistent with the findings of Paegelow et al. (I977) and demonstrates a reduction of a relaxation associated with a decrease in the cyclic AMP level. The addition of indomethacin together with imidazole to the mucosal perfusate of the duodenum, further reduced the bradykinin-induced relaxation. This reduction of the bradykinin-induced relaxation was similar to that obtained in the presence of indomethacin alone. However, the bradykinin-induced relaxation of the serosal perfused duodenum in the presence of imidazole, was unaffected by the addition of indomethacin. Thus, during mucosal perfusion, inhibition of cyclo-oxygenase activity appears to override the effect of imidazole.

Similar studies were performed on the rat isolated perfused ileum. It was observed that theophylline had no effect upon contractions to bradykinin or potassium chloride during perfusion over either ileal surface. The reason for this apparent resistance of the ileum to the action of theophylline is unclear. However, during mucosal but not serosal perfusion of the ileum with imidazole, there was a marked increase in contractions to bradykinin but not to potassium chloride. Thus, it appears that during mucosal perfusion, imidazole had an effect upon phosphodiesterase activity which was involved in the response to bradykinin. Furthermore, since the increased contractions to bradykinin in the presence of imidazole, were abolished by indomethacin, this again implies an interrelationship between cyclic nucleotides and cyclo-oxygenase products in the response of

the mucosal perfused rat intestine to bradykinin.

Although there appears to be an interrelationship between the cyclo-oxygenase system and cyclic nucleotides in the response of the mucosal perfused duodenum and ileum to bradykinin, the results give little indication of the level at which cyclic nucleotides are involved in the response. Cyclic AMP has been shown to stimulate prostaglandin biosynthesis in cultured mammalian cells, Graafian follicles, thyroid cells, adrenal cortex and adipocytes (see Samuelsson et al.,I978b). Alternatively, the contractile response of various tissues to prostaglandins has been suggested to be mediated by cyclic nucleotides (see pp.22-23). Thus, it is possible that cyclic AMP may be involved with activation of, or as a consequence of activation by, cyclo-oxygenase products. However, since cyclic AMP would appear to be involved in the bradykinin-induced relaxation of both surfaces of the duodenum, whilst indomethacin only reduces responses during mucosal perfusion, it seems likely that cyclo-oxygenase products stimulate cyclic AMP formation during mucosal perfusion. If such a situation exists then cyclo-oxygenase products are probably acting as intracellular regulators of cyclic AMP activity as discussed by Silver and Smith (I975). However, these suggestions are purely speculative, since the results of the present study have been obtained by an indirect method, and the involvement of cyclic AMP is inferred as a consequence of the effects of imidazole and theophylline. If cyclo-oxygenase products are to be considered as intracellular messengers, which increase or inhibit the intracellular accumulation of cyclic nucleotides, it is essential to determine the exact sequence of

events and to monitor the concentration of each mediator.

The possible involvement of cyclic GMP must also be considered in the action of bradykinin reported in the present study. It has been shown that the rat small intestine contains relatively large amounts of cyclic GMP, in comparison to other rat tissues (Ishikawa et al., I969). It may be that cyclic GMP plays an important role in the contraction of the rat ileum, since it has been suggested that increases in cyclic GMP levels may promote contractions in a variety of smooth muscles (see Diamond, I978). This is of particular importance to the consideration of the results obtained in the mucosal perfused ileum with imidazole, since there is at least one situation, the rat brain, where imidazole enhances cyclic AMP-phosphodiesterase activity and inhibits that of cyclic GMP-phosphodiesterase (O'Dea et al., I970; Goldberg et al., I970). However, there is little evidence to support a direct role for cyclic GMP in the contractile response of rat small intestine. Although cyclic AMP has been found in the smooth muscle of rat small intestine, cyclic GMP was only found in the villus brush border and in the crypt areas (Ong et al., I975).

(i) Interpretation of Results on the Involvement of Cyclic AMP and Cyclo-Oxygenase Products in the Response to Bradykinin

Although the interpretation of the results may seem straightforward, some caution is necessary. The methyl xanthines, of which theophylline is a member, have been shown to behave pharmacologically as competitive antagonists of prostaglandin actions at concentrations lower than those

required to inhibit phosphodiesterase (Beavo et al., I970; Horrobin et al., I977). Similarly, whilst imidazole can activate a number of phosphodiesterases (Jost and Rickenberg, I970; O'Dea et al., I970; Roberts and Simonsen, I970; Chasin and Harris, I977), it can also inhibit thromboxane synthesis (Moncada et al., I977) at the same low concentration. Furthermore, indomethacin has been shown to inhibit phosphodiesterase activity (Flores and Sharp, I972), although it does this generally at concentrations higher than those required to inhibit cyclo-oxygenase activity (see Flower, I974). Therefore, the simultaneous addition of theophylline or imidazole, together with indomethacin, may inhibit the synthesis and/or action of the cyclo-oxygenase products more effectively than indomethacin alone. This may be of relevance to the effect of imidazole on the response of the rat intestine to bradykinin, particularly if a low concentration of a cyclo-oxygenase product is necessary for phosphodiesterase activity in a permissive manner as suggested by Horrobin, (I978).

In view of these points some caution may be applied to the interpretation of the results. Moreover, the general hypothesis that increases in cyclic AMP levels promote smooth muscle relaxation, whilst increases in cyclic GMP promote contraction, appears to be no longer tenable in the light of recent evidence (see Diamond, I978). However, the results presented are compatible with the view that cyclic AMP may be involved in the relaxation of the mucosally or serosally perfused duodenum to bradykinin. Cyclic AMP may also be involved in the response of the mucosal perfused ileum to bradykinin, although the additional possibility

of an involvement of cyclic GMP must be considered. Furthermore, the results suggest that there is an interrelationship between cyclic nucleotides and cyclo-oxygenase products, in the response of the mucosal perfused rat small intestine to bradykinin. However, although there is evidence in the literature for an interrelationship between cyclic nucleotides and cyclo-oxygenase products (see pp.2I-24) it appears to be of an extremely complex nature. Thus, the preliminary nature of the experiments performed, and the possible non-specific actions of the drugs used, do not permit more than the most tentative general conclusions to be drawn with regard to the mechanism involved.

B. The Interrelationship between Nerves and Cyclo-Oxygenase Products in the Response of the Rat Ileum to Bradykinin

The contractile action of bradykinin on intestinal smooth muscle has been postulated to be direct and not to involve a neuronal component, although an indirect cholinergic mechanism has been suggested (see pp.3I-33). However, all these studies were presumably concerned with the action of bradykinin on the serosal surface. The present study has demonstrated that during mucosal perfusion of the rat small intestine the action of bradykinin is dependent upon a functional cyclo-oxygenase pathway. There is considerable evidence in the autonomic nervous system indicating an involvement of cyclo-oxygenase products in neurotransmission (see Hedqvist, I977 and Westfall, I977). Whilst recently, in the rabbit isolated heart and pulmonary artery, Starke et al. (I977) have demonstrated that bradykinin promotes the

biosynthesis of PGE_1 and/or PGE_2, and that these prostaglandins mediate bradykinin-induced inhibition of noradrenaline release. It was possible that a similar interrelationship between bradykinin and prostaglandins might exist in the contractile response of the rat isolated perfused ileum, although it appeared more likely that the cholinergic system would be involved. Preliminary studies were therefore performed to investigate this interrelationship. The study used tetrodotoxin as a specific inhibitor of sodium-dependent action potentials in autonomic (and other) nerves (Kuriyama et al., I966; Gershon, I967; Evans, I972).

During serosal perfusion the contraction of the ileal longitudinal muscle to bradykinin, or potassium chloride, was unaffected by the addition of either tetrodotoxin and/or indomethacin to the perfusate. However, during mucosal perfusion the contraction to bradykinin, but not to potassium chloride, was markedly reduced in the presence of tetrodotoxin and/or indomethacin. The addition of either tetrodotoxin or indomethacin to the mucosal perfusate reduced the response to bradykinin by similar amounts, whilst the simultaneous addition of indomethacin and tetrodotoxin produced no further reduction above that seen with either drug alone. This suggested that both indomethacin and tetrodotoxin were acting on the same component of the bradykinin-induced contraction.

The evidence presented, suggests the involvement of a neuronal mechanism in the contractile response of the rat ileum to bradykinin during mucosal perfusion. Initially it was considered that this neuronal component might be of a cholinergic nature, since both prostaglandins and bradykinin have been implicated to play a role in cholinergic

transmission. Wiegershausen et al. (I964) demonstrated that the response of the guinea-pig ileum to bradykinin involved a cholinergic mechanism, whilst studies on the same tissue by Potter and Walaszeck (I972), have shown the effect of bradykinin to be potentiated by facilitation of acetylcholine release from nerve endings. In addition, several prostaglandins have been suggested to play a role in pre- or post-synaptic cholinergic transmission in the gastrointestinal tract (see pp.I7-I9). However, the present study demonstrated that the action of bradykinin did not involve stimulation of intramural ganglia or release acetylcholine from nerve endings.

Bradykinin has been shown to liberate adrenaline from the suprarenal glands (Feldberg and Lewis, I964), to stimulate sympathetic ganglia (Lewis and Reit, I965) and to stimulate noradrenaline release from sympathetic nerve endings in the rabbit ear (Guth et al., I966). It has also been suggested that bradykinin may play the role of an agent acting similarly to β-adrenergic receptor blocking agents in the rat isolated heart (Moniuszko-Jackoniuk, I978), whilst a sympathicomimetic mechanism for bradykinin has been postulated in the relaxation of the rat duodenum (Turker et al., I964; Montgomery, I968) and circular muscle of human ileum (Fishlock, I966). There have been numerous studies which suggest a role for prostaglandins in adrenergic transmission in the gastrointestinal tract (see pp.20-2I). Thus, in view of this evidence it was necessary to investigate the possible involvement of catecholamines in the contractile response to bradykinin. However, the addition of either α or β-adrenergic antagonists to the mucosal perfusate

had no effect upon the contractions to bradykinin.

Several workers have suggested that 5-hydroxytryptamine or a similar substance might be an intestinal neurotransmitter (Bulbring and Gershon, I967; Costa and Furness, I976; Gershon, I977). In the present study an inhibitor of 5-hydroxytryptamine was found to have no effect upon the response to bradykinin. Furthermore, contractions of the ileum to bradykinin were also unaffected by a histamine antagonist.

Thus, if the concentrations of the antagonists studied were sufficient to reduce the effect of their respective agonist, and if the action of tetrodotoxin was selective to inhibition of nervous conduction, then it is reasonable to suggest that the response of the mucosal perfused ileum to bradykinin involves a non-adrenergic, non-cholinergic excitatory mechanism. Recently, Daniel et al. (I979) has demonstrated, for the first time in a mammalian tissue, the release of or response to a non-adrenergic, non-cholinergic inhibitory mediator which involves prostaglandins. Perhaps prostaglandins may also play a role in excitatory transmission. However, it would be premature to speculate too strongly on the role of a neuronal mechanism in the action of bradykinin, on the rat ileum, until further experiments have been performed.

C O N C L U S I O N S

An involvement of prostaglandins in the contractile response of the longitudinal muscle of the rat isolated ileum was suggested by Crocker and Willavoys (I976). The purpose of the first part of this study, was to investigate whether bradykinin could stimulate the release of prostaglandins from the rat isolated ileum. It was shown that PGE-like material was the main prostaglandin released by the rat intestinal tract, but following incubation with bradykinin, PGF-like material was the predominant prostaglandin produced. These findings provide further evidence for an interrelationship between bradykinin and prostaglandins in the rat intestinal tract. It appears that bradykinin not only stimulates an overall increase in prostaglandin synthesis, but may also alter the profile of prostaglandin release. The likely mechanism involved has been suggested to be activation of the enzyme PGE 9-ketoreductase. However, the possibility that bradykinin might stimulate some alternate metabolic pathway, particularly the regulation of endoperoxide metabolism, must also be considered. Furthermore, cofactor and substrate concentration might also have influenced the preferred biosynthetic pathway. Therefore, it is necessary to investigate these possibilities before any firm conclusions can be made regarding the bradykinin-induced release of prostaglandins from the rat ileum.

Studies were also performed to measure, by direct bioassay, the release of prostaglandins from the rat isolated ileum after exposure to bradykinin. However, it became apparent that measurement of the bradykinin-induced release

of prostaglandins by this method was difficult, since the rat isolated perfused ileum has been shown to possess a continuous basal release of prostaglandin-like material, which appears to potentiate contractions to bradykinin on the assay tissue. It is therefore concluded that direct bioassay is unsuitable for the measurement of the bradykinin -induced release of prostaglandins, from a tissue which exhibits a continuous basal release of prostaglandin-like material.

The study was extended to investigate the role of cyclo-oxygenase products in the contractile response of the rat ileum to bradykinin. These studies demonstrate for the first time, that bradykinin has a dual action on the rat isolated ileum. During mucosal perfusion the action of bradykinin has been shown to be dependent upon a functional cyclo-oxygenase pathway, whilst during serosal perfusion the action of bradykinin is more direct, or at least independent of the cyclo-oxygenase system. Furthermore, a similar interrelationship between bradykinin and cyclo-oxygenase products has been demonstrated in the relaxation of the rat duodenum to bradykinin. Again it was observed that the response of the ileal longitudinal muscle to bradykinin was dependent upon cyclo-oxygenase products during mucosal, but not serosal perfusion. Thus, cyclo-oxygenase products appear to be common biochemical mediators in the response of the mucosal perfused rat small intestine to bradykinin. However, although extensive evidence has been presented to support a role for cyclo-oxygenase products in the response of the longitudinal muscle of the rat small intestine to bradykinin, much of the evidence is by necessity indirect.

It must also be considered that inhibition of cyclo-oxygenase may result in the re-direction of cyclo-oxygenase substrate into the lipoxygenase pathways. Thus, the reduction of responses to bradykinin, by indomethacin, during mucosal perfusion of the rat small intestine, may primarily represent an increase of lipoxygenase products and not a reduction of cyclo-oxygenase products. Therefore, if cyclo-oxygenase products are to be considered as mediators of the response to bradykinin, it is essential in future studies to identify the cyclo-oxygenase product(s) involved and to elucidate the activation sequence.

Since cyclo-oxygenase products appeared to be mediators during mucosal perfusion in both the bradykinin-induced relaxation and contraction, it was considered relevant to investigate the involvement of an additional effector mechanism. The possible involvement of cyclic nucleotides, or a neuronal mechanism has been investigated in the response to bradykinin. Evidence is presented which supports an interrelationship between bradykinin, cyclo-oxygenase products and cyclic nucleotides during mucosal, but not serosal perfusion of the rat duodenum and ileum. Furthermore, preliminary studies have revealed that there is possibly also a non-adrenergic, non-cholinergic excitatory mechanism involved with cyclo-oxygenase products, in the contractile response of the mucosal perfused rat ileum to bradykinin. However, it is premature to speculate on the significance of this latter observation, and its relationship to cyclic nucleotides is unclear. The involvement of cyclo-oxygenase products with cyclic nucleotides and/or a neuronal mechanism, suggest that perhaps the main role of cyclo-oxygenase products is as

intercellular or intracellular regulators in the response of the rat small intestine to bradykinin.

Therefore, it has been demonstrated that the response of the longitudinal muscle of the rat small intestine to bradykinin is dependent upon a functional cyclo-oxygenase pathway. At the present time the great majority of studies on cyclo-oxygenase products have assumed that they are the controlling factors and have therefore sought the direct effects of the cyclo-oxygenase products. However, the results of this study suggest that the main role of cyclo-oxygenase products, in the mucosal perfused rat small intestine, may be to permit and to modulate the response to bradykinin. Although at present the nature of this role is far from clear, the results presented provide promising foundations for further studies.

A P P E N D I C E S

This thesis is based on the following publications:

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