ADRENOCEPTOR INFLUENCES ON FLUID AND ELECTROLYTE TRANSPORT IN THE RAT INTESTINE

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SUMMARY

An investigation of rat jejunal and distal colonic electrolyte transport *in-vitro* was undertaken using an Ussing chamber preparation. Selective α_2-adrenoceptor stimulation in the jejunum was found to depress theophylline elevated anion secretion, as evidenced by decreases in short-circuit current (SCC). α_1-Adrenoceptor stimulation, after α_2-adrenoceptor antagonism in the jejunum, evoked transient increases in basal anion secretion, as reflected by transient increases in basal SCC. The use of the neurotoxin tetrodotoxin indicated that this was a direct epithelial secretory effect. 5-hydroxytryptamine (5-HT) on the jejunum elicited transient increases in basal anion secretion, as demonstrated by transient increases in basal SCC. The use of tetrodotoxin, reserpine and α_1-adrenoceptor antagonists, indicated that a major component of this epithelial secretory effect by 5-HT, was associated with activation of intramural nervous pathways of the sympathetic nervous system, ultimately stimulating α_1-adrenoceptors. This might represent an important secretory mechanism by 5-HT in the jejunum. β_2-Adrenoceptor stimulation in the distal colon was found to decrease basal SCC, as evidenced by the metoprolol resistant effect of the selective β_2-adrenoceptor agonist salbutamol, and lack of effect of the selective β_1-adrenoceptor agonist prenalterol.

An investigation of rat distal colonic fluid and electrolyte transport *in-vivo* was undertaken using a colonic loop technique. Although a basal colonic absorption of Na^+ and Cl^-, and a secretion of K^+ were observed, these processes were not under tonic α-adrenergic regulation, as evidenced by the lack of effect of selective α-adrenoceptor antagonism. The secretory effects of prostaglandin-E_2 were inhibited by α-adrenoceptor activation, whereas such stimulation did not evoke pro-absorptive responses upon basal transport, unlike noradrenaline.

Key words:- Adrenoceptor Intestinal Jejunum Colon Transport

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DEDICATION

I would like to dedicate this Ph. D., Thesis to my Mother and Father, whose support and sacrifices over many years made my education possible.

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INTRODUCTION TO THE THESIS

The aims of the present study were to investigate adrenergic influences upon electrolyte transport in the rat jejunum and distal colon using the Ussing chamber *in-vitro* technique, and also evaluate adrenergic influences upon distal colonic fluid and electrolyte transport *in-vivo*. Knowledge about the function of adrenoceptor sub-types which influence intestinal transport is a prerequisite for the development of adrenergic agents for treatment of intestinal dysfunction.

It was established in the 1940s that the sympathetic and parasympathetic nervous systems are pro-absorptive and pro-secretory, respectively (Florey *et al*, 1941; Wright *et al*, 1940). The sympathetic nervous system was later found to promote intestinal absorption and inhibit secretion via the release of noradrenaline (Field and McColl, 1973), a response which is mediated through activation of α-adrenoceptors (Dietz and Field, 1973; Racusen and Binder, 1979).

Noradrenaline in the small intestine *in-vivo* is suggested to stimulate fluid absorption through activation of α_1-adrenoceptors under basal conditions (Levens *et al*, 1981a; Levens, 1983) or in conditions of dehydration or haemorrhage (Levens, 1984a and 1984b). In addition based on *in-vitro* evidence using everted intestinal sacs, a similar α_1-adrenoceptor mediated pro-absorptive response upon basal transport, has been suggested by Parsons *et al* (1983) and Cotterell *et al* (1983). In contrast α_2-adrenoceptor activation is suggested to mediate the reversal of secretagogue evoked intestinal fluid secretion *in-vivo* (Nakaki *et al*, 1982a and 1982b; Bunce and Spraggs, 1983a and 1983b). Furthermore *in-vitro* Ussing chamber studies using both the small and large intestine have shown that α_2-adrenoceptor activation produces an antisecretory response upon electrogenic ion transport processes (Chang *et al*, 1982; Dettmar *et al*, 1986a; Dharmsathaphorn *et al*, 1984; Durbin *et al*, 1982; Fondacaro *et al*, 1988). To further complicate the

situation, the β-adrenoceptor antagonist propranolol, is reported to evoke pro-absorptive and anti-secretory effects in the rat jejunum *in-vivo* under basal (Levens, 1983) and secretory (Donowitz and Charney, 1979) conditions respectively. In contrast Morris and Turnberg (1981) have proposed the presence of β-adrenergic drive promoting absorption in the human jejunum and ileum. In addition Racusen and Binder (1979) have reported that β-adrenoceptor stimulation in the rat colon *in-vitro* evokes an antisecretory response upon electrogenic electrolyte transport.

The original objective of the present study was to characterise adrenoceptor regulation of fluid and electrolyte transport in both the rat jejunum and distal colon. However as the project progressed it became evident that time restraints would not allow a comprehensive investigation of colonic transport. Therefore the study in the rat distal colon was limited to an assessment of the role of β-adrenoceptor sub-types in the regulation of basal electrogenic electrolyte transport *in-vitro,* and adrenergic influences upon fluid and electrolyte transport under secretory and basal conditions *in-vivo*. The latter investigation also addressed the possible existence of any ongoing adrenergic absorptive tone to the colon, which if present would have suscitating implications in the treatment of chronic constipation, i.e., by the development of adrenoceptor antagonists with intestinal selectivity.

CHAPTER 1: LITERATURE REVIEW.

1. INNERVATION OF THE INTESTINAL MUCOSA

In the mid 19th century it was first noticed that removal of the solar ganglia induced a fluid secretion in the intestine (Bernard,1859). The concept that the sympathetic nervous system promotes absorption, whilst the parasympathetic nervous system stimulates secretion was established in the early 1940s (Florey *et al,* 1941; Wright *et al,* 1940). However it is now accepted that the enteric division of the autonomic nervous system is an independent, integrative system that differs in its structure and function from the sympathetic and parasympathetic divisions of the autonomic nervous system (figure 1). But even though the enteric nervous system is capable of autonomous regulation, extrinsic innervation appears to be important in setting the overall "tone" of the regulatory systems. The enteric nervous system consists of the myenteric plexus (Auerbach's plexus) located between the longitudinal and circular muscle layers and the submucosal plexus (Meissner's plexus) which lies within the submucosa (Cooke, 1986). The ganglionated myenteric and submucosal plexuses are interconnected into a single functional system. Nerve fibers from the submucosal ganglia project to the mucosa to form the nonganglionated mucosal plexus adjacent to the muscularis mucosae, surrounding the crypts and subjacent to the villus cells, suggesting that the submucosal plexus functions to control and co-ordinate secretory and absorptive function, blood flow, and contractility of the muscularis mucosae (Furness and Costa, 1980). Other fibers can be found in the submucosa that are derived from myenteric ganglia or extrinsic ganglia (Feher, 1976; Feher *et al,* 1978; Feher and Csanyi, 1974; Furness and Costa, 1980).

Although a blood-ganglionic barrier to small proteins that is similar to the blood-brain barrier in the central nervous system has been suggested for the

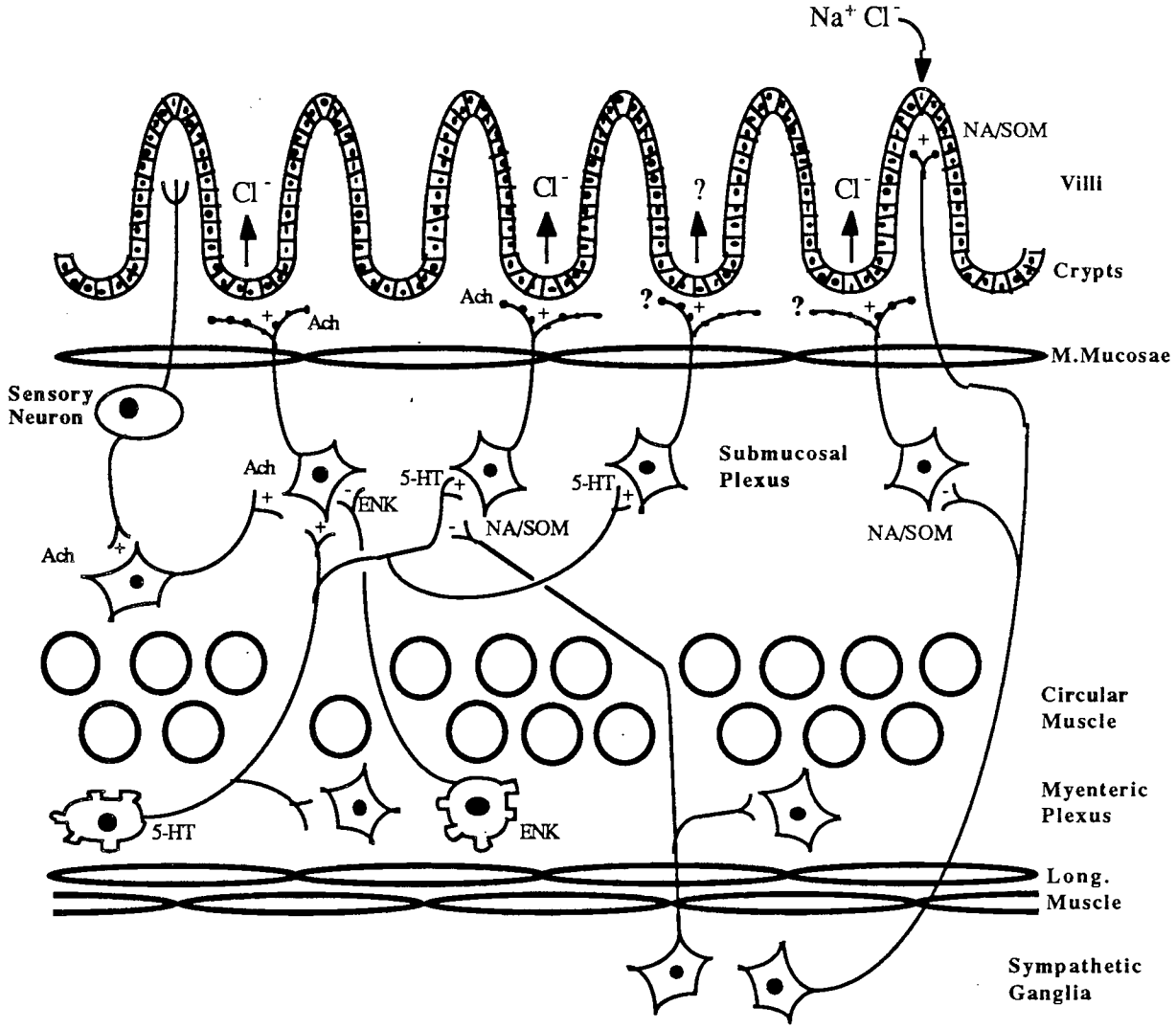

FIGURE 1: Model of neuromodulation of intestinal mucosal transport. Mechanical or chemical stimuli within the lumen activate sensory neurones. Acetylcholine (Ach) is released and acts at nicotinic cholinergic synapses within the submucosal plexus to excite (+) interneurones and/or motor neurones, innervating the crypt cells. Ach released from enteric motor neurones evokes chloride secretion by stimulating (+) muscarinic cholinoceptors on the crypt cells. It is suggested that cholinergic motor activity may be modulated by excitatory (+) input from 5-hydroxytryptaminergic neurones (5-HT) or inhibitory (-) input from enkaphalinergic neurones (ENK) whose cell bodies originate in the myenteric ganglia. 5-HT may have some other modulatory action upon secretory processes. Extrinsic sympathetic nerves arising from sympathetic ganglia also modulate the enteric nervous system. Sympathetic activity releases noradrenaline (NA)/somatostatin (SOM), which evokes inhibition (-) of neuronal activity by postsynaptic (and possibly presynaptic) mechanisms within the submucosal ganglia, as well stimulating (+) absorption by direct action on the enterocytes. Abbreviations: M. Mucosae (Muscularis Mucosae); Long. Muscle (Logitudinal Muscle).

myenteric plexus, submucosal capillaries appear to be permeable, and this raises the possibility that humoral agents circulating in the blood could reach synaptic sites in the submucosal ganglia and modulate neuronal activity (Gershon and Bursztajn, 1978).

Immunocytochemical studies have helped to determine particular submucosal neurones, however little work has been done in humans, pigs, dogs, cats, and rats, whilst guinea-pigs have been extensively used (Bishop *et al*, 1984; Christofides *et al*, 1984; Costa *et al*, 1980; Furness *et al*, 1983b, 1983c and 1984; Itoh *et al*, 1983). The total population of the guinea-pig submucosal plexus appears to be identified as either cholinergic (50%-54%) or vasoactive intestinal peptidergic (46%-50%). Cholinergic neurones were found to contain substance P (11%) or cholecystokinin-octapeptide, somatostatin, and neuropeptide Y (29%), whilst some contained none of the above peptides (14%). It has been suggested that galanin, neuropeptide Y, or dynorthin may coexist with vasoactive intestinal peptide however this is uncertain (Costa *et al*, 1985; Ekblad *et al*, 1984; Melander *et al*, 1985). The estimation that the total population of neurones in the submucosal plexus are either cholinergic or VIPergic is not absolute due to experimental errors. In this respect approximately 11% of the total population of the cell bodies within the submucosal ganglia contain an intrinsic amine that is neither noradrenaline, adrenaline, dopamine, nor 5-hydroxytryptamine (5-HT) (Furness and Costa, 1978). In addition some submucosal neurones in the rat are reported to contain both adrenocorticotropin and β-endorphin (Wolter, 1985).

The submucosal plexus also contains nerves that originate from either the myenteric plexus or an extrinsic origin. In the guinea-pig ileum submucosa, nerves derived from the myenteric plexus contain substance P (Furness *et al*, 1981), 5-HT (Furness and Costa, 1982), enkephalins (Furness *et al*, 1983a; Schultzberg *et al*,1980), and gastrin-releasing peptide (GRP)/bombesin immunoreactivity (Buffa *et al*, 1982; Costa *et al*, 1984) and possibly others.

Wade and Wood (1988), have reported that application of 5-HT or substance P on myenteric neurones of the guinea-pig distal colon results in a depolarization. The sparse distribution of enkephalinergic, GRP/bombesin-immunoreactive and 5-hydroxytryptaminergic fibers in the mucosa and their presence in the submucosal ganglia reduces the possibility that these neurones influence mucosal function directly by releasing neurotransmitters at the neuroenterocyte junction (Keast *et al*, 1984). Furthermore Gaginella *et al* (1983), using a receptor ligand binding technique were unable to detect any 5-HT-receptors on rat intestinal epithelial cell membranes. It is possible that 5-hydroxytryptaminergic neurones are involved in synaptic interactions with interneurones in the submucosal ganglia and with motor neurones that innervate the mucosal effectors. The morphology of 5-hydroxytryptaminergic neurones with their single long axons that project over relatively long distances down the intestine suggests that they are involved in transmitting information down the gut to the musculature and the submucosal plexus. Wood (1984) has hypothesised that activation of 5-hydroxytryptaminergic neurones excites a network of synaptically coupled neurones that function to ensure the simultaneous excitation or inhibition of the musculature and secretory epithelium around the circumference of the intestine. A cholinergic component has been suggested to be associated with the 5-hydroxytryptaminergic responses (Cassuto *et al*, 1982a, 1982b and 1982c).

Nerve fibers of extrinsic origin include parasympathetic vagal and pelvic fibers, sympathetic postganglionic fibers and sensory afferents with cell bodies in the dorsal root ganglia. It is presumed that the vagal efferent fibers are cholinergic, but it is unclear whether other neurotransmitters are also present (Bulbring and Gershon, 1967; Campbell, 1970; Lundberg *et al*, 1979; Malmfors *et al*, 1981). Fluorescence histochemical techniques have identified noradrenergic nerve fibers that follow the arteriolar vascular system supplying the intestine, and do not send ascending or descending branches

within the wall of the gut (Ahlman *et al*, 1973; Costa and Furness, 1984; Jacobowitz, 1965; Keast *et al*, 1984; Scheuermann and Stach, 1984; Schultzberg *et al*, 1980; Thomas and Templeton, 1981). There are three subclasses of noradrenergic fibers that innervate the intestine via the mesentery. There are (i) the fibers that project to the myenteric ganglia, which contain noradrenaline (Costa and Furness, 1984), (ii) the fibers that project to the submucosal ganglia and mucosa, which contain noradrenaline/somatostatin, and (iii) the fibers that innervate intestinal blood vessels, which contain noradrenaline/NPY (Costa and Furness, 1984; Furness *et al*, 1981, 1983c and 1984; Keast *et al*, 1984). Occasionally adrenergic fibers run directly below the villus epithelium, therefore there is the possibility that noradrenaline/somatostatin released during sympathetic stimulation, may exert an indirect effect via the submucosal plexus or a direct effect on the epithelium to modulate epithelial transport (Keast *et al*, 1984). This is further supported by the work of Thomas and Templeton (1981), who found that noradrenergic varicose fibers originating in the plexus associated with the arterioles of the sub-mucosa of the rat jejunum, run close to the central lacteal and extend to the villus tip. Again there is the suggestion that these nerves may be involved in the control of absorption. In addition there are some substance P fibers surrounding the submucosal blood vessels which are extrinsic, especially in the cat and are thought to be sensory (Brodin *et al*,1983; Furness *et al*,1981; Llewellyn-Smith *et al*, 1984; Schultzberg *et al*, 1980).

2. MECHANISMS OF ACTIVE INTESTINAL ABSORPTION AND SECRETION OF ELECTROLYTES.

Although the present study is mainly an investigation into the adrenoceptor control of intestinal electrolyte transport, it is important to recognise the different active transport mechanisms, since active transcellular transport governs fluid movement.

When one measures the electrical properties of intestinal epithelia as described later in Chapter 2, Section 1A, it is observed that there is a difference in tissue resistance and SCC, between e.g., the small and large intestine, the latter having higher values for both parameters. The terms "tight" and "leaky" epithelia have been used to describe the small and large intestine epithelia respectively, and reflect properties of transepithelial ionic conductance pathways in parallel with paracellular pathways (Armstrong, 1987). The extracellular or paracellular shunt pathway lies in the junctional-LIS (lateral intercellular space) region between adjacent absorptive cells (Erlij *et al*, 1979). In leaky epithelia the total ionic conductance of the paracellular pathway is much greater than that of the transcellular pathway (Armstrong and Garcia-Diaz 1984). In the tight epithelia especially the amphibian skin and urinary bladder, the ionic conductance of the paracellular shunt pathway is much smaller relative to the transcellular pathway. The paracellular shunt pathway is purely a passive route for ionic movement and requires a concentration gradient between both sides of the cell. Although it is important to be aware of the paracellular ionic pathway, especially since Na^+ and fluid follow secretagogue stimulated transcellular Cl^- secretion by the paracellular pathway (Munck and Schultz, 1974), the following discussion will concentrate on transcellular ion transport, since it is this which is directly affected by adrenergic mechanisms.

Transmembrane ionic transport processes in general can be divided into two broad classes, (i) those in which the ion passes across the membrane by diffusing through an aqueous channel or pore (electrodiffusion), and (ii) those in which the ion interacts with a specific membrane-bound carrier or transporter molecule. Carrier mediated transport processes in the intestine virtually always involve coupling between two or more ions or as is the case in the small intestine coupling of Na^+ and some electroneutral molecule such as glucose. Electrodiffusive ionic transport generates a net flow of electrical charge (an ionic current) across the membrane, i.e., it is electrogenic

or rheogenic. Coupled ionic transport can be electrogenic or electroneutral (i.e., involving no net charge movement), depending on, the directions of the ionic transport, the nature of the ionic species, and the stoichiometry of the transport process. However of course, if the ion is coupled to a neutral molecule, the transport process will always be electrogenic.

A) Sodium Chloride Absorption.

Sodium absorption can be both electrogenic and electroneutral. Coupled sodium chloride absorption is an electrogenically neutral process involving the 1:1 absorption of Na^+ and Cl^-. In the rabbit gallbladder NaCl absorption is entirely due to this electrically silent mechanism. However the coupled transport of NaCl is also present in many intestinal epithelia, along with other electrogenic transport processes. Cholera toxin, prostaglandins, cyclic adenosine monophosphate (cAMP) and theophylline eg., inhibit coupled NaCl absorption (Frizzell and Schultz, 1979). Intracellular calcium $[Ca^{2+}]_i$ levels also have a regulatory role over electrically silent NaCl absorption. Raised levels of $[Ca^{2+}]_i$ decrease absorption, whilst reduced levels of $[Ca^{2+}]_i$ result in increased absorption. The mechanism of action is unknown, however it does appear to be at the brush border level (Fan *et al*, 1983). There is no evidence of electroneutral coupled NaCl absorption in the rabbit distal colon (Frizzell and Schultz, 1979), however coupled NaCl absorption is present in the rabbit proximal colon (Sellin and DeSoignie, 1984). There is also evidence for coupled electroneutral NaCl absorption, as well as electrogenic Na^+ absorption in the rat distal colon (Binder, 1978), as is the case in the small intestine.

A large amount of evidence now supports the hypothesis that, in the absence of actively transported nutrients such as sugars and amino acids from the mucosal bathing solution, a coupled electroneutral entry of Na^+ and Cl^- across the apical membrane is the major mucosal step in Na^+ and Cl^-

absorption by the small intestine (Nellans *et al*, 1973 and 1974).

Na^+ plays a central role in the energetics of intestinal absorption via the transcellular route. The process of electrogenic Na^+ absorption has been extensively studied using the *in-vitro* rabbit colon. Figure 2, illustrates a model of electrogenic sodium absorption in the rabbit distal colon, adapted from that proposed by Frizzell and Schultz (1979). At both the apical and basolateral boundaries of the absorptive cell, the interior is electrically negative with respect to the extracellular environment. So there is normally a steep inwardly directed gradient of electrochemical potential for Na^+ across the brush border (apical membrane) and the basolateral membrane. Thus net Na^+ entry into the cell across either of these membranes can occur passively down the electrochemical gradient. However the precise nature of the mechanisms of apical Na^+ entry are still not fully understood. Na^+ exit from the cell must occur against a large energy gradient. The transmembrane sodium gradient is maintained by the operation of the ouabain sensitive Na^+/K^+ ATPase pump situated in the basolateral membrane, which actively 'pumps' Na^+ from the cell into the serosal compartment.

Apical Na^+ entry processes in the intestinal absorptive cell can be divided into three major categories; i) electrodiffusion, ii) transport processes in which Na^+ entry is coupled to the entry (symport) or exit (antiport) of another ion, and iii) processes in which Na^+ entry is coupled to the uptake, into the cell, of an organic nonelectrolyte (Armstrong, 1987).

Electrodiffusion was the original process described by Ussing and Zerahn (1951) for Na^+ and Cl^- transport in frog skin. This model is illustrated in figure 2. Na^+ is supposed to enter the absorptive cell by electrodiffusion down its gradient of electrochemical potential and to be extruded across the basolateral membrane by the action of the Na^+/K^+ ATPase pump. K^+ that enters the cell via the Na^+/K^+ ATPase pump is suggested to leave the cell

through basolateral K^+ channels (Hardcastle and Hardcastle, 1986). The transepithelial flow of Na^+ generates a serosal-positive transepithelial potential difference, which, in turn, is responsible for the net mucosal to serosal movement of Cl^- observed under open-circuit conditions.

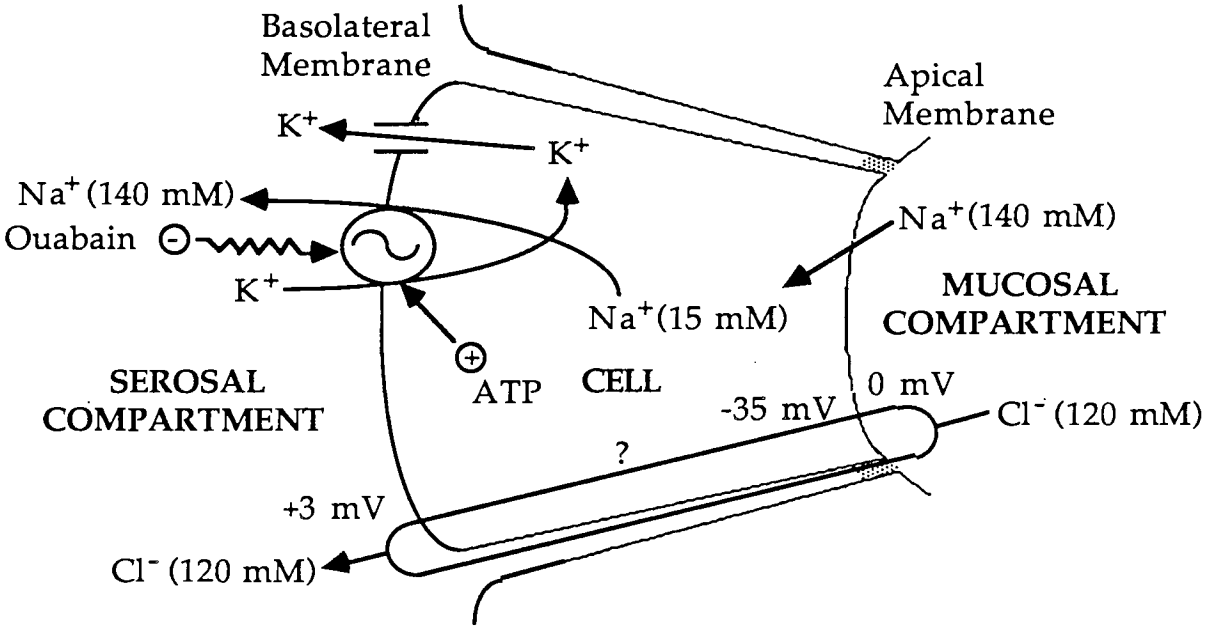

FIGURE 2: Model for NaCl absorption in the intestine based on electrodiffusive entry of Na^+ across the apical membrane. ⊕ facilitatory and ⊖ inhibitory. Originally proposed by Frizzell (1979), for the rabbit distal colon.

Transcellular Cl^- movement is supposed to occur via the low resistance paracellular shunt, in this model (figure 2). This model no longer provides an adequate explanation for transapical Na^+ and Cl^- absorption by the small intestine, mainly because there is now compelling evidence that a large fraction of the Na^+ and Cl^- that enters the cell does so by coupled transport. Although apical electrodiffusion in the small intestine and in the rat colon, constitutes a minor contribution to transapical Na^+ absorption (Nellans *et al*, 1973 and 1975), this is not the case in the rabbit colon, were electrodiffusion plays a more predominate role.

A 1:1 symport, via a common carrier, of Na^+ and Cl^- for apical absorption

is illustrated in figure 3A. Frizzell *et al* (1979), suggested that the electrically silent absorption of Na^+ and Cl^- across the apical membrane of epithelial cells

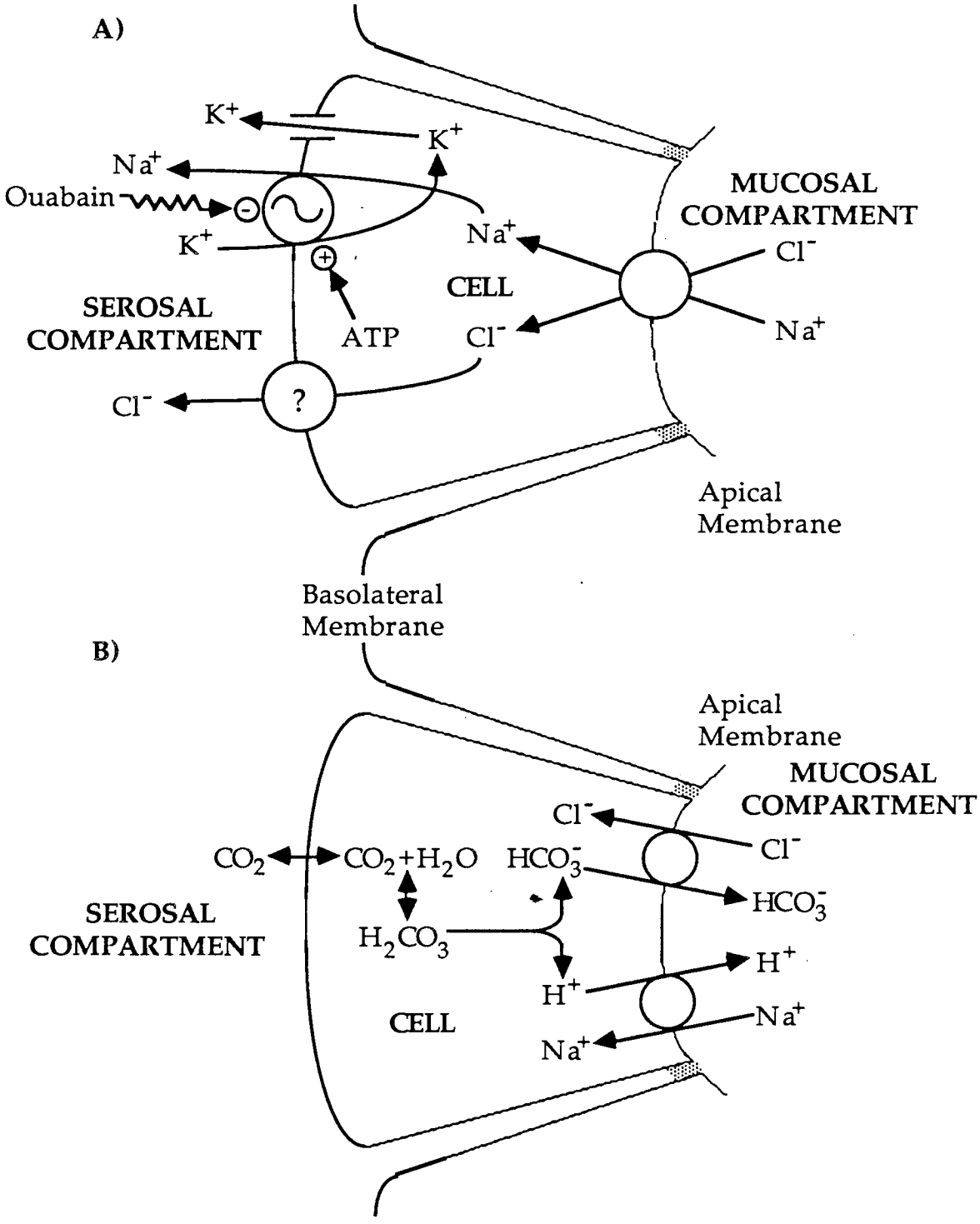

FIGURE 3: Models for NaCl absorption in the small intestine that involve electroneutral mucosal Na^+ entry. **A)** Single-entry process in the apical membrane that involves direct coupling between Na^+ and Cl^- entry. **B)** Na^+ and Cl^- entry occur via parallel exchange mechanisms. These may be indirectly coupled through the mediation of intracellular components (e.g., H^+). N.B., that, for simplicity, basolateral Na^+ and Cl^- exit mechanisms are omitted from B. ⊕ facilitatory and ⊖ inhibitory. Adapted from models described by Armstrong (1987).

is mediated through a single carrier mechanism. More recently two alternatives to the single-carrier symport model for Na^+ and Cl^- have been proposed (Armstrong, 1987). The first hypothesised the existence of separate carriers for Na^+ and Cl^- in the apical membrane (figure 3B). The second is similar to the model illustrated in figure 3A, being a single carrier hypothesis, but suggests a more complex entry mechanism. It involves the simultaneous coupled transport of Na^+, K^+, and $2Cl^-$ ions.

In the separate carrier model, the mucosal Na^+ carrier operates as a 1:1 Na^+/H^+ exchange or antiport. The Cl^- carrier operates by a 1:1 antiport of Cl^- with either HCO_3^- or OH^- (Knicklebein *et al*, 1985). When both systems operate in parallel across the apical membrane, the overall entry of Na^+ and Cl^- into the cell is electroneutral. Intracellular pH has been suggested to have a regulatory effect upon the kinetics of Na^+/H^+ and Cl^-/ HCO_3^- (or OH^-) antiporters, and so accounting for the interdependence of Na^+ and Cl^- apical transport (Reuss, 1984). Workers using small intestinal brush border vesicles have reported evidence for Na^+/H^+ and Cl^-/ OH^- antiporters (Liedke and Hopfer, 1982; Murer and Hildmann, 1984; Murer, *et al*, 1976). Interestingly, the loop diuretic frusemide, has been shown to inhibit Cl^-/ OH^- exchange in brush border vesicles from the rat intestine (Liedke and Hopfer, 1982).

In recent years, the $Na^+/K^+/2Cl^-$ symport model has been suggested to play a major role in the regulation of apical Na^+ and Cl^- entry by a number of epithelial systems (Greger, 1985; Musch *et al*, 1982), however, its existence in the mammalian small intestine is not yet established.

The Na^+/K^+ ATPase pump located in the basolateral membrane is thought to act as the energy source for the coupled transport of NaCl. The exit of Cl^- across the basolateral membrane is as yet unclear, an electroneutral K^+/Cl^- symport, Cl^-/HCO_3^- exchange or electrodiffusion, all remain

possibilities (Armstrong and Youmans, 1980; Corcia and Armstrong, 1983; Field *et al*, 1978b; Greger, 1985; Turnberg *et al*, 1970a; White and Imon, 1981).

Edmonds and Marriott (1970) have demonstrated that 90% of the SCC recorded in the rat colon *in-vivo*, can be attributed to Na^+ absorption.

B). Active Electrogenic Chloride Secretion.

Active Cl^- secretion has again been extensively studied using the rabbit colon *in-vitro*. Figure 4, illustrates a model of active Cl^- secretion by the rabbit distal colon.

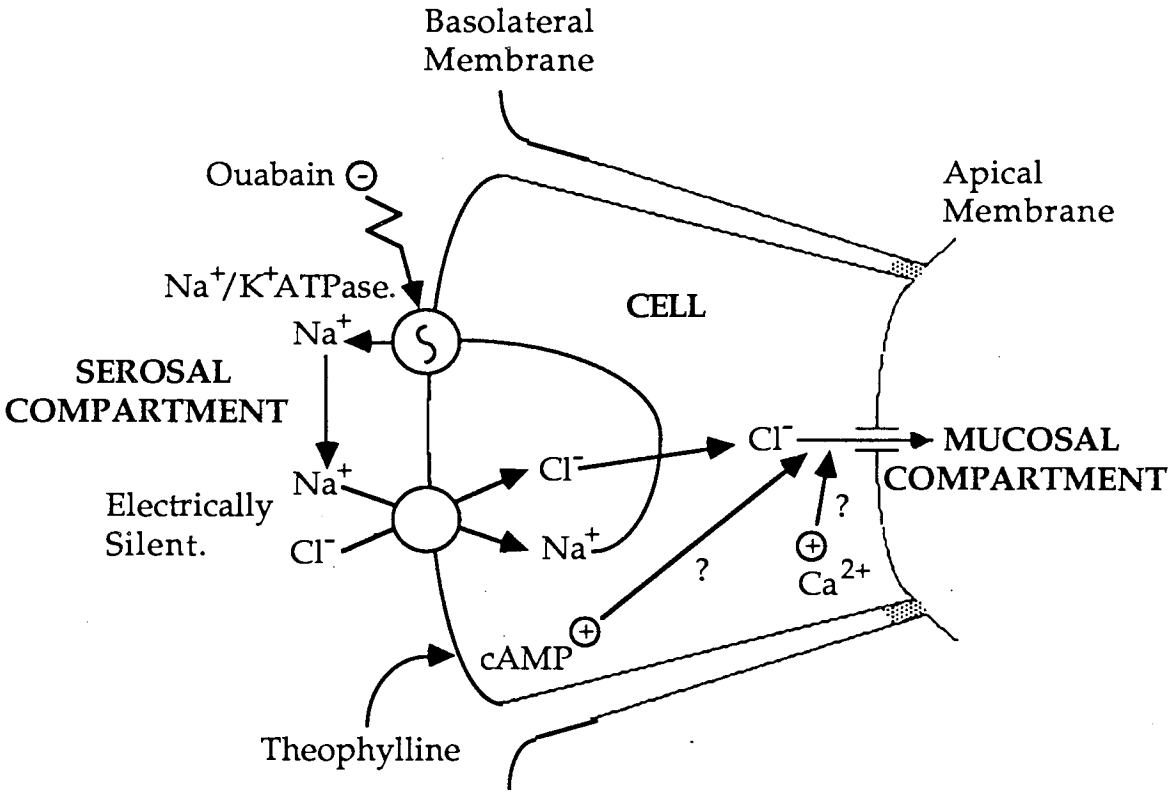

FIGURE 4: A model of electrogenic Cl^- secretion by the rabbit distal colon. ⊕ facilitatory and ⊖ inhibitory. Adapted from Frizzell and Schultz (1979).

There are many secretagogues which can elicit Cl^- secretion, eg cholinergic drugs, vasoactive intestinal peptide (VIP), cholera toxin, bile salts, gastrointestinal hormones, long chain and hydroxy fatty acids, cAMP,

theophylline and intracellular calcium etc (Frizzell and Schultz, 1979; Schwartz *et al*, 1974).

Active Cl^- secretion depends on the presence of Na^+ in the serosal bathing medium and an active Na^+ extrusion at the basolateral membrane. Cl^- secretion is inhibited by ouabain and by Na^+ replacement, i.e., removal of Na^+ from the serosal surface, thus Na^+ links the metabolic process via the Na^+/K^+ATPase in the basolateral membrane. NaCl enters the cell from the serosal bathing medium through neutral coupled transport. Na^+ is therefore simply recycled during Cl^- secretion. Cl^- leaves the cell down its electrochemical gradient through apical membrane conductive channels.

Intracellular Ca^{2+} has a regulatory role in Cl^- secretion, however the actual mechanism of action for Ca^{2+} is unknown, although it is well established that raised levels of intracellular Ca^{2+} evoke Cl^- secretion (Bolton and Field, 1977; Dharmsathaphorn and Pandol, 1985 and 1986; Dharmsathaphorn *et al*, 1985a; Field, 1981; Frizzell, 1977; Frizzell *et al*, 1979). Originally the effect of Ca^{2+} was thought to be due to a direct effect upon apical conductance of Cl^-, but more recent evidence suggests that at least part of the response is mediated by an increase in prostaglandin production (Martens *et al*, 1985; McCabe and Smith, 1985; Welsh *et al*, 1982a). In fact there has been no clear demonstration of Ca^{2+} causing a direct change in intestinal apical membrane permeability, although it might occur. There is however evidence for direct Ca^{2+} regulation of K^+ apical channels as discussed later in Section D. There is no conclusive evidence for Ca^{2+} effecting the basolateral Cl^- entry or the basolateral Na^+ exit step (Na^+/K^+ATPase pump).

Theophylline (a phosphodiesterase inhibitor) and other agents that increase the intracellular levels of cAMP, evoke a Cl^- secretion as well as inhibiting coupled NaCl absorption. The magnitude of the Cl^- secretory process induced by agents acting through cAMP is greater than that caused by

agents acting through Ca^{2+} or cyclic guanosine monophosphate (cGMP) (Bolton and Field, 1977; Field, 1981; Rao, 1985), but the reason for this is unknown.

PGE (which increases cAMP levels) has been shown in colonic cells to cause a cellular depolarization, decreased transepithelial resistance, and stimulated Cl^- secretion (Racusen and Binder, 1980; Welsh *et al*, 1982a). These changes are consistent with an increase in apical membrane Cl^- permeability. VIP and cAMP have also been shown to increase Cl^- secretion in a T-84 colonic cell line (Dharmsathaphorn *et al*, 1985a and 1985b; Dharmsathaphorn and Pandol, 1985). In addition to an effect on the apical Cl^- conductance, cAMP may regulate two of the basolateral membrane transport processes. In the T-84 cell line, VIP stimulates sodium and potassium dependent chloride uptake into the cell. It has been suggested that cAMP stimulates the $Na^+/K^+/2Cl^-$ co-transporter.

There is some evidence of synergism between cAMP and Ca^{2+}, with respect to Cl^- secretion in the T-84 cell line (Dharmsathaphorn *et al*, 1985a and 1985b; Dharmsathaphorn and Pandol, 1985). In contrast in the rabbit ileum no such synergism has been observed (Donowitz *et al*, 1980a and 1980b).

cGMP is reported in the small intestine to produce similar effects to those described for cAMP, with respect to secretory processes (Field *et al*, 1978a; Murad *et al*, 1979; Rao, 1985; Rao and Field, 1984; Rao *et al*, 1979; Rao *et al*, 1980). The secretory mechanism of cGMP is unknown and furthermore no regulatory role has been demonstrated to date under normal regulatory conditions. However in certain pathological conditions, a role has been demonstrated (e.g., as with *Escherichia coli* heat stable enterotoxin infection) (Field *et al*, 1978a).

It was originally accepted that the columnar vaculated cells of the crypts of Lieberkühn are responsible for Cl^- secretion (Welsh *et al*, 1982b), and that

these crypt cells are the progenitors of the more mature villus cells responsible for Na^+ absorption (Frizzell and Schultz, 1979). However more recent evidence from microelectrode studies, has suggested that villus cells also have the capability of Cl^- secretion (Stewart and Turnberg, 1989).

C). Bicarbonate Absorption And Secretion.

The mammalian jejunum absorbs HCO_3^- against steep electrochemical gradients, in part by an electically neutral process, which is sodium dependent, especially in the human and rat (Fordtran *et al*, 1968; Hubel, 1973; Podesta and Mettrick, 1977; Powell *et al*, 1971; Sladen and Dawson, 1968; Turnberg *et al*, 1970b). Furthermore Na^+ and HCO_3^- have a mutually stimulatory effect on the absorption of each other. This electically silent $NaHCO_3$ absorption could be due to either Na^+/H^+ exchange (figure 5) or cotransport of Na^+ with HCO_3^-. Na^+/H^+ exchange has been shown in rabbit jejunal brush border vesicles (Gunther and Wright, 1983).

However not all HCO_3^- absorption in the mammalian jejunum is Na^+ dependent (Blair *et al*, 1975; Hubel, 1973; Lucas, 1976; Powell *et al*, 1971). There is some evidence in the rat proximal jejunum of an electrogenic HCO_3^- absorptive process, in which acidification takes place concomitantly with mucosal K^+ uptake, and is inhibited by both ouabain and theophylline (Lucas, 1976). A K^+ - HCO_3^- symport or K^+/H^+ exchange remain possibilities for the mechanism of K^+ and HCO_3^- absorption (Imon and White, 1984). It has been reported that abolishing HCO_3^-/Cl^- exchange in the mammalian ileum and colon uncovers an HCO_3^- absorptive process (Davis *et al*, 1983; Garcia *et al*, 1984; Smith *et al*, 1985).

The major mechanism of HCO_3^- secretion by the mammalian ileum and colon is an HCO_3^-/Cl^- exchange process (Davis *et al*, 1983; Hubel, 1967; Hubel, 1969; Phillips and Schmalz, 1970; Turnberg *et al*, 1970a). It is reported that removal of Cl^- from the luminal bathing media abolished all or part of the

HCO_3^- secretion. This HCO_3^-/Cl^- exchange process has been demonstrated in the rabbit colon *in vitro,* where inhibition of electrogenic Na^+ absorption by amiloride, induces an electrically silent exchange of HCO_3^- and Cl^- (Frizzell *et al,* 1976). A basal electrogenic HCO_3^- secretion is reported in the rabbit ileum *in vitro* (Dietz and Field, 1973; Field *et al,* 1971; Hubel, 1974; Smith *et al,* 1985), whilst a basal secretion is also reported in the human ileum *in vivo* (Turnberg, 1970a). Basal HCO_3^- secretion is not totally explained by HCO_3^-/Cl^- exchange (Donowitz and Welsh, 1987). Electroneutral sodium dependent HCO_3^- secretion may in part be a contributory factor. However the coupling mechanism between HCO_3^- and Na^+ is as yet unexplained.

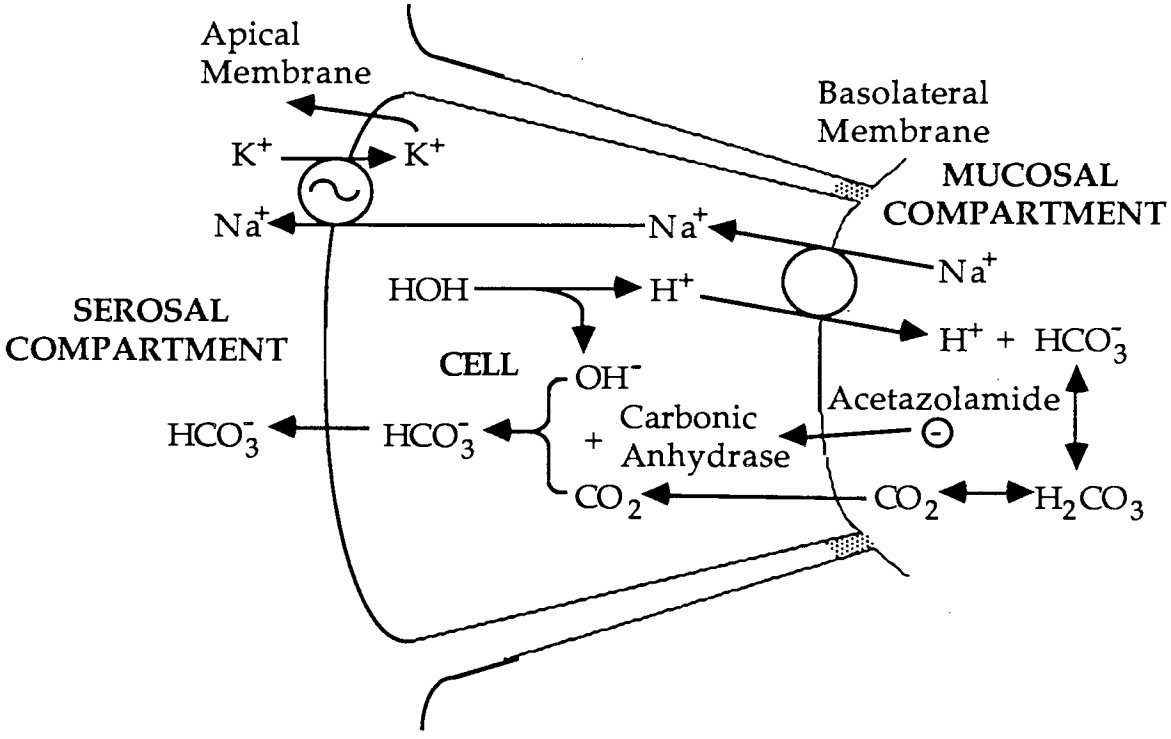

FIGURE 5: A model illustrating Na^+/H^+ exchange, which accounts for part of jejunal HCO_3^-absorption. Adapted from the diagram described by Rector (1983). ⊖ inhibitory.

D). Potassium Absorption And Secretion.

It has been reported that Na^+ depletion in rats stimulates net colonic potassium (K^+) secretion (Edmonds, 1967a and 1967b) and furthermore cAMP

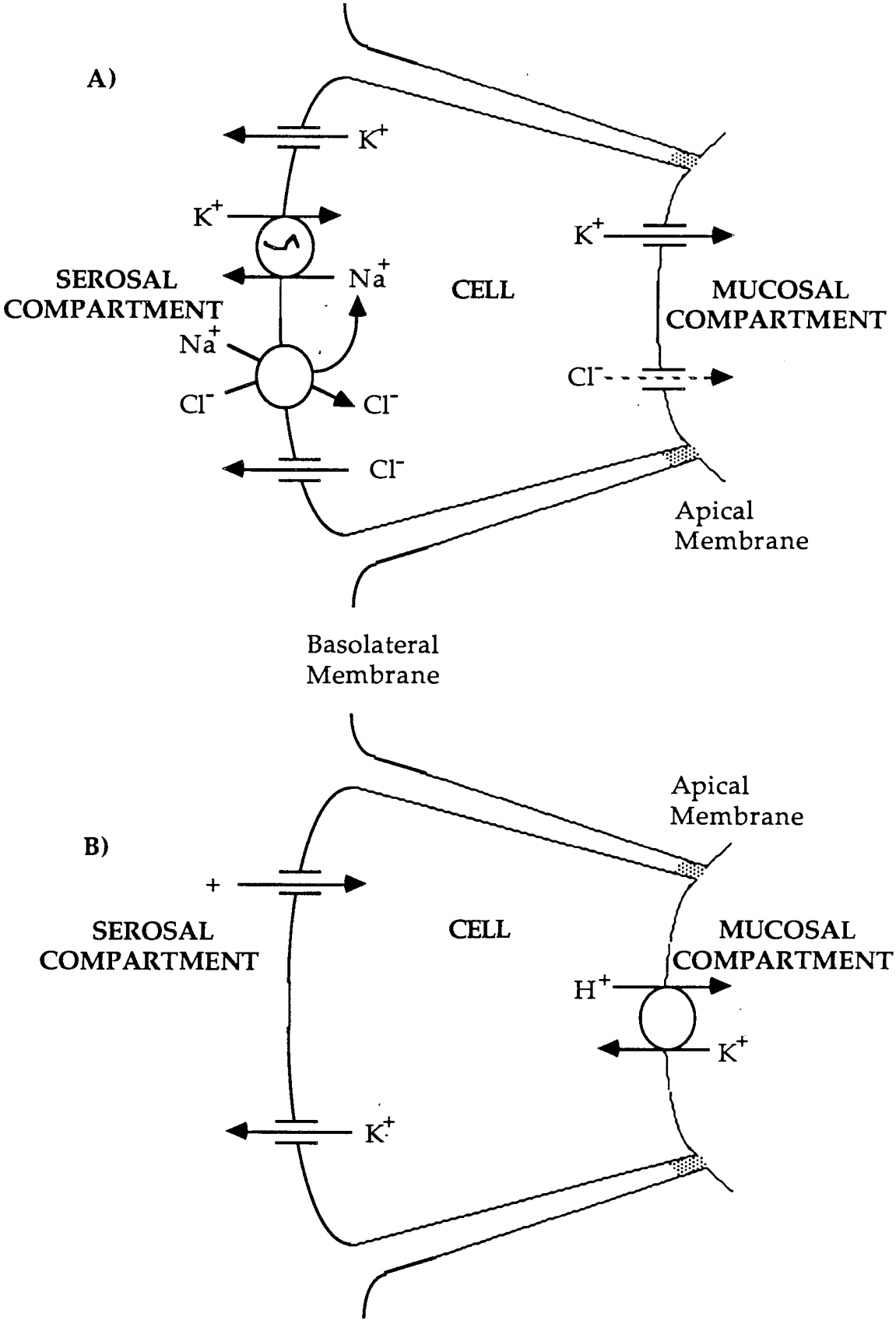

FIGURE 6: A cellular model for K^+ transport in the rabbit distal colon: **A)** Secretion; exit of Cl^- across the apical membrane is shown as a dashed line to emphasise that K^+ secretion may occur with or without net Cl^- secretion. **B)** Electrically silent absorption. Adapted from Halm and Frizzell (1986).

evoked K^+ secretion in the rat proximal colon has been reported by Foster *et al*, (1983). Interestingly K^+ absorption has been reported in the rat distal colon *in-vivo* (Kliger *et al*, 1981), and *in-vitro* (Foster *et al*, 1981). Active electrogenic K^+ secretion evoked by adrenaline and prostaglandin-E_2, in the rabbit distal colon has been reported by Halm and Frizzell (1986). They proposed a model for K^+ absorption and secretion (figure 6), which was an extension of that suggested by Smith and McCabe (1984). The transport processes that underlie absorption and secretion are shown in figure 6, in separate cells for the sake of simplicity, however they may possibly reside within the same cell type. During absorption or secretion, K^+ enters the cell via the Na^+/K^+ ATPase pumps. The basolateral membrane Na^+/K^+ ATPase pump drives the secretory flow while an apical membrane pump, possibly K-H exchange is responsible for K^+ absorption. Both processes lead to cellular accumulation of K^+ in excess of equilibrium so that subsequent K^+ exit can occur via K^+ channels in the basolateral membrane which are possibly Ca^{2+} dependent (Hardcastle and Hardcastle, 1986).

During secretion the supply of Na^+ for continued turnover of the Na^+/K^+ATPase is maintained by a coupled NaCl entry process located in the basolateral membrane. Cl^- that enters with Na^+, exits through the basolateral membrane Cl^- channels down its electrochemical gradient. According to this model SCC associated with K^+ secretion is generated by apical K^+ exit and basolateral Cl^- exit. Because of the similarities between Cl^- and K^+ secretion it has been suggested that the epithelial crypt cells are responsible for K^+ secretion (Halm and Frizzell, 1986). Potassium secretion is stimulated by raised levels of intracellular Ca^{2+}, which is thought to activate basolateral K^+ channels. (Dharmsathaphorn *et al*, 1985a and 1985b; Dharmsathaphorn and Pandol, 1986; Mandel *et al*, 1986; McRoberts *et al*, 1985). It has also been suggested that cAMP may regulate K^+ conductive channels and efflux out of the cell, but it is unclear if the K^+ effects are secondary to Ca^{2+}.

The overall process of K^+ absorption appears to be electroneutral since net

K^+ movement from mucosa to serosa is observed after inhibition of K^+ secretion when SCC is zero. Neutral K^+ - H^+ exchange would satisfy the requirement for zero SCC. Basolateral K^+ exit would need to be balanced by a conductive cation entry or anion exit to maintain electroneutrality. Then in addition if K^+ absorption is coupled to proton secretion then an acid equivalent must enter across the basolateral membrane. Conductive entry of a proton or exit of either bicarbonate or hydroxyl across the basolateral membrane would satisfy both these requirements, but this is not proven yet. It has been suggested that the mature villus cells may be responsible for K^+ absorption (Halm and Frizzell, 1986).

3. INTESTINAL AUTONOMIC TONE.

Tonic or spontaneous activity refers to ongoing neuronal activity, not directly experimentally induced. Large fluid volumes are transported across the gut mucosa during normal digestion. It has been calculated that the maximal absorptive capacity of the human small bowel is 2 l/h when absorbing from a solution containing sodium and glucose at optimal concentrations. However, in diarrhoea very large quantities of fluid may be lost in the stools. In Asiatic cholera, for example, patients may lose 1 l/h of fluid (Sjövall *et al*, 1987). Obviously, a physiological regulation of these potent transport mechanisms would be advantageous for body fluid homeostasis. Thus it might be expected that there may be a level of adrenergic tone to the mammalian intestine, however there is a lot of uncertainty whether any actually exists.

The term 'diarrhoea of diabetes' was first used by Bargen *et al* (1936) to describe unexplained diarrhoea associated with severe diabetes. Chang *et al* (1983a) have suggested the concept of adrenergic tone based on circumstantial evidence. They proposed that diabetic diarrhoea may be due to the

destruction of sympathetic nerve endings supplying the ileum and colon. Diabetic rats were reported to exhibit a malabsorption of ions. Loss of adrenergic innervation may play a role in impaired intestinal fluid and electrolyte absorption in diabetic patients with autonomic neuropathy, since clonidine is effective in treatment of patients with 'idiopathic' diabetic diarrhoea after other treatments have failed (Fedorak *et al,* 1985). Diarrhoea has also been noticed in rats with Streptazocin-induced chronic diabetes, which have decreased levels of mucosal noradrenaline. Sympathetic denervation by 6-hydroxydopamine (6-OHDA) in nondiabetic rats, produced the same pattern of impaired fluid absorption as that seen in chronically diabetic rats. *In-vitro* studies showed that tyramine-induced decreases in SCC were greater in chronically diabetic and 6-OHDA treated rats than controls. Exogenously administered adrenaline induced a greater change in SCC in the jejunum of the chronically diabetic rats than the other groups, suggesting the development of a denervation supersensitivity (Chang *et al,* 1985). Furthermore denervation supersensitivity has also been demonstrated in the ileum of chronically diabetic rats (Chang *et al,* 1986). It has been suggested that the supersensitivity is due to an increase in the number of α_2-adrenoceptors on the surface of the enterocytes rather than to changes in adrenoceptor binding affinity (Chang *et al,* 1986). It would appear that clonidine is able to correct the specific impairment of electrolyte and fluid absorption *in-vivo,* and also correct the denervation supersensitivity seen in the chronically diabetic animals. In contrast, chronically diabetic rats do not develop a cholinergic denervation supersensitivity (Perdue and Davison, 1988).

It is likely that some side effects of drugs may be explained by their effects upon intestinal electrolyte and fluid transport, eg clonidine is known to cause constipation (Schmitt, 1977). Furthermore clonidine was shown to inhibit castor oil-induced diarrhoea (Lal *et al,* 1981; Spraggs and Bunce, 1983) and to prevent naloxone-precipitated morphine withdrawal diarrhoea in rats

(Nakaki *et al*, 1981; Schreier and Burks, 1980), and acute opiate-withdrawal diarrhoea in humans (Gold, *et al*, 1978). Drug induced diarrhoea can also be produced with reserpine or adrenergic neurone blocking drugs such as guanethidine (Bowman and Rand, 1980).

Increases in fluid and electrolyte absorption in the rat small intestine in response to haemorrhage or dehydration are thought to be mediated through reflex sympathetic nervous activity (Levens, 1984a and 1984b).

There is also some evidence for a level of secretory tone to the mammalian intestine. Ussing chamber and histochemical studies on the rat colon have suggested the presence of spontaneously active cholinergic neurones in the submucosal plexus, which are antiabsorptive in nature (Andres *et al*, 1985). However activation of intramural reflexes are thought to constitute a major proportion of the stimulation of secretory processes in response to cholera toxin and some other secretagogues, whilst the remaining fraction is mediated by a direct action on the enterocytes (Cassuto *et al*, 1981a, 1981b, 1982a, 1982b, 1982c).

The neurotoxin tetrodotoxin, which blocks neuronal sodium conductance channels and prevents the action potential-dependent release of neurotransmitters (Hubel, 1978), was found in the rabbit ileum to decrease baseline short-circuit current and increase sodium and chloride transport. Work using the guinea-pig ileum suggested that tetrodotoxin-evoked changes in basal transport rates reflect the inhibition of ongoing neural activity rather than direct effects upon the transporting cells, since the presence of the submucosal ganglia was a requirement for these responses (Carey *et al*, 1985; Cooke *et al*, 1983b). Carey and Cooke (1989), have reported that tetrodotoxin in the guinea-pig ileum, also increased both sodium and chloride absorption, whilst chloride absorption exceeded that of sodium and was associated with the decreases in short-circuit current. They suggested that tonically active non-cholinergic submucosal neurones limit the absorptive capacity of the

ileum.

Basal release of acetylcholine and VIP from submucosal neurones, and 5-HT, acetylcholine, VIP, and substance P from myenteric neurones, has been reported in the large or small intestine (Baron *et al,* 1983; Gaginella, *et al,* 1981; Gershon and Tamir, 1981; Pfeuffer-Friederich and Kilbinger, 1984; Wood and Mayer, 1978; Wu *et al,*1982; Yau *et al,* 1983).

4. THE NEUROHUMORAL CONTROL OF INTESTINAL ELECTROLYTE TRANSPORT.

It has been reported that the glucocorticoid hormones and aldosterone enhance electrogenic sodium absorption by an action on specific transport proteins for sodium. These are thought to have an important role in conditions of salt depletion and excess, however the effects appear to be limited to the colon (Turnberg, 1983). Furthermore aldosterone is suggested to have a homeostatic role in colonic potassium adaption, evoking potassium secretion during potassium loading (Edmonds and Willis, 1987; Martin *et al,* 1986; McGlone and Sandle, 1988).

The neuroendocrine control of intestinal transport is believed to involve a complex matrix of interelationships between the parasympathetic and sympathetic nervous systems, the enteric nervous system and neuroendocrine agents released from intestinal epithelial endocrine cells. *In-vitro* investigations appear to indicate that neuroendocrine agents which promote secretion generally seem to stimulate electrogenic chloride secretion, however some may also exert an antiabsorptive effect on neutral sodium chloride absorption. Neuroendocrine agents which promote absorption generally appear to stimulate neutral sodium chloride absorption, whilst some also exert antisecretory effects on chloride secretion (Gaginella, 1984; Hubel, 1985; Tapper, 1983; Turnberg, 1984). It is the general consensus of opinion that the adrenergic nervous system promotes absorption whereas the

cholinergic nervous system promotes secretion (refer Section 1).

The secretory effects of cholinoceptor agonists have been antagonised by atropine, and are thought to be mediated through muscarinic-cholinoceptor stimulation (Hubel, 1976; Tapper *et al*, 1978). However high concentrations of carbachol stimulate sodium chloride absorption in the rabbit ileum, an effect thought to be mediated by pre-synaptic nicotinic-receptor stimulation on sympathetic nerve terminals (Tapper *et al*, 1978).

Low doses of angiotensin stimulate fluid absorption whilst high doses inhibit fluid absorption in the rat distal colon and jejunum *in-vivo*, (Levens *et al*, 1981a; Levens, 1983). Davies *et al* (1972) have attributed the angiotensin stimulated fluid transport in the rat distal colon as being due to stimulation of *de novo* protein synthesis, however it has been more recently suggested that the enhancement of fluid and electrolyte absorption by angiotensin is due to enhanced release of noradrenaline from sympathetic nerve terminals (Levens *et al*, 1979, 1981b and 1981c; Levens, 1983 and 1985). Reflex increases in fluid and electrolyte absorption in the rat small intestine in response to haemorrhage or dehydration are thought to be mediated by angiotensin (Levens, 1984a and 1984b). Secretion evoked by high doses of angiotensin is thought to be mediated through stimulation of prostaglandin biosynthesis (Levens *et al*, 1981c; Levens, 1983). The indirect sympathomimetic tyramine is reported to decrease SCC and stimulate fluid absorption in the rat jejunum (Munday *et al*, 1980; Tapper *et al*, 1981; Young and Levin, 1990). It has also been shown that K^+-depolarization of the rat colonic mucosa *in-vitro* can evoke [^{3}H]-noradrenaline release (Wu and Gaginella, 1980 and 1981b).

Perdue and Davison (1985) have reported that electrical transmural stimulation of the guinea-pig jejunum *in-vitro*, releases a non-adrenergic,

non-cholinergic endogenous neurotransmitter which increases SCC. Electrical field stimulation increases SCC and induces or increases chloride secretion without significantly altering net sodium absorption in the mouse (Carey and Cooke, 1984), guinea-pig (Cooke *et al*, 1983b), and rabbit ileum (Hubel, 1978), but the human caecum does show a reduction in sodium absorption with a concomitant stimulated chloride secretion (Hubel *et al*, 1987). The human ileum (Hubel and Shirazi, 1982), sigmoid colon (Hubel *et al*, 1983 and 1987) and guinea-pig proximal colon (Kuwahara and Radowicz-Cooke, 1988) respond to electrical field stimulation by an inhibition of chloride absorption, whilst the human transverse colon shows no change in transport function. Furthermore it has been shown that electrical field stimulation resulting in secretory or antiabsorptive responses of the guinea-pig ileum, human ileum and colon, are caused in part by acetylcholine, however some other unknown agent also contributes to these responses (Hubel, 1984; Hubel *et al*, 1987). Electrical field stimulus-induced changes in SCC are calcium dependent and can be prevented by the addition of cadmium and elevated levels of magnesium, which interfere with neurotransmitter release (Cooke *et al*, 1983b; Hubel, 1978; Hubel and Callanan, 1980). The chloride secretory responses to electrical field stimulation can be abolished by prior depolarization of neurones with scorpion venom or high concentrations of extracellular potassium or veratrine (Cooke *et al*, 1983b; Hubel, 1981 and 1983).

The fact that nerve fibers can be found in association with numerous endocrine cells distributed throughout the intestinal tract (Table 1) leaves the possibility that neural stimulation may release messengers from these cells either (i) locally into the interstitial space, (ii) into the blood, or (iii) into the intestinal lumen (Lundberg *et al*, 1978; Wade and Westfall, 1985). Of the intestinal hormones and autocoids found in the intestine, many act as secretagogues (Barbezat and Reasbeck, 1981; Donowitz *et al*, 1980b; El Masri *et al*, 1977; Helman and Barbezat, 1977; Hicks and Turnberg, 1973; Hubel, 1984; Kachel *et al*, 1984; Kachur *et al*, 1982; Mitchenere *et al*, 1981; Wade and

Wood, 1988).

Functional studies have also suggested a role for the local autocoids histamine, bradykinin and prostaglandins in the modulation of intestinal electrolyte and water transport. The local autocoid histamine has been reported to evoke secretory responses in the small intestine of the dog (Lee and Silverberg, 1976), rabbit (Fromm and Halpern, 1979; Linaker *et al*, 1981), guinea-pig (Cooke *et al*, 1984b) and rat (Hardcastle and Hardcastle, 1987).

TABLE 1: Hormonal or putative paracrine messengers identified in enteroendocrine cells (Cooke, 1986).

Furthermore histamine evoked secretion has also been observed in the rat proximal colon (Hardcastle and Hardcastle, 1988) and in a human colonic T_{84} cell line (Wasserman *et al*, 1988). The kinin bradykinin is reported to evoke electrogenic chloride secretory responses in the rat jejunum, proximal colon

(Hardcastle *et al*, 1978), distal colon (Cuthbert and Margolius, 1982; Diener *et al*, 1988a; Perkins *et al*, 1988; Phillips and Hoult, 1988), guinea pig ileum and rabbit distal colon (Musch *et al*, 1983; Phillips and Hoult, 1988). Diarrhoea is one of the major side effects occuring in patients treated with prostaglandins of the E series (PGE) and stable analogs (Konturek and Pawlik, 1986). Nakaki *et al*, (1982c) have reported that PGE_1 induced a fluid secretion in the rat jejunum, whilst Diener *et al*, (1988b) have observed that PGE_2 and the stable prostacyclin derivative iloprost, evoke a chloride secretion in the rat distal colon. Furthermore Hill *et al*, (1988) have suggested that PGD_2 has an opposite action than PGE_2 on the rat distal colon, ie PGD_2 acts to inhibit chloride secretion.

Other neuroendocrine secretagogues include; γ-aminobutyric acid, ATP/adenosine, bombesin (ileum only), cholecystokinin, gastric inhibitory peptide, gastrin, glucagon, 5-HT, motilin, neurotensin, pancreatic polypeptide (ileum only), secretin, substance P, thyrotropin releasing hormone and vasoactive intestinal polypeptide. Other neuroendocrine agents which promote absorption include; dopamine, enkephalins, neuropeptide Y, pancreatic polypeptide (jejunum and colon only), peptide YY and somatostatin (Cox *et al*, 1988; Fondacaro, 1986; Gaginella, 1984; Hubel, 1985; Tapper, 1983; Turnberg, 1983 and 1984).

The physiological significance of many of the neurotransmitters and neuromodulators in the enteric nervous system, is poorly understood. It is however believed that the enteric nervous system in association with local autocoids provide an intricately fine control of the electrolyte transport within distinct areas of the gastrointestinal tract.

5. SECRETORY STIMULI AND THE EFFECTS ON INTESTINAL ELECTROLYTE TRANSPORT.

A large number of secretagogues have been shown to stimulate active intestinal chloride secretion and/or inhibit sodium chloride absorption.

Interestingly in the rabbit, the distal colon responds to secretagogue stimulation, by chloride secretion (Frizzell and Heintzek, 1979), whereas the proximal colon responds by inhibition of electrically silent sodium chloride absorption (Sellin and DeSoignie, 1984).

During the intestinal secretory process there appears to be raised levels of cAMP and intracellular calcium (Berridge, 1979; Frizzell and Heintze, 1979; Frizzell, 1977) (refer to Section 2).

Active potassium secretion in the rat proximal colon can also be elicited by raised intracellular cAMP (Foster *et al*, 1983), whereas β-adrenoceptor stimulation by adrenaline produces a similar response in the rabbit distal colon, whilst also inhibiting active chloride secretion (Halm *et al*, 1983; Halm and Frizzell, 1986; Smith and McCabe, 1986). Stimulation of secretion by β-adrenoceptor agonists, without concomitant chloride secretion presents a pharmacological paradox, since the intracellular mediator of such β-adrenoceptor stimulation is cAMP. Two explanations are possible; i) intracellular cAMP is compartmentalised, or ii) cAMP is not the actual mediator of potassium and chloride secretion (Halm and Frizzell, 1986).

5-HT is reported to act as a secretagogue in the rabbit ileum *in-vivo* (Donowitz *et al*, 1977), and to increase SCC in the rabbit ileum *in-vitro* (Donowitz *et al*, 1979). 5-HT is located in both the enterochromaffin cells and in some neurones of myenteric plexus in the gastrointestinal tract (refer to Sections 1 and 4). Activation of intramural reflexes are thought to constitute a major proportion of the stimulation of secretory processes in response to cholera toxin and some other secretagogues, whilst the remaining fraction is mediated by a direct action upon the enterocytes (Cassuto *et al*, 1981a, 1981b, 1982a, 1982b, 1982c and 1983). These workers have suggested that the neural effect to cholera toxin in cats and rats is mediated by the release of 5-HT from mucosal endocrine cells, which then depolarizes afferent nerves of a reflex circuit that eventually release secretory neurotransmitters. Although the

cholera toxin evoked responses were antagonised by the ganglionic nicotinic-receptor antagonist, hexamethonium, they were unaffected by atropine, suggesting a nicotinic cholinergic link but no involvement of muscarinic cholinoceptors. In contrast Hardcastle *et al* (1981), were unable to antagonise the secretory SCC response to 5-HT in the rat jejunum, with either atropine or hexamethonium, suggesting no cholinergic link. Keast *et al* (1985), reported that 5-HT or electrical field stimulation in the guinea-pig small intestine increases SCC. Furthermore they observed that electrical field stimulation or high concentrations (>5 X 10^{-7} M) of 5-HT stimulated both cholinergic and non-cholinergic nerves, whilst lower concentrations of 5-HT (10^{-8} - 5 X 10^{-7} M) preferentially stimulated non-cholinergic nerves, and that the nicotinic ganglionic stimulant 1, 1-dimethyl-4-phenylpiperazinium (DMPP) preferentially stimulated cholinergic nerves. In contrast Cooke and Carey (1985), suggested that the secretory response evoked by electrical field stimulation in the guinea-pig ileum, is not mediated by release of 5-HT from submucosal motor neurones nor from enterochromaffin cells.

Baird and Cuthbert (1987) have suggested that 5-HT evoked secretory responses in the guinea-pig ileum and colon are mediated through neuronal 5-HT_3-receptor stimulation. Ball *et al* (1988b), have suggested that two types of 5-HT-receptors mediate secretory responses in the rat ileum, high concentrations of 5-HT (>10^{-6} M) stimulate neuronal 5-HT_3-receptors, whilst low concentrations (<10^{-6} M) stimulate non-neuronal benzamide-sensitive 5-HT-receptors which are not 5-HT_1, 5-HT_2 or 5-HT_3-like in nature, similar to the single population of 5-HT-receptors in the rat distal colon which promote secretion (Allbee and Gaginella, 1985; Ball *et al*, 1988a; Zimmerman and Binder, 1984). In contrast Beubler and Burg (1989) have suggested that 5-HT released by cholera toxin in the rat jejunum, does stimulate 5-HT_2 - as well as 5-HT_3 -receptors.

Beubler, *et al* (1986) have proposed that 5-HT secretory responses in the rat small intestine are mediated by an indirect humoral link via the production of PGE_2 which stimulates the epithelial secretory cells directly. They hypothesised that PGE_2 at physiological concentrations mediates intestinal secretion not through cAMP, but through Ca^{2+}. This was based on the evidence of Bukhave and Rask-Madsen (1980), who showed that secretion in human jejunum could be evoked by concentrations of PGE_2 far below those required for activation of adenylate cyclase, and their own observation that the responses of the rat small intestine were verapamil sensitive. It is suggested that PGE_2 may increase calcium entry and that 5-HT may facilitate calcium gating properties.

Donowitz *et al* (1980a and 1980b), have suggested that 5-HT induces changes in the movement of sodium and chloride across sheets of rabbit ileal mucosa, whilst chloride secretion has also been attributed to the 5-HT evoked SCC responses in the rat jejunum (Hardcastle *et al*, 1981) and guinea-pig ileum (Cooke and Carey, 1985). In contrast Sheerin (1979), could not demonstrate any changes in the movement of sodium and chloride across sheets of rabbit ileal mucosa, although a transient increase in SCC was obtained when 5-HT was added.

The local autocoid histamine has been reported to evoke a secretory response in the small intestine of the dog (Lee and Silverberg, 1976). Fromm and Halpern, (1979) reported that histamine-receptor antagonists on the rabbit ileum inhibit residual alkali secretion associated with HCO_3^-, whilst not affecting NaCl transport. In contrast Linaker *et al* (1981) have suggested that histamine in the rabbit ileum inhibited net chloride absorption. Cooke *et al* (1984), using the guinea-pig ileum found that histamine evoked a net chloride secretory response which was partly nerve mediated. However prostaglandin production has been suggested to mediate histamine evoked electrogenic chloride secretory responses in the small intestine (Hardcastle and Hardcastle, 1987) and proximal colon (Hardcastle and Hardcastle, 1988) of the rat. Furthermore Wasserman *et al* (1988), have observed a histamine

stimulated chloride secretion in a human colonic T_{84} cell line. All of the secretory responses evoked by histamine appear to be associated with histamine H_1-receptor stimulation. There are many other endogenous neuroendocrine secretagogues (refer to Section 4).

6. ADRENOCEPTOR SUB-TYPES IN THE CONTROL OF INTESTINAL WATER AND ELECTROLYTE TRANSPORT.

Field and McColl (1973) found that the adrenergic agonists, adrenaline and noradrenaline, evoked concentration dependent decreases in net electrogenic ionic secretion, associated with changes in basal and stimulated SCC in tissues *in-vitro*. These responses were partially antagonised by the non-selective α-adrenoceptor antagonist phentolamine, but not by the non-selective β-adrenoceptor antagonist propranolol. Furthermore the non-selective β-adrenoceptor agonist isoprenaline, evoked a transient increase in SCC. Addition of theophylline, cAMP, or dibutyryl cAMP (Db-cAMP) evoked increases in SCC, but did not prevent the response to noradrenaline. However, the noradrenaline induced SCC response was greater in tissues pretreated with theophylline than those pretreated with Db-cAMP, a result consistent with α-adrenoceptor regulated inhibition of cAMP accumulation. The absence of HCO_3^- and CO_2 from the mucosal Ringer solution abolished the agonists' effects on SCC, whilst their absence from the serosal Ringer solution had no effect. Adrenaline and noradrenaline stimulate net sodium and chloride absorption and decreased residual ion flux, presumably reflecting net HCO_3^- secretion. Subsequent work has confirmed that α-adrenoceptor stimulation increases sodium chloride absorption with a concomitant decrease in HCO_3^- secretion in the rabbit ileum (Dietz and Field, 1973), rabbit proximal colon (Chang *et al*, 1985), and rat colon (Racusen and Binder, 1979). In contrast Smith *et al*, (1985) suggest that in the rabbit ileum adrenaline stimulates sodium-dependent bicarbonate absorption, although

the same net effect is a reduced luminal alkalinization.

A). The Small Intestine.

Hubel (1976) has shown that fluid and sodium chloride absorption is enhanced by noradrenaline in the rat jejunum and ileum *in-vivo*. α_2-Adrenoceptor stimulation in the rabbit ileum *in-vitro*, is reported to stimulate neutral sodium chloride absorption as well as depressing electrogenic Cl^- secretion, whereas α_1-adrenoceptors show no physiological transport function (Chang *et al*, 1982; Dharmsathaphorn *et al*, 1984; Durbin *et al*, 1982). This is in agreement with binding study evidence in the rat ileum and jejunum (Nakaki *et al*, 1982a), rabbit ileum (Chang *et al*, 1983b) and guinea-pig ileum (Tanaka and Starke, 1979). Fondacaro *et al* (1988), have observed that decreases in SCC evoked by the selective α_2-adrenoceptor agonist B-HT 920 in the rabbit ileum, were antagonised by the selective α_2-adrenoceptor antagonist rauwolscine. Furthermore Dettmar *et al* (1986a) and Williams (1986) have also proposed that α_2-adrenoceptors may regulate intestinal electrolyte transport in the rat jejunum *in-vitro*. Cotterell *et al* (1982; 1983 and 1984) however have the contrary view that α_1-adrenoceptors control intestinal transport, based on binding study evidence again in the rat jejunum. It has also been reported that in the rat jejunum *in-vivo*, an α_1-adrenoceptor mechanism mediates increases in basal fluid and electrolyte transport (Levens, 1983; Levens *et al*, 1981b), and reflex increases in fluid absorption in response to haemorrhage (Levens, 1984a) or dehydration (Levens, 1984b). Increases in fluid absorption in response to haemorrhage or dehydration are believed to be mediated through the release of angiotensin, which in turn enhances noradrenaline release from sympathetic nerve terminals, synapsing on α_1-adrenoceptors. Munday *et al* (1980), have shown that the indirect sympathomimetic tyramine can stimulate fluid absorption in the rat jejunum *in-vivo*, and that this response can be antagonised by the

α-adrenoceptor antagonist, phentolamine. Workers using the rat jejunum *in-vitro,* have suggested that a "pool" of noradrenaline in intestinal neurones, released by tyramine, affects intestinal ion transport by stimulating α-adrenoceptors, but does not alter basal ion transport (Tapper *et al,* 1981). Young and Levin (1990) have shown that tyramine will decrease SCC in the fasted rat jejunum, a response suggested to be mediated through release of noradrenaline and α_2-adrenoceptor stimulation, however they proposed that there was little endogenous noradrenaline in the fed jejunum. Secretagogue evoked fluid secretion in the rat jejunum *in-vivo,* has been shown to be reversed by α_2-adrenoceptor stimulation (Bunce and Spraggs, 1983a and 1983b; Nakaki *et al,* 1982b and 1982c). Sjöqvist (1989), has reported that in the rat jejunum naloxone will increase cholera toxin induced secretion, an effect antagonised by phentolamine. Furthermore naloxone-precipitated morphine withdrawal diarrhoea in the rat has been prevented again by α_2-adrenoceptor stimulation (Nakaki *et al,* 1981; Schreier and Burks, 1980). α_2-Adrenoceptor stimulation also induces active transcellular Ca^{2+} transport in the rabbit ileum *in-vitro* (Hyun *et al,,* 1985).

Dopamine is reported to promote fluid absorption, be electrogenically antisecretory and to stimulate active sodium chloride absorption in the ileum of the rabbit (Donowitz *et al,* 1982) and the rat (Donowitz *et al,* 1983). These responses to dopamine were mediated through both stimulation of dopamine-receptors and α_2-adrenoceptors. In the rabbit ileum, dopamine appears to act by lowering Ca^{2+} entry across the basolateral membrane and causes a decrease in total ileal calcium content.

Morris and Turnberg (1981) have proposed the presence of a β-adrenergic drive promoting absorption in the human jejunum and ileum. In contrast Levens (1983) found that the β-adrenoceptor antagonist propranolol stimulated fluid and electrolyte absorption in the rat jejunum *in-vivo,* which agrees with the findings of Donowitz and Charney (1979), that

propranolol inhibited secretagogue evoked secretion in the rat jejunum *in-vivo*. Furthermore it has been proposed that in the rat jejunum *in-vitro*, β_2-adrenoceptors mediate a portion of secretory tone and also increases in secretory processes (Dettmar *et al*, 1986b; Williams, 1986).

B). The Large Intestine.

In-vitro, studies in rat colon suggest that catecholamines affect electrolyte transport directly, in that their effects were not inhibited by tetrodotoxin, reserpine, naloxone, or atropine (Racusen and Binder, 1979). It therefore appears that no known neurotransmitter, opioid, or cholinergic intermediate are involved.

Numerous studies have supported a physiological role for adrenergic regulation of large intestinal electrolyte transport, although there is a lot of uncertainty about the adrenoceptor sub-types, which probably arises from species variation and the heterogeniety of this transporting epithelium. In rat colonic mucosa, noradrenergic nerve endings, identified directly by their uptake of [^{3}H] noradrenaline (Chang, 1988; Wu and Gaginella, 1981a), appeared to be subjacent to the intestinal epithelia. Adrenaline administered to the rabbit colon *in-vitro* (Sellin and DeSoignie, 1984), and to the rat colon *in-vitro* (Racusen and Binder, 1979), stimulates coupled sodium chloride absorption. Furthermore in the rat colon adrenaline evokes an antisecretory decrease in SCC, a response attributed to both α and β-adrenoceptor stimulation. Dharmsathaphorn *et al* (1984), found that decreases in SCC and secretory processes were evoked in the rat colon *in-vitro*, with α_2-adrenoceptor agonists. α_2-Adrenoceptor stimulation is also reported to abolish spontaneous calcium secretion in the rabbit proximal colon *in-vitro* (Hyun *et al*, 1985). Albin and Gutman (1980) found that α-adrenoceptor stimulation in the rabbit distal colon *in-vitro*, enhanced net sodium

absorption through the mucosa. Boige *et al* (1984) have shown that stimulation of α_2-adrenoceptors in human colonic crypts inhibited VIP-induced cAMP accumulation. They suggested that colonic cAMP metabolism undergoes a dual control: VIP-ergic, activator and adrenergic, inhibitor. Furthermore Donowitz *et al* (1983) have found that VIP induced a net fluid and electrolyte secretion in the human colon which was associated with a reduction in active sodium absorption and an enhancement active chloride secretion. In contrast Durbin *et al*, (1982) reported that α_2-adrenoceptor stimulation had no influence on sodium chloride transport in the rabbit colon, which agrees with the findings of Bunce and Spraggs (1983a), who failed to demonstrate any effect of α_2-adrenoceptor stimulation upon secretagogue induced secretion in the rat colon *in-vivo*.

Dopamine is reported to stimulate intestinal water absorption, a response which might be due to stimulation of both dopamine-receptors and α_2-adrenoceptors, however this needs to be confirmed in the colon (Donowitz *et al*, 1983).

Angiotensin is reported to stimulate electroneutral sodium absorption in the rat distal colon *in-vivo* (Levens *et al*, 1981a), whilst also inhibiting VIP evoked fluid secretion in the entire rat colon *in-vivo* (Bhaskar *et al*, 1984). It is suggested that both these responses to angiotensin may be due to enhanced noradrenaline release from sympathetic nerve terminals, synapsing on α-adrenoceptors. Davies *et al* (1972), however have attributed these responses by angiotensin to stimulation of *de novo* protein synthesis.

Coyne *et al* (1974 and 1976), reported that propranolol inhibited bile acid stimulation of rabbit colonic adenylate cyclase *in-vitro*, and furthermore Conley *et al* (1976), have reported that propranolol inhibited adenylate cyclase and secretion by deoxycholic acid in the rabbit colon *in-vivo*. In

contrast Hall *et al* (1981) found that propranolol was ineffective in the treatment of bile acid induced diarrhoea in humans. β_1-Adrenoceptor stimulation in the rabbit distal colon stimulates active potassium secretion (Smith and McCabe, 1986).

It would appear that there is still no clear understanding of the involvement of adrenoceptor mediated mechanisms in the control of small and large intestinal electrolyte and fluid transport.

C). Cellular Mechanisms of Adrenoceptor Regulation of Intestinal Electrolyte Transport.

The cellular mechanisms regulating ion transport mediated by α_2-adrenoceptor stimulation remain ambiguous. Field *et al* (1975), using the rabbit ileal mucosa *in-vitro*, showed that adrenaline elicited no change in tissue intracellular cAMP levels and a small reduction of questionable significance, in tissues pretreated with theophylline, whilst decreases in HCO_3^- or stimulated chloride secretion was observed. However they also showed that adrenaline reduced cAMP levels that were augmented by PGE_1 and cholera toxin, which is consistent with the findings of Birnbaumer *et al*, (1985) and Limbird, (1984), who demonstrated an inhibition of adenylate cyclase associated with α_2-adrenoceptor stimulation. But these latter workers could only show a marginal reduction in intestinal secretion. In contrast Nakaki *et al*, (1982b and 1982c) using the rat jejunum *in-vivo*, found that adrenaline did not reduce PGE_1- or cholera toxin-augmented cAMP levels, although fluid secretion was significantly reduced. It has been suggested that gross intracellular mucosal cAMP levels may not fully reflect small changes in compartmentalized cAMP levels evoked by α_2-adrenoceptor stimulation (Chang *el al*, 1982; Nakaki *et al*, 1982b and 1982c). Furthermore some evidence suggests that α-adrenoceptor agonists may exert their effects distal to cAMP generation (Nakaki *et al*, 1982c).

The relationship between GTP-binding proteins (G-proteins) and adrenoceptor systems has become increasingly important. The transmembrane signal transduction system comprises of three components: receptor (e.g., α_2-adrenoceptor or β-adrenoceptor), transducer (e.g., $G_i[N_i]$, or $G_s[N_s]$), and catalytic subunit (e.g., adenylate cyclase) (Chang, 1988). G_s and G_i are members of a family of G-proteins that act as hormonal receptor transmembrane signal transducers. In many cellular systems, G_i interacts with the α_2-adrenoceptor, whereas G_s interacts with the β-adrenoceptor. Each G-protein is composed of α, β and γ subunits. The specificity of each G-protein appears to depend on the α subunit. Upon receptor activation and GTP binding by the α subunit, the α-β-γ trimer dissociates to α-GTP and β-γ complexes. α_s-GTP stimulates adenylate cyclase, while the β-γ subunits from both G_i and G_s inhibit the catalytic subunit by several mechanisms. After hydrolysis of the α_s-GTP to α_s-GDP, the increased size of the β-γ pool promotes release of the GDP with the subsequent reformation of the α-β-γ trimer (Birnbaumer *et al*, 1985). More recent evidence suggests that the β-γ complex and α_i-GTP may also directly inhibit adenylate cyclase through a separate distinct binding site from that of activated α_s, and through competition for the α_s binding site respectively (Katada *et al*, 1986).

Two enterocyte α_2-adrenoceptor states appear to exist, the low-affinity and the high-affinity (Chang *et al*, 1983b; Cotterell *et al*, 1982; Tanaka and Starke, 1979; Tsai *et al*, 1985). It has been suggested that sodium and GTP convert the receptors from the high-affinity state to low-affinity state (Chang *et al*, 1983b). Work using human platelet membranes show that the high-affinity state may be a stable receptor-G-protein complex, whereas the low affinity state is the receptor dissociated from the G-protein (Limbird, 1984). Thus the binding of the GTP-binding site by GTP destabilizes the receptor-G-protein complex and converts the high-affinity receptor to a low-affinity state. In contrast sodium acts on the receptor directly, reducing its affinity for the agonist. Furthermore

it has been suggested that sodium may also reduce the number of high affinity receptors, but this needs to be fully established (Tanaka and Starke, 1979; Tsai *et al*, 1985).

As previously mentioned stimulation of rat jejunal α_2-adrenoceptors may inhibit intestinal secretion by affecting mechanisms distal to cAMP generation (Nakaki *et al*, 1982b and 1982c). These workers showed that clonidine inhibited Db-cAMP-induced secretion *in-vivo*, but PGE_1- and cholera toxin-induced secretion was also inhibited without concomitant alterations of mucosal cAMP levels. Field *et al* (1975) have also suggested that other mechanisms may exist for ion transport regulation by the α_2-adrenoceptors independent of cAMP. So there is the possibility that α_2-adrenoceptors may couple to other effector systems in addition to adenylate cyclase (Limbird, 1984). Many workers have hypothesised that stimulated α_2-adrenoceptors may interfere with the mobilization of intracellular free calcium (Ca_i). Chang *et al* (1984), have reported that the secretory effects of the Ca_i-dependent agonists carbachol, substance P, and calcium ionophore A23187, in the rabbit and chicken ileum *in-vitro*, were inhibited by a prior addition of adrenaline, whilst cytosolic Ca_i was unaltered by the pretreatment. It was therefore suggested that α_2-adrenoceptor agonists interfere with calcium-dependent mechanisms but do not alter calcium entry or free cytosolic calcium.

McRoberts *et al* (1985), have shown that there are cAMP- and Ca^{2+}-dependent K^+ channels located in the basolateral membranes of cells derived from a human colonic carcinoma cell line (T84). Recently G-proteins in several receptor and tissue systems have also been implicated as direct regulators of Ca^{2+} and K^+ ion channels (Breitwieser and Szabo, 1985; Hescheler *et al*, 1987). G-proteins may be involved in α_2-adrenoceptor regulation of intestinal ion secretion, however this needs to be established. It is therefore possible, that α_2-adrenoceptors regulate intestinal ion transport through several possible mechanisms: 1) by inhibiting adenylate cyclase via

activation of G_i, 2) by inhibiting a Ca^{2+} -dependent system, 3) by inhibiting apical Cl^- conductance, and 4) by a direct coupling of G-proteins to ion channels.

7. CLINICAL IMPLICATIONS OF THE PHARMACOLOGICAL MANIPULATION OF THE CONTROL OF INTESTINAL ELECTROLYTE AND FLUID TRANSPORT.

The manipulation of the control mechanisms influencing intestinal secretion and absorption has obvious therapeutic potential in the treatment of diarrhoeal diseases especially in "third world countries" where associated childhood mortality is high (Holmgren and Svennerholm, 1982), and in the treatment of constipation, both conditions also being a major source of morbidity in western society. The condition of cystic fibrosis has also been associated with a dysfunction of epithelial sodium chloride and fluid secretion resulting in a dehydration of the intestinal mucus layer (Taylor *et al*, 1988). It has been suggested that the dysfunction is due to insensitivity of epithelial chloride channels.

The most commonly used first line antidiarrhoeal therapeutic treatments, are oral rehydration solutions and opiate based compounds, however the mechanism of the antisecretory effects of the latter are still poorly understood. It was originally thought that opiates exerted their antidiarrhoeal effects by an inhibition of gastrointestinal motility, but it is now hypothesised that they act directly to depress intestinal secretory processes (Kachur *et al*, 1980) and, for those which can penetrate the blood brain barrier, by central stimulation of efferent sympathetic activity (Brown and Miller, 1984). The use of opiate therapy can sometimes lead to social and physical problems such as addiction and tolerance, which limits their use. Oral rehydration solutions often contain glucose which stimulates small intestinal glucose coupled sodium absorption. The pathophysiology of constipation is unclear and therapy is often indirect, commonly involving the use of bowel stimulants or bulking

agents. Idiopathic constipation has been associated with reduced activity of colonic cholinergic nerves (Burleigh, 1988). The development of more selective compounds for the treatment of secretory diarrhoea and constipation is an area which invites further exploration.

Evidence is now available to suggest that α-adrenoceptor compounds may possess a considerable therapeutic potential for managing intestinal transport dysfunction. α-Adrenoceptor agonists could possibly have a wide spectrum of action in that they may be effective against many secretagogues having different mechanisms of action (e.g., mediation through cAMP, Ca^{2+}, etc.).

Several authors have confirmed that α-adrenoceptor stimulation also alters intestinal motility and transit time, but questions still remain regarding the central versus peripheral sites of action of anti-motility responses to α-adrenoceptor agonists (Doherty and Hancock, 1983; Galligan and Burks, 1981; Chang, 1988). In contrast Greenwood *et al* (1987) suggested that separate but parallel neural mechanisms control the smooth muscle and epithelial responses of the rabbit ileum; the inhibition of motility is mediated by β-adrenoceptors, while α-adrenoceptors alter fluid and electrolyte transport.

Clinical trials using small patient numbers have shown that clonidine and other α_2-adrenoceptor agonists may help patients suffering from diabetic diarrhoea. Goff (1984) reported that the α_2-adrenoceptor agonist, lidamidine, in three patients with chronic, poorly controlled diabetic diarrhoea and varying degrees of steatorrhoea, significantly decreased the frequency of bowel movements (30-60%) and improved stool consistency, with minimal adverse side-effects. Furthermore Fedorak *et al* (1985) investigated the effects of clonidine, again in three patients with diabetic diarrhoea. There was an improvement in stool consistency and a significant reduction in stool volume, although bowel movement frequency did not decrease, clustering of

bowel movements did occur. Stool volume increased when the drug was discontinued, but decreased again when the therapy was reinstated. There were no orthostatic hypotensive side effects in these patients, which was probably due to the presence of severe autonomic neuropathy. Diabetic diarrhoea is suggested to occur when the sympathetic nervous system in the intestine fails, allowing the parasympathetic nervous system's antiabsorptive effects to predominate (refer to Section 3).

Some evidence exists suggesting that α_2-adrenoceptor agonists are also beneficial in the management of other forms of secretory diarrhoea such as that associated with bronchogenic carcinoma (McArthur *et al*, 1982). Both lidamidine and clonidine significantly decreased total stool weight by 35% and 53% respectively, in one patient with high VIP levels in the tumor. However it is unclear whether the diarrhoea was secondary to VIP since serum levels were not elevated.

Diarrhoea is often associated with opiate withdrawal (Henry, 1974; Jaffe, 1980). Brown and Miller (1983 and 1984) have shown that the stable enkephalin analog, [D-Ala^2, Met^5] enkephalinamide (DAMA), exerts an antisecretory action in rats, partially via sites within the central nervous system. Furthermore they found that depletion of the sympathetic nerve terminals of the intestine by pretreatment with guanethidine blocked the centrally mediated antisecretory effects of DAMA, as did antagonism by phentolamine. These results suggest that opiates may exert some of their effects via the central and sympathetic nervous systems. The diarrhoea associated with opiate withdrawal may be a result of gastrointestinal motility disturbances (Ehrenpreis *et al*, 1975; Kramer and Woinoff, 1980), as well as alterations in ion transport (Chang *et al*, 1983b). The relative contributions of the motility and secretory mechanisms in stimulating the diarrhoea have yet to be fully established. Clinical trials of clonidine in small numbers of narcotic-dependent patients have suggested that α_2-adrenoceptor agonists

may help to reduce the symptoms of opiate withdrawal, such as abdominal pain and diarrhoea (Gold *et al*, 1978 and 1980).

Unfortunately unpleasant and intolerable side effects such as orthostatic hypotension, lethargy, and α_2-adrenergic withdrawal syndromes, have been associated with treatment of diarrhoea with α_2-adrenoceptor agonists. Obviously the development of greater gastrointestinal selectivity would be advantageous in reducing the unwanted side effects. To this end Dharmsathaphorn *et al* (1984) looked at the structure activity relationships of different imidazoline derivatives, and found that various substitutions on the phenyl ring (e.g., halide) increased agonist activity, while methoxy substitutions were related to gut verses brain selectivity.

CHAPTER 2: METHODS AND MATERIALS.

1. THE MEASUREMENT OF ELECTROGENIC ION TRANSPORT IN THE RAT INTESTINE *IN-VITRO*.

A). The Ussing Chamber Technique And The Measurement Of Transepithelial Potential Difference And Short-Circuit Current.

The Ussing chamber technique was originally used to investigate transepithelial ion fluxes and transepithelial electrical parameters across sheets of isolated frog skin (Ussing and Zerahn, 1951). The technique was modified later to measure the same parameters across isolated intestinal sheets (Schultz and Zalusky, 1964).

In the present investigation the modified Ussing chamber technique was adapted to measure changes in transepithelial potential difference (PD) and short-circuit current (SCC) across sheets of isolated rat jejunum and distal colon. The technique involved mounting the sheet of tissue vertically between two half chambers, the exposed area being 0.64 cm^2. The volume of solution in each half chamber was adjusted separately to avoid any hydrostatic pressure difference across the intestinal sheet. Each side of the tissue was oxygenated and circulated by a bubble lift mechanism. Both the half chambers were surrounded by water jacketed reservoirs which maintained the bathing solution temperature at 37 ± 0.5°C. A diagrammatic representation of the Ussing chamber used is illustrated in figure 7.

Transepithelial PD was measured through narrow tipped salt agar bridges situated close to either side of the tissue. These contained 4% agar in physiological salt solution of the same ionic composition (refer to Section B) as the solution bathing the tissue. The agar bridges were connected to a high impedence digital voltmeter and an automatic voltage clamp, via

silver/silver chloride electrodes. Prior to tissue mounting the combined electrode and bridge potential was backed off using a potentiometer. The automatic voltage clamps were built in the department by D. Briggs and were based on a modification of the design described by Rothe *et al* (1969). A comprehensive description of there operation and design by D. Briggs is included in appendix I. Fluid resistance was determined prior to tissue

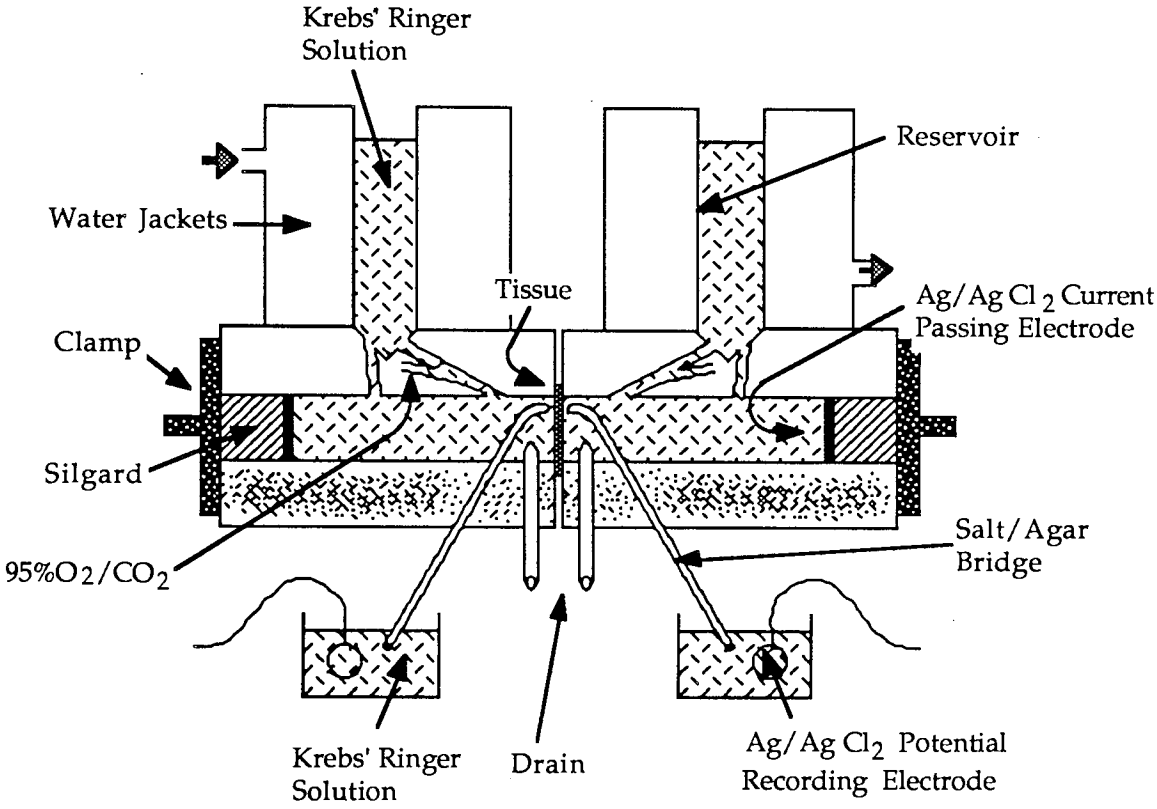

FIGURE 7: A diagrammatic representation of the Ussing chamber used in the present investigation. The equipment comprised of two interlocking perspex half chambers with bathing fluid reservoirs surrounded by water jackets (refer to text for details).

mounting by monitoring voltages generated by current flow through the physiological salt solution between the bridge tips. Current flow was induced using a hand adjusted potentiometer which passed a varying current through the bath. A multiturn potentiometer which was built into the SCC circuit, was then adjusted to cancel out voltages injected into the bath, and so the proportion of the voltage signal resulting from fluid resistance was exactly

cancelled out. The automatic voltage clamp was used to apply a current across the tissue which was equal and opposite to the spontaneous tissue PD. Under SCC conditions transepithelial PD produced by a particular ion flux in the bathing media is abolished. The recorded SCC therefore corresponds to the sum of the net fluxes of those ions which are electrogenically transported. Changes in SCC evoked by pharmacological agents therefore relate to changes in electrogenic ion transport. SCC was displayed on a Teckman flatbed recorder.

B) The Preparation Of The Isolated Jejunal Sheets.

Male Wistar rats of weight range 200-240g were killed by cervical dislocation and exsanguination. The abdomen was opened by a midline incision and a 2-3 cm segment of jejunum was taken from an area 20-30 cm from the pyloric sphincter. The segment was flushed through with Krebs' Ringer solution (composition mM: NaCl 118.3, KCl 4.7, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ $7H_2O$ 1.2, $CaCl_2$ 2.5 and glucose 10), which was gassed with 5% CO_2 in O_2. The jejunal segment was then slid over a glass rod (diameter 4 mm) and ligated at either end with cotton ligatures. A partial thickness incision was made next to the remaining mesentery using a blunt scalpel blade, and the outer muscle layers were stripped away with fine forceps. The stripped jejunal segment was removed from the glass rod, cut along its length and mounted between two Ussing-type half chambers, the exposed area being 0.64 cm^2. The muscle layers were removed to reduce tissue resistance which was not due to the epithelium and also to improve oxygenation of the serosal side of the tissue. To minimize deterioration of the preparations during the dissection, only one preparation was used from each rat.

The mucosal and serosal surfaces of the tissue were bathed in Krebs' Ringer solution which was oxygenated and circulated by a gas lift and

maintained at 37 ± 0.5°C by water jacketed reservoirs. Normal Krebs' Ringer solution was present in the serosal bathing medium, whereas the mucosal bathing medium contained 10 mM mannitol replacing glucose. This reduced the contribution of glucose-coupled electrogenic Na^+ transport to the tissue's spontaneous PD. All bathing solutions contained 10^{-5} M EDTA and 10^{-4} M ascorbic acid, to reduce the degradation of noradrenaline. Both these agents were without effect upon SCC at the concentrations stated.

C) The Preparation Of The Isolated Distal Colonic Sheets.

Male Wistar rats of weight range 300-320g were starved over-night and anaesthetised with pentobarbitone sodium (60 mg/Kg; i.p.). Starvation for 24 hours is reported to have little effect upon intestinal electrolyte transport (Young and Levin, 1990). The abdomen was opened by a midline incision and a 2-3 cm segment of distal colon was taken from an area 4-5 cm from the anus. The colonic segment was flushed through with Krebs' Ringer solution (composition; refer to Section B) at room temperature, gassed with 5% CO_2 in O_2. The colon was slid over the narrow terminal of a tapered glass rod (diameter range 4-8 mm). Then the colonic segment was gently slid towards the thick terminal of the glass rod until the tissue was stretched taut, and secured with cotton ligatures. The outer muscle layers were stripped away and the colonic sheet was mounted in the Ussing chamber, as described previously for the jejunum. To minimize deterioration of the preparations during the dissection, only one preparation was used from each rat. The mucosal and serosal tissue surfaces were bathed in normal Krebs' Ringer solution which was again oxygenated and circulated by a gas lift, and maintained at 37 ± 0.5°C by water jacketed reservoirs. All bathing solutions contained 10^{-4} M ascorbic acid and 10^{-5} M EDTA., to reduce the degradation of noradrenaline.

2. THE MEASUREMENT OF RAT COLONIC ELECTROLYTE AND FLUID TRANSPORT *IN-VIVO*.

A) Operative Procedure.

Male Wistar rats of weight range 250-300g were starved for 24 hours, whilst having free access to drinking water containing 4 % Liquid Lectade® electrolyte supplement. Anaesthesia was induced with pentobarbitone sodium (60 mg/Kg; i.p.), which is reported to be the most suitable anaesthetic for absorption/secretion studies (Coupar, 1985). Body temperature was maintained at 37°C by means of a thermostatically regulated heat generating

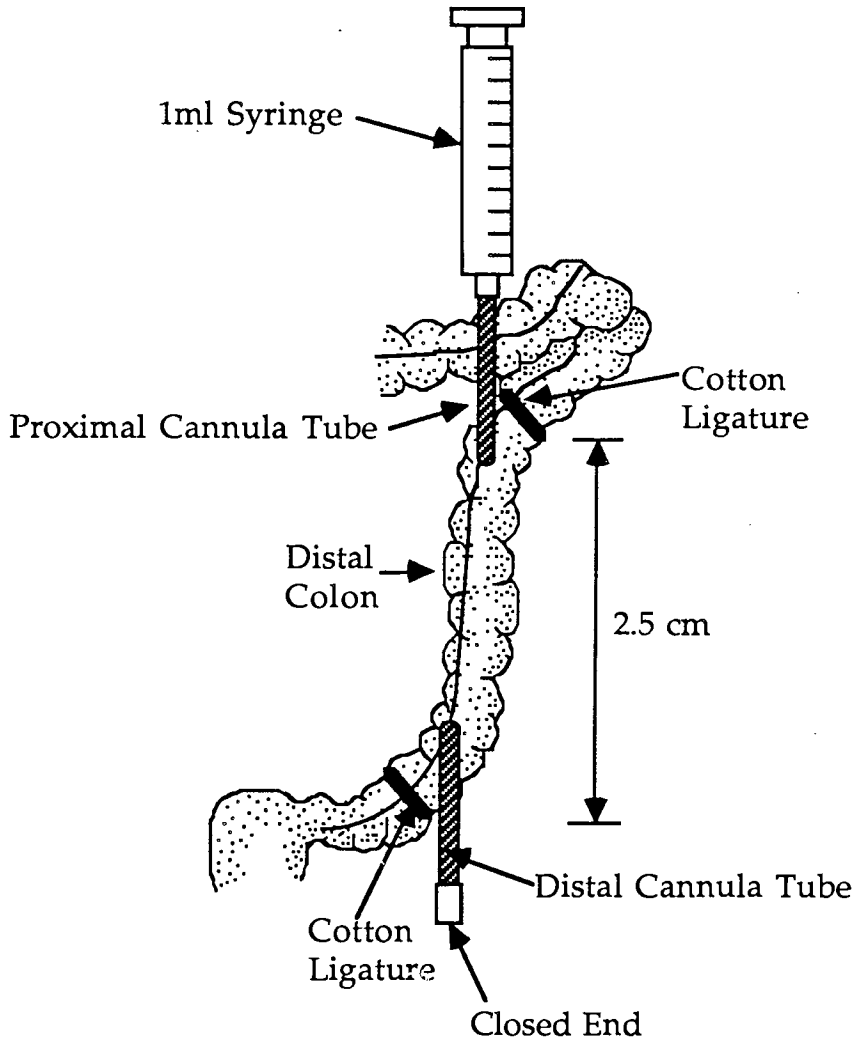

FIGURE 8: A diagrammatic illustration of the cannulated sealed distal colonic loop of the rat *in-vivo*.

lamp. The trachea was intubated, and the left carotid artery and left femoral vein were cannulated. Anaesthesia was maintained by an i.v. infusion of 6 mg/Kg/hour pentobarbitone sodium, using a slow infusion pump (Scientific & Research Instruments Ltd.), set at an infusion rate of 1.5 ml/hour. The abdomen was opened by a midline incision, and a 2.5 cm distal colonic loop was prepared by ligating the colon 4-5 cm and 6.5-7.5 cm from the anus. Figure 8 illustrates a diagrammatic representation of the completed operation. Extreme care was taken to maintain the enteric innervation and vascular supply to the isolated colonic loop. The loop was then cannulated at the proximal terminal and a small hole made near the distal terminal. The section was rinsed out of any contents by introducing 5 ml of 300 mM isotonic mannitol at 37 ± 0.5°C, via the proximal cannula. Following this, any residual fluid was removed by flushing the section out with 5 ml of air. Once the loop was cleaned of any excess debris or fluid, a second cannula was introduced and secured into the small hole at the distal terminal of the section. This distal cannula could be sealed during the experimental incubation period, but could be reopened at the end of such a period, to act as a drain for the loop. A 1 ml syringe was used to introduce 0.25 ml of electrolyte solution of known composition and concentration. At the end of the incubation period the contents of the lumen was collected for analysis by opening the distal cannula and injecting air into the proximal cannula to drain the colonic loop.

B). The Measurement Of Fluid Transport In The Rat Distal Colonic Loop.

The colonic loop was prepared as described previously. At time zero 0.25 ml of isotonic electrolyte solution (composition mM: NaCl 145 and KCl 5) was introduced into the colonic loop. The colon was then sealed and left for 1 hour time period, within which any drugs to be tested were infused via the femoral vein. After the 1 hour incubation period the content of the lumen

was collected into an Epidorth centrifuge tube and weighed using a Oertling HB63 balance. The samples were then centrifuged at 1000g using a Eppendorf Zentrifuge 3200 bench centrifuge for 10 minutes, after which they were analysed for electrolyte concentration as described in Section 2C, following this they were freeze-dried using a Virtis Unitrap II freeze dryer. Once the sample tubes were fully dry they were once again weighed and the weight of the electrolyte solution was calculated, accounting for the weight of any solid debris. The volume of each sample was calculated gravimetrically with the assumption that 1 ml of fluid is equivalent to 1 g. Since the initial electrolyte sample volume was known the change in volume could be calculated and expressed as a function of time.

Under basal conditions it was observed that there was negligible recordable mucus in the samples collected from the lumen of the colon. However in those experiments which had the secretagogue PGE_2 present in the lumen, there was a large stimulation of mucus secretion. This mucus in secretagogue treated rats could introduce an error in the gravimetric determination of fluid volume, and so with these experiments the mucolytic N-acetyl-L-cysteine (1% w/v) (Norris *et al*, 1983) was added to the fluid samples after collection, and incubated for 15 minutes before centrifugation, in order to degrade the mucus.

C). The Measurement Of Electrolyte Transport In The Rat Distal Colonic Loop.

After the centrifugation stage in the sampling procedure described in Section 2B above, the sodium and potassium concentration in the samples were determined using an Instrumentation Laboratory 943 flame photometer. The total amount of a particular ion was calculated by multiplying the concentration of that ion by the lumenal fluid volume determined after the incubation period. The chloride concentration was determined using a

Corning Chloride Analyzer 925. Unfortunately in those samples which had the mucolytic, N-acetyl-L-cysteine present, the chloride concentration could not be determined because of lack of specificity of the chloride analyzer, which also titrated the N-acetyl-L-cysteine along with chloride ions.

3. STATISTICAL ANALYSIS.

Results have been expressed as a mean ± standard error of the mean (SEM) and statistical comparisons have been made by application of an unpaired Student's t-test ($P<0.05$ being taken as the limit of significance). Comparisons between control and test results were as indicated in the text. In experiments where SCC was elevated by theophylline, responses to each α-adrenoceptor agonist was expressed as a percentage of the maximum response obtained to that agonist in each preparation, and EC_{50} values were derived graphically from log-concentration response curves and have been expressed as the geometric mean with 95% confidence limits. Agonist concentration ratio values were determined from the EC_{50} values in the presence and absence of each concentration of antagonist, and plots were constructed of the logarithm of (concentration-ratio - 1) against the negative logarithm of the molar concentration of the antagonist according to the method of Arunlakshana and Schild (1959). Antagonism was considered to be competitive if the 95% confidence limits for the slope of the Schild plot, drawn by linear regression, overlapped unity. In those instances when the α-adrenoceptor blockade was found to be competitive, K_B values were also calculated at each antagonist concentration by the dose ratio method of Furchgott (1972).

4. MATERIALS.

The compounds used in the present study were obtained from the following sources:-

Acetazolamide	- Sigma
Adrenaline hydrogen tartrate	- BDH
Amiloride hydrochloride	- Sigma
Atropine sulphate	- BDH
BRL 24924	- Beecham
Carbamylcholine chloride (Carbachol)	- BDH
CH 38083	- Chinoin
Cirazoline hydrochloride	- Synthelabo
Corynanthine hydrochloride	- Sigma
1,1-Dimethyl-4-phenyl-piperazinium iodide (DMPP)	- Sigma
5-Hydroxytryptamine creatine sulphate	- Sigma
ICI 118,551	- ICI
ICS 205,930	- Sandoz
Idazoxan	- Reckitt & Colman
Isoprenaline sulphate	- Sigma
Liquid Lectade ®	- Beecham
Methysergide hydrogen malate	- Sandoz
Metoprolol	- Geigy
N-acetyl-L-cysteine	- Sigma
Noradrenaline bitartrate	- Sigma
Phenylephrine hydrochloride	- Sigma
Piretanide	- Hoechst
Prenalterol	- Astra
Prazosin hydrochloride	- Pfizer
Propranolol hydrochloride	- Sigma
Prostaglandin E_2	- Sigma
Salbutamol	- BDH
Reserpine	- Sigma
Tetrodotoxin	- Sigma
Theophylline hydrate	- BDH

Timolol maleate	- Sigma
UK-14,304	- Pfizer

All concentrations referred to in the text are expressed as the base. Stock solutions of drugs were freshly prepared in distilled water and then diluted with in the appropriate Krebs' bicarbonate Ringer solution, except the following: Prazosin (10^{-3} M) was prepared in 5% glucose/5% glycerol, and then diluted in Krebs' bicarbonate Ringer solution. Amiloride (10^{-2} M), acetazolamide (10^{-2} M) and theophylline (10^{-2} M) were prepared in Krebs' bicarbonate Ringer solution. Piretanide (10^{-2} M) was prepared in Krebs' bicarbonate Ringer solution at 37° C.

RESULTS CHAPTER 3.

α_2-ADRENOCEPTOR CONTROL OF SECRETAGOGUE AUGMENTED SHORT-CIRCUIT CURRENT IN THE RAT JEJUNUM *IN-VITRO*.

1. INTRODUCTION.

α_2-Adrenoceptor agonists have been reported to to reverse secretagogue-induced fluid secretion but have no effect on basal levels of fluid absorption in the rat jejunum *in-vivo* (Bunce and Spraggs, 1983a and 1983b; Nakaki *et al*, 1982a and 1982b). Radioligand binding studies have revealed the presence of both α_2-adrenoceptors (Chang *et al*, 1983b; Cotterell *et al*, 1984; Nakaki *et al*, 1983) and α_1-adrenoceptors (Cotterell *et al*, 1984) on basolateral membranes of mucosal cells.

Cotterell *et al* (1983), and Parsons *et al* (1983), have reported a stimulation of fluid absorption in everted sacs of rat jejunum following α_1-adrenoceptor activation by noradrenaline. However, Dettmar *et al* (1985), were unable to identify the receptor mechanism involved in the stimulation of absorption by noradrenaline in the rat jejunum, since the response was resistant to antagonism of both α_1- and α_2-adrenoceptors.

It has been reported that α_2-adrenoceptor stimulation in the rabbit ileum *in-vitro*, stimulates neutral sodium chloride absorption as well as depressing electrogenic Cl^- secretion (Chang *et al*, 1982; Dharmsathaphorn *et al*, 1984; Durbin *et al*, 1982; Fondacaro *et al*, 1988). Furthermore Dettmar *et al*, (1986a) have suggested that α_2-adrenoceptors may have a regulatory role over basal electrolyte transport in the rat jejunum *in vitro*.

The primary driving force for fluid absorption in the mammalian small

intestine is the active transport of Na^+ and Cl^- which may be electrically silent (Frizzell *et al*, 1979) or which may involve electrogenic Na^+ transport (Esposito, 1984). In addition, there is a well documented active secretion of Cl^- accompanied by Na^+ and water (Rao and Field, 1983). Thus net fluid and electrolyte movement is the balance of absorptive and secretory influences (refer to Chapter 1, Section 2).

In the present investigation the Ussing chamber technique (refer to Chapter 2, Section 1) was used to determine the role of α-adrenoceptors in the regulation of jejunal electrolyte transport, after secretagogue challenge, since α-adrenoceptor activation in this tissue is reported to be anti-secretory upon basal ion transport (Dettmar *et al*, 1986a).

2. THE EFFECT OF THEOPHYLLINE UPON SCC.

It is well documented that secretagogues stimulate electrogenic Cl^- secretion and therefore increase transepithelial PD and SCC in the small intestine.

In the present investigation theophylline was used to elevate SCC in the rat jejunum. Theophylline inhibits the phosphodiesterase enzyme which cleaves cAMP, thus elevating intracellular cAMP levels which in turn elicits Cl^- secretion (Frizzell and Schultz, 1979; Rao and Field, 1983). Preliminary experiments showed that a bilateral addition of theophylline to the rat jejunal sheets evoked a concentration dependent increase in SCC (figure 9). Theophylline (4×10^{-3} M) produced a maximum elevation of SCC, and was therefore used in the following experiments to raise SCC, 30 minutes after tissue mounting. The total SCC in the presence of 4×10^{-3} M theophylline was 77 ± 4 μ Amps/cm^2, n=5, compared with a basal level of 25 ± 2 μ Amps/cm^2, n=5.

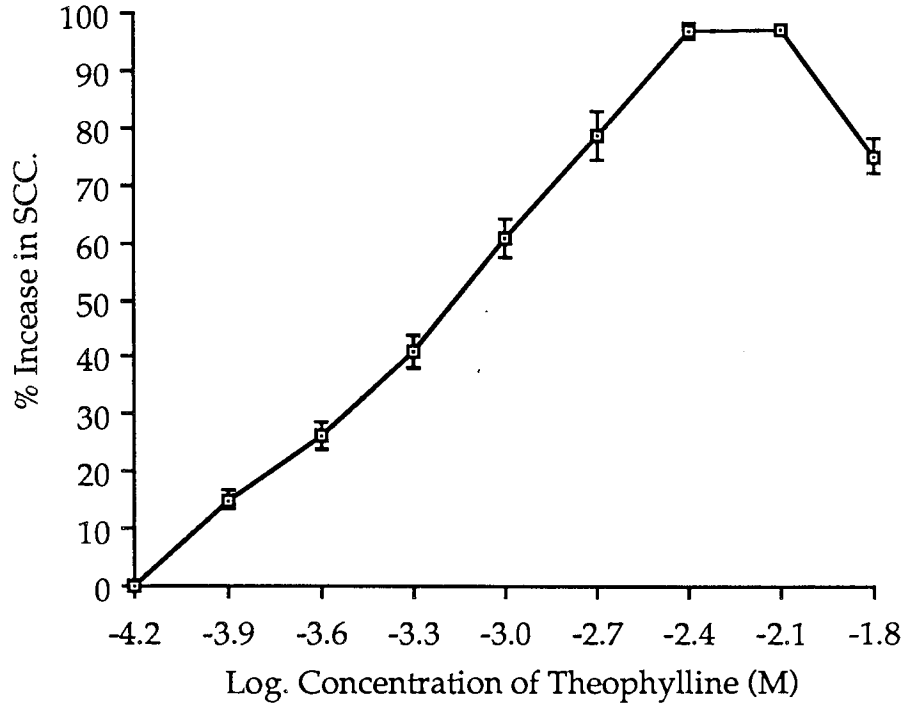

FIGURE 9: A log. concentration-percentage-response curve for theophylline with respect to increasing SCC in the rat jejunum *in-vitro*. Each point represents the mean of five experiments and the vertical bars indicate the standard error of the mean (SEM).

Preliminary investigations showed that the increase in SCC evoked by theophylline (4 X 10^{-3} M) was resistant to a subsequent addition of either the sodium transport inhibitor amiloride (10^{-3} M) (Cuthbert *et al*, 1979) or the carbonic anhydrase inhibitor acetazolamide (10^{-3} M) (Waygood, 1955), but addition of the chloride uptake inhibitor piretanide (10^{-3} M) (Zeuthen *et al*, 1978) completely reversed the response (figure 10), which supports the hypothesis that theophylline increases SCC by a stimulation of electrogenic Cl^- secretion. These concentrations of amiloride, piretanide and acetazolamide have been used to study ion transport (Baird and Margolius, 1987).

piretanide (10^{-3} M).

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3. THE EFFECT Of NORADRENALINE, UK-14,304 AND PHENYLEPHRINE UPON THEOPHYLLINE AUGMENTED SCC.

It was considered essential to investigate the effects of the non-selective adrenoceptor agonist, noradrenaline, the selective α_2-adrenoceptor agonist, UK-14,304 (Cambridge, 1981), and the selective α_1-adrenoceptor agonist, phenylephrine (McGrath, 1982), upon theophylline augmented SCC. Agonists were administered serosally 60 minutes after tissue mounting when the SCC was stable, using a half logarithmic cumulative progression of the final bath concentration (Van Rossüm, 1963). With every monolateral drug addition an equal volume of Krebs' Ringer solution was added to the bathing medium at the opposite side of the tissue.

A). The Effect Of Noradrenaline On Theophylline Augmented SCC.

Theophylline (4 mM) elevated SCC, a response which is well established to be associated with a stimulation of electrogenic Cl^- secretion. This theophylline elevated SCC was decreased by noradrenaline (10^{-9}-10^{-5} M) in a concentration dependent manner. Figure 11 shows the effect of noradrenaline in the rat jejunum.

Figure 12 shows the log. concentration-percentage-response curve for noradrenaline with respect to decreasing SCC. The threshold concentration of noradrenaline was 10^{-9} M, whereas 10^{-5} M noradrenaline evoked a maximal 64.6 ± 3.7 % inhibition of the total SCC, which is expressed as a 100% response in the log concentration-percentage-response curve (figure 12). The EC_{50} was found to be 1.7 X 10^{-7} M (95% confidence limits: 8.9 X 10^{-8} to 3.2 X 10^{-7} M, n=5).

FIGURE 11: A trace of the noradrenaline (10^{-9}-10^{-5} M) evoked decreases in secretagogue augmented (4 mM theophylline)SCC in the rat jejunum *in-vitro*.

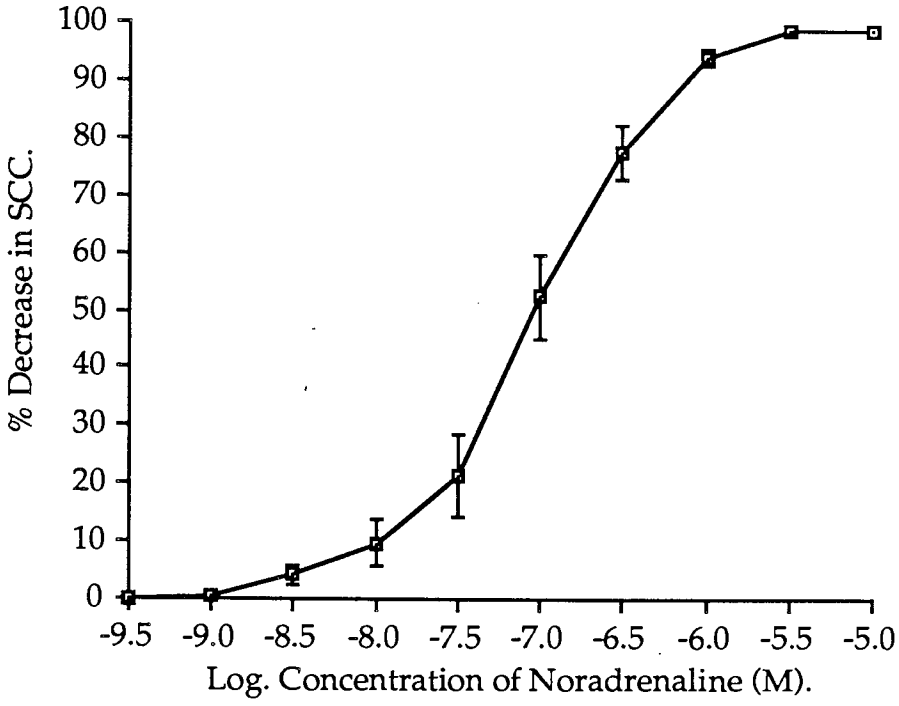

FIGURE 12: A log. concentration-percentage-response curve for noradrenaline with respect to decreasing SCC augmented by 4 X 10^{-3} M theophylline, in the rat jejunum *in-vitro*. Each point represents the mean of five experiments and the vertical bars indicate the SEM.

B). The Effect Of The Selective α_2-Adrenoceptor Agonist UK-14,304 Upon Theophylline Augmented SCC.

The selective α_2-adrenoceptor agonist UK-14,304 (10^{-9}- 3 X 10^{-6} M) also evoked concentration dependent decreases in theophylline elevated SCC. Figure 13 shows the log. concentration-percentage-response curve for UK-14,304 with respect to decreasing SCC. The threshold concentration was found to be 10^{-9} M, whereas 3 X 10^{-6} M evoked a maximal 64.4 ± 4.1 % inhibition of the total SCC, which is expressed as a 100% response in the log concentration-percentage-response curve. The EC_{50} was found to be 2.1 X 10^{-8} M (95% confidence limits: 10^{-8} to 4.2 X 10^{-8} M, n=5).

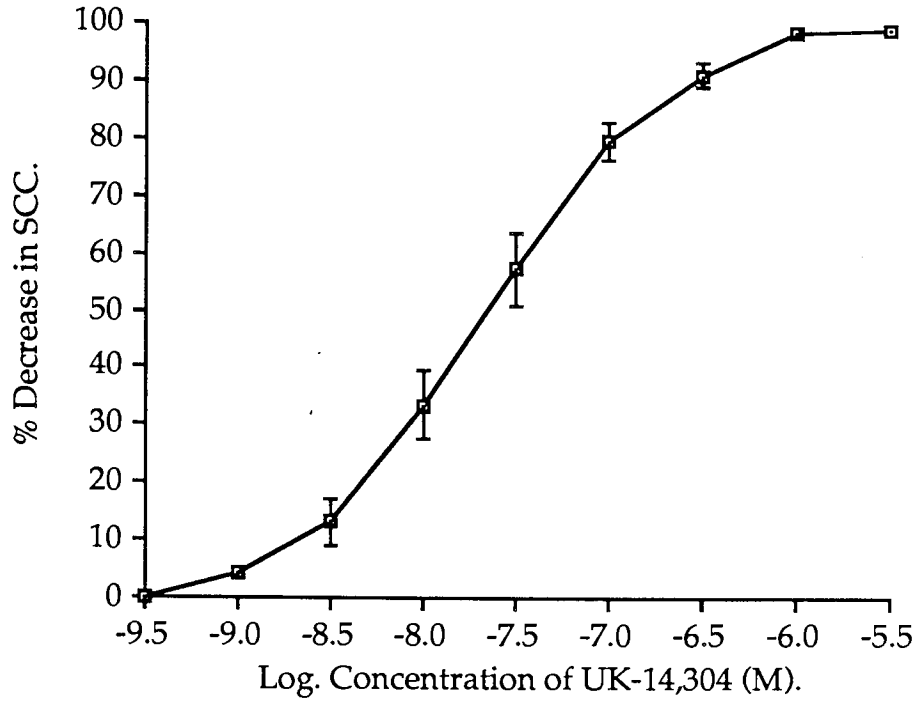

FIGURE 13: A log. concentration-percentage-response curve for UK-14,304 with respect to decreasing SCC augmented by 4 X 10^{-3} M theophylline, in the rat jejunum *in-vitro*. Each point represents the mean of five experiments and the vertical bars indicate the SEM.

C). The Effect Of The Selective α_1-Adrenoceptor Agonist Phenylephrine Upon Theophylline Augmented SCC.

The selective α_1-adrenoceptor agonist phenylephrine (McGrath, 1982) at high concentrations was also found to produce concentration dependent decreases in secretagogue elevated SCC. Figure 14 shows the log. concentration-percentage-response curve for phenylephrine with respect to decreasing SCC. The threshold concentration was found to be 10^{-6} M, whereas 10^{-4} M evoked a maximal 20.4 ± 4.4 % inhibition of the total SCC, which is expressed as a 100% response in the log concentration-percentage-response curve. The EC_{50} was found to be 1.6 X 10^{-6} M (95% confidence limits: 1.2 X 10^{-6} to 2.2 X 10^{-6} M, n=5).

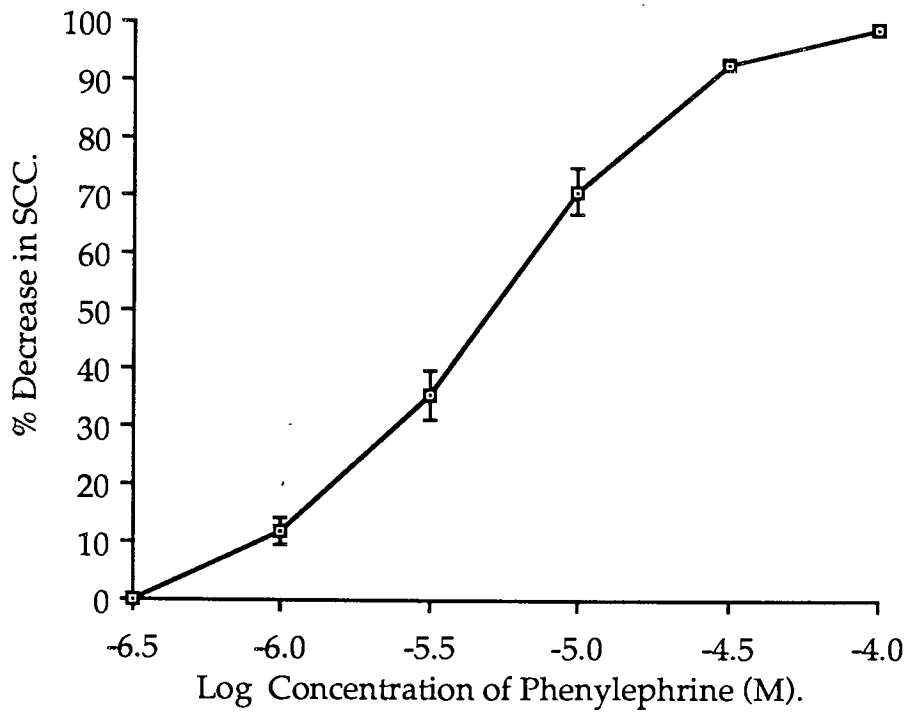

FIGURE 14: A log. concentration-percentage-response curve for phenylephrine with respect to decreasing SCC augmented by 4 X 10^{-3} M theophylline, in the rat jejunum *in-vitro*. Each point represents the mean of five experiments and the vertical bars indicate the SEM.

D). Rank Order Of Potency.

The rank order of pontency was as follows UK14,304>Noradrenaline>>Phenylephrine. UK-14,304 was found to be X8.1 more potent than noradrenaline and X76.2 more potent than phenylephrine. Table 2 shows the EC_{50}s, the maximum percentage decrease of the total theophylline elevated SCC and the relative intrinsic activities for the three agonists tested. Noradrenaline and UK-14,304 produced similar maximum percentage decreases of total SCC, thus were treated as full agonists and given a relative intrinsic activity of 1. However phenylephrine was found to have a relative intrinsic activity of only 0.32 and thus appeared to act as a partial agonist in this tissue.

Agonist and Concentration	Maximum % Decrease of Total SCC	Relative Intrinsic Activity	EC_{50} (M) with 95 % confidence limits.
Noradrenaline (10^{-5} M)	64.6 ± 3.7	1	1.7×10^{-7} (8.9×10^{-8} to 3.2×10^{-7})
UK-14,304 (3×10^{-6} M)	64.4 ± 4.1	1	2.1×10^{-8} (1.0×10^{-8} to 4.2×10^{-8})
Phenylephrine (10^{-4} M)	20.4 ± 4.4	0.32	1.6×10^{-6} (1.2×10^{-6} to 2.2×10^{-6})

TABLE 2: Showing the maximum % decrease of the total theophylline elevated SCC (± SEM), the relative intrinsic activities and the geometric meaned EC_{50} values (with 95% confidence limits) for the three agonists tested.

These results suggest that the decreases in secretagogue elevated SCC by noradrenaline, UK-14,304 and phenylephrine may be mediated through α_2-adrenoceptor stimulation.

4. THE EFFECT OF IDAZOXAN UPON NORADRENALINE, PHENYLEPHRINE AND UK-14,304, EVOKED DECREASES IN THEOPHYLLINE AUGMENTED SCC.

Since the previous study using noradrenaline, UK-14,304 and phenylephrine suggests that the decreases in secretagogue elevated SCC may be mediated through α_2-adrenoceptor stimulation, it was considered worthwhile to investigate the effect of the selective α_2-adrenoceptor antagonist idazoxan (Doxey *et al*, 1983) upon these responses.

A). The Effect Of Idazoxan Upon The Noradrenaline Evoked Responses.

The same method of drug addition was used, as that described in Section 3A. Antagonists when used were added serosally 15 minutes before the agonist addition. The effect of various concentrations of the selective α_2-adrenoceptor antagonist, idazoxan were investigated upon the noradrenaline evoked decreases in theophylline (4 X 10^{-6} M) augmented SCC. Figure 15

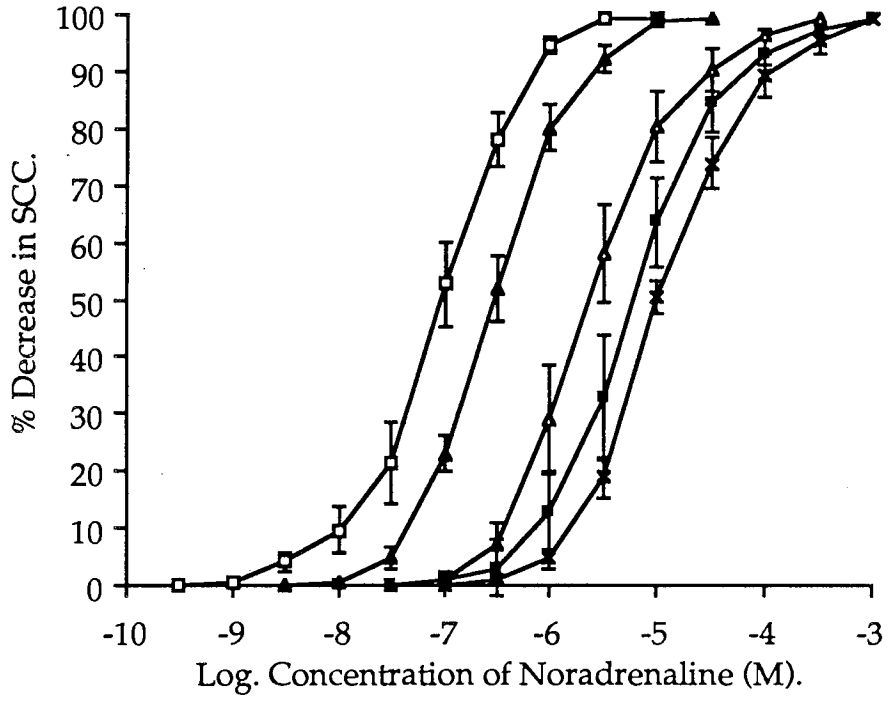

FIGURE 15: Log. concentration-percentage-response curves for the reduction by noradrenaline of theophylline (4 X 10^{-6} M) augmented SCC, in the absence (□) and presence of either 10^{-8} M (▲), 10^{-7} M (△), 3 X 10^{-7} M (■) or 10^{-6} M (✕) idazoxan. Each point represents the mean value of five experiments and vertical bars indicate SEM.

shows the effect of idazoxan on these noradrenaline responses. It can be seen that idazoxan produced rightward, parallel displacements of the noradrenaline concentration-response curve in a concentration dependent manner. Table 3 shows the EC_{50} values and concentration ratios meaned at

the EC_{50}, for noradrenaline in the presence of the various concentrations of idazoxan.

Idazoxan Concentration (M).	EC_{50} for Noradrenaline (M) with 95 % confidence limits.	Concentration Ratio
0	1.7×10^{-7} (3.2×10^{-8} to 8.9×10^{-7})	-
10^{-8}	2.8×10^{-7} (1.9×10^{-7} to 4.2×10^{-7})	1.7
10^{-7}	2.7×10^{-6} (9.6×10^{-7} to 5.4×10^{-6})	15.9
3×10^{-7}	4.8×10^{-6} (1.4×10^{-6} to 1.6×10^{-5})	28.2
10^{-6}	9.2×10^{-6} (5.9×10^{-6} to 1.4×10^{-5})	54.1

TABLE 3: Showing the EC_{50} and mean concentration ratio measured at the EC_{50} (n=5), for the noradrenaline evoked decreases in SCC, in the absence and in the presence of different concentrations of idazoxan.

The antagonist dissociation constant (K_B) for idazoxan was found to be $1.43 \pm 0.32 \times 10^{-8}$ M. The Arunlakshana and Schild plot (1959) of log. concentration of idazoxan (antagonist) against log. (dose ratio-1) yielded a straight line with a correlation coefficient of 0.98. The slope was not significantly different from 1 (0.99 with 95% confidence limits of ± 0.68), suggesting competitive antagonism (figure 16). The pA_2 value (the log. concentration of antagonist which produced a concentration ratio of 2) for idazoxan was calculated to be 7.87. These results suggest that idazoxan was acting as a competitive antagonist at the α_2-adrenoceptors to antagonise the noradrenaline induced decreases in SCC.

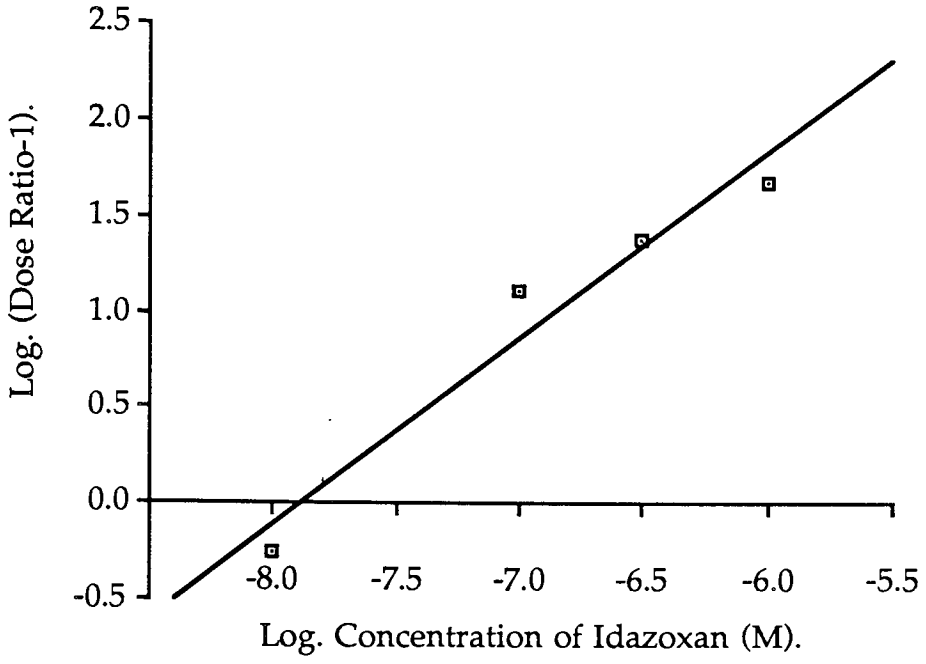

FIGURE 16: An Arunlakshana and Schild plot (1959) of log. concentration of idazoxan (antagonist) against log. (dose ratio-1), for noradrenaline on the rat jejunum. The intercept on the abscissa gives the pA_2 value or the log. concentration of idazoxan which produced a dose ratio of 2.

B). The Effect Of Idazoxan Upon The UK-14,304 Evoked Responses.

The same method of drug addition was used, as that described in Section 3A. Antagonists when used were added serosally 15 minutes before the agonist addition.

The effect of various concentrations of the selective α_2-adrenoceptor antagonist idazoxan were investigated upon the UK-14,304 evoked decreases in theophylline (4×10^{-6} M) augmented SCC. Figure 17 shows the effect of idazoxan on these UK-14,304 responses. It can be seen that idazoxan produced rightward parallel displacements of the UK-14,304 concentration response curve in a concentration dependent manner. Table 4 shows the mean EC_{50} values and concentration ratios measured at the EC_{50}, for UK-14,304 in the presence of the various concentrations of idazoxan.

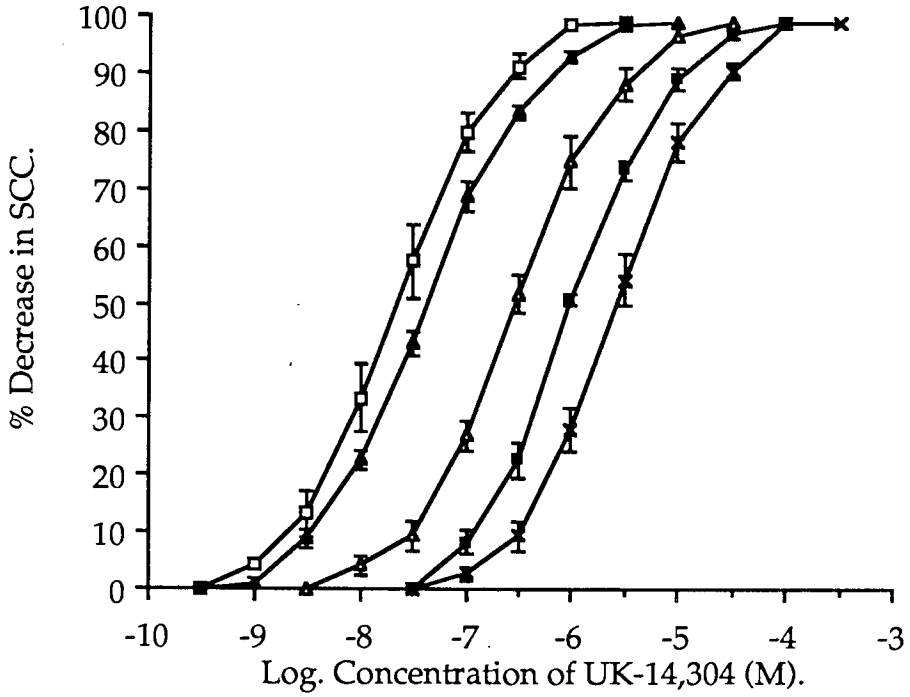

FIGURE 17: Log. concentration-percentage-response curves for the reduction by UK-14,304 of theophylline (4 X 10^{-6} M) augmented SCC, in the absence (□) and presence of either 10^{-8} M (▲), 10^{-7} M (△), 3 X 10^{-7} M (■) or 10^{-6} M (×) idazoxan. Each point represents the mean value of five experiments and vertical bars indicate SEM.

The antagonist dissociation constant (K_B) for idazoxan was found to be 9.5 ± 1.5 X 10^{-9} M. The Arunlakshana and Schild plot yielded a straight line with a correlation coefficient of 1.00. The slope was not significantly different from 1 (1.10 with 95% confidence limits of ± 0.24), suggesting competitive antagonism (figure 18). The pA_2 value for idazoxan was calculated to be 8.02. These results suggest that idazoxan was acting as a competitive antagonist at the α_2-adrenoceptors to antagonise the UK-14,304 decreases in SCC.

Idazoxan Concentration (M).	EC_{50} for UK-14,304 (M) with 95 % confidence limits.	Concentration Ratio
0	2.1×10^{-8} (1.0×10^{-8} to 4.2×10^{-8})	-
10^{-8}	4.2×10^{-8} (3.4×10^{-8} to 5.2×10^{-8})	2.0
10^{-7}	3.0×10^{-7} (1.9×10^{-7} to 4.7×10^{-7})	14.3
3×10^{-7}	9.7×10^{-7} (8.7×10^{-7} to 1.1×10^{-6})	46.2
10^{-6}	2.6×10^{-6} (1.7×10^{-6} to 4.0×10^{-6})	123.8

TABLE 4: Showing the EC_{50} and mean concentration ratio measured at the EC_{50} (n=5), for the UK-14,304 evoked decreases in theophylline (4×10^{-6} M) augmented SCC, in the absence and in the presence of different concentrations of idazoxan.

FIGURE 18: An Arunlakshana and Schild plot (1959) of log. concentration of idazoxan (antagonist) against log. (dose ratio-1), for UK-14,304 on the rat jejunum.

C). The Effect Of Idazoxan Upon The Phenylephrine Evoked Responses.

Since high concentrations of phenylephrine were required to construct concentration response curves, and the magnitude of the decreases in SCC were relatively small (refer to Section 3, previous), no attempt was made to construct concentration response curves to phenylephrine in the presence of various concentrations of idazoxan, however the effect of 10^{-6} M idazoxan was unsurmountable.

5. DISCUSSION.

The present study has shown that noradrenaline, when added to the serosal surface of rat jejunal sheets, evoked decreases in transepithelial SCC, which had been raised by addition of the secretagogue theophylline, which is reported to increase SCC by stimulation of anion secretion (Rao and Field, 1983). Noradrenaline has also been reported to produce similar effects upon basal transepithelial SCC (Dettmar *et al*, 1986a), a response which was confirmed later in Chapter 4, Section 4.

Evidence has been presented for an α_2-adrenoceptor mediated inhibition of theophylline stimulated SCC. UK-14,304 was approximately 8 times more potent than noradrenaline and 76 times more potent than phenylephrine, furthermore, both UK-14,304 and noradrenaline were full agonists whereas phenylephrine had a relative intrinsic activity of only 0.32. These results are similar to those reported by Cambridge (1981), who showed that UK-14,304 was 8.7 times more potent than noradrenaline in stimulating pre-junctional α_2-adrenoceptors in the rabbit pulmonary artery. In the present study the responses to noradrenaline and UK-14,304 were antagonised in a competitive manner by the selective α_2-adrenoceptor antagonist idazoxan. The calcuated values of pA_2 for idazoxan against noradrenaline and UK-14,304 were in each

case approximately 8.0, which compares favourably with the potency of this antagonist at pre-junctional α_2-adrenoceptors in the rat vas deferens (Doxey *et al*, 1983; Langer and Shepperson, 1982) and post-junctional α_2-adrenoceptors in rat and cat vascular smooth muscle (Medgett *et al*, 1984).

Williams (1986) has reported that the reduction of theophylline augmented SCC by the non-selective α-adrenoceptor agonist noradrenaline was resistant to antagonism by the selective α_1-adrenoceptor antagonist prazosin, suggesting that the response was not in fact mediated through α_1-adrenoceptor stimulation. Furthermore this worker also reported that the response to noradrenaline was resistant to the neurotoxin tetrodotoxin, and therefore was probably due to a direct effect on the transporting epithelium.

The present *in-vitro* finding that activation of α_2-adrenoceptors in the rat jejunum decreased elevated transepithelial SCC, is consistent with the report of Dettmar *et al* (1986), who showed that α_2-adrenoceptors also mediate decreases in basal secretory SCC in this tissue. They found that the effect of noradrenaline was antagonised by the selective α_2-adrenoceptor antagonists idazoxan and yohimbine but unaffected by the selective α_1-adrenoceptor antagonists corynanthine and prazosin. In addition a similar decrease in SCC was observed to UK-14,304, but only a very high concentration of the selective α_1-adrenoceptor agonist phenylephrine.

Stimulation of α_2-adrenoceptors with adrenaline or selective α_2-adrenoceptor agonists such as clonidine also decreases SCC in rabbit ileum and this effect correlates well with a reduction in electrogenic chloride secretion (Chang *et al*, 1982; Durbin *et al*, 1982). Munck (1972), has suggested that in the rat jejunum most of the recorded basal SCC represents electrogenic chloride secretion. Furthermore Williams (1986) reported that the decrease in basal SCC in the rat jejunum, evoked by noradrenaline was abolished by chloride replacement, whereas bicarbonate replacement was

without effect, which is consistent with the suggestion that in this preparation electrogenic chloride secretion is depressed by α_2-adrenoceptor stimulation. These findings are also in agreement with the present study, in that α_2-adrenoceptor activation decreased theophylline stimulated SCC, as did the chloride transport inhibitor piretanide.

The present *in-vitro* finding of a jejunal α_2-adrenoceptor antisecretory regulation of electrolyte transport is also consistent with *in-vivo* evidence for a similar regulatory role over fluid and electrolyte transport (Bunce and Spraggs 1983a and 1983b; Nakaki *et al,* 1982a and 1982b). In addition these observations in the rat are consistent with those made in the rabbit by Fondacaro *et al* (1988) who suggested that α_2-adrenoceptor agonists may be useful in converting the hypersecreting mammalian small bowel to its normal absorptive state.

RESULTS CHAPTER 4.

α_1-ADRENOCEPTOR CONTROL OF BASAL SHORT-CIRCUIT CURRENT IN THE RAT JEJUNUM *IN-VITRO*.

1. INTRODUCTION.

Evidence has been presented in Results Chapter 3 for an antisecretory regulatory role of α_2-adrenoceptors in the rat jejunum *in-vitro*. Although radioligand binding studies have identified both α_2- (Cotterell *et al*, 1982 and 1984; Nakaki *et al*, 1983) and α_1-adrenoceptors (Cotterell *et al*, 1982, 1983 and 1984) on rat jejunal epithelial cell membranes, most of the functional evidence favours a predominate α_2-adrenoceptor regulatory role over electrolyte and water transport (Chapter 1, Section 6A and Chapter 3). There have, however, been some reports of an α_1-adrenoceptor regulation (Cotterell *et al*, 1984; Parsons *et al*, 1983).

High doses of the preferential α_1-adrenoceptor agonist phenylephrine have been shown to evoke antisecretory responses upon fluid absorption in the rat jejunum *in-vivo* through activation of α_2-adrenoceptors (Bunce and Spraggs, 1983b). In addition it has been reported by Dettmar *et al* (1986a), that in the rat jejunum *in-vitro*, high concentrations ($>10^{-4}$ M) of phenylephrine evoke transient increases in SCC after α_2-adrenoceptor antagonism. Furthermore β-adrenoceptor agonists have been reported to cause a similar transient increase in SCC, which was attributed to β_2-adrenoceptor stimulation (Dettmar *et al*, 1986b). Therefore the possibility arises that high concentrations of phenylephrine may be stimulating β_2-adrenoceptors, since phenylephrine at high concentrations is documented to have β-adrenoceptor agonist activity (Lefevre, 1977).

In the present study the increase in SCC elicited by a high concentration (10^{-4} M) of phenylephrine after α_2-adrenoceptor antagonism by idazoxan was further investigated, to determine whether the effects were neurally mediated and/or associated with α_1- or β-adrenoceptor, muscarinic-cholinoceptor, or 5-HT-receptor activation. The ionic nature of the response to phenylephrine was investigated by pharmacological inhibition of electrogenic ion transport processes. The effect of a high concentration of the sympathetic neurotransmitter noradrenaline (10^{-4} M), after α_2-adrenoceptor antagonism by idazoxan, was also investigated to see if a similar pharmacological profile to that of phenylephrine could be obtained.

2. THE EFFECTS OF HIGH A CONCENTRATION OF PHENYLEPHRINE ON BASAL SCC.

A). The Effects Of A High Concentration Of Phenylephrine And Antagonism Of α_2- And β-Adrenoceptors Upon The Basal SCC Responses.

Basal SCC (and PD) in the rat jejunal sheets initially declined with time, but levelled off 30 minutes after tissue mounting. Tissue resistance was calculated according to Ohm's law, and a level of $62 \pm 5\ \Omega.cm^{-2}$, n=6, was determined. Phenylephrine was added serosally 30 minutes after tissue mounting when resting SCC was stable. An equal volume of Krebs' Ringer solution was simultaneously added to the opposite side of the Ussing chamber with every monolateral drug addition. Measurements were made in changes of basal SCC evoked by phenylephrine in the absence and presence of various antagonists. Antagonists were generally added 15 minutes after tissue mounting, which allowed a 15 minute equilibration period before the addition of the agonist. An exception to this method of antagonist addition was made when the effects of a subsequent addition of idazoxan were

investigated upon the decrease in SCC to phenylephrine, the antagonist being added when a sustained response to phenylephrine was achieved. Preliminary studies showed that a bilateral addition of glucose (10^{-2} M), evoked an increase in SCC of 20.5 ± 3.7 μ Amps/cm^2, n=6. Tissue viability was checked at the end of each experiment by a bilateral addition of this concentration of glucose.

Phenylephrine (10^{-4} M) evoked a sustained decrease in basal SCC of 9.9 ± 1.3 μ Amps/cm^2, n=6, (figure 19A). Subsequent addition of the selective α_2-adrenoceptor antagonist idazoxan (10^{-6} M) antagonised this response (figure 20). Idazoxan (10^{-6} M) alone had no effect upon basal SCC. The decrease in SCC evoked by phenylephrine (10^{-4} M) was also antagonised by a prior addition of idazoxan (10^{-6} M), and changed to a transient increase in SCC of 8.2 ± 2.0 μ Amps/cm^2, n=6 (figure 19B). The transient increase in SCC obtained in the presence of idazoxan, peaked in 1.8 ± 2.0 minutes, and the half life for the decay of the peak was 2.0 ± 0.3 minutes (n=6). A second addition of phenylephrine (10^{-4} M), after the first response had subsided did not evoke any further change in SCC. In the presence of idazoxan, no consistent increases in SCC in response to phenylephrine were observed with concentrations below 10^{-4} M.

Following addition of idazoxan (10^{-6} M), there was no significant difference ($P>0.05$) between the increases in SCC evoked by phenylephrine in the presence and absence of 10^{-6} M propranolol (figure 21). In addition propranolol alone was without effect upon basal SCC. This would suggest that there was no involvement of β-adrenoceptors in this transient secretory response of a high concentration of phenylephrine.

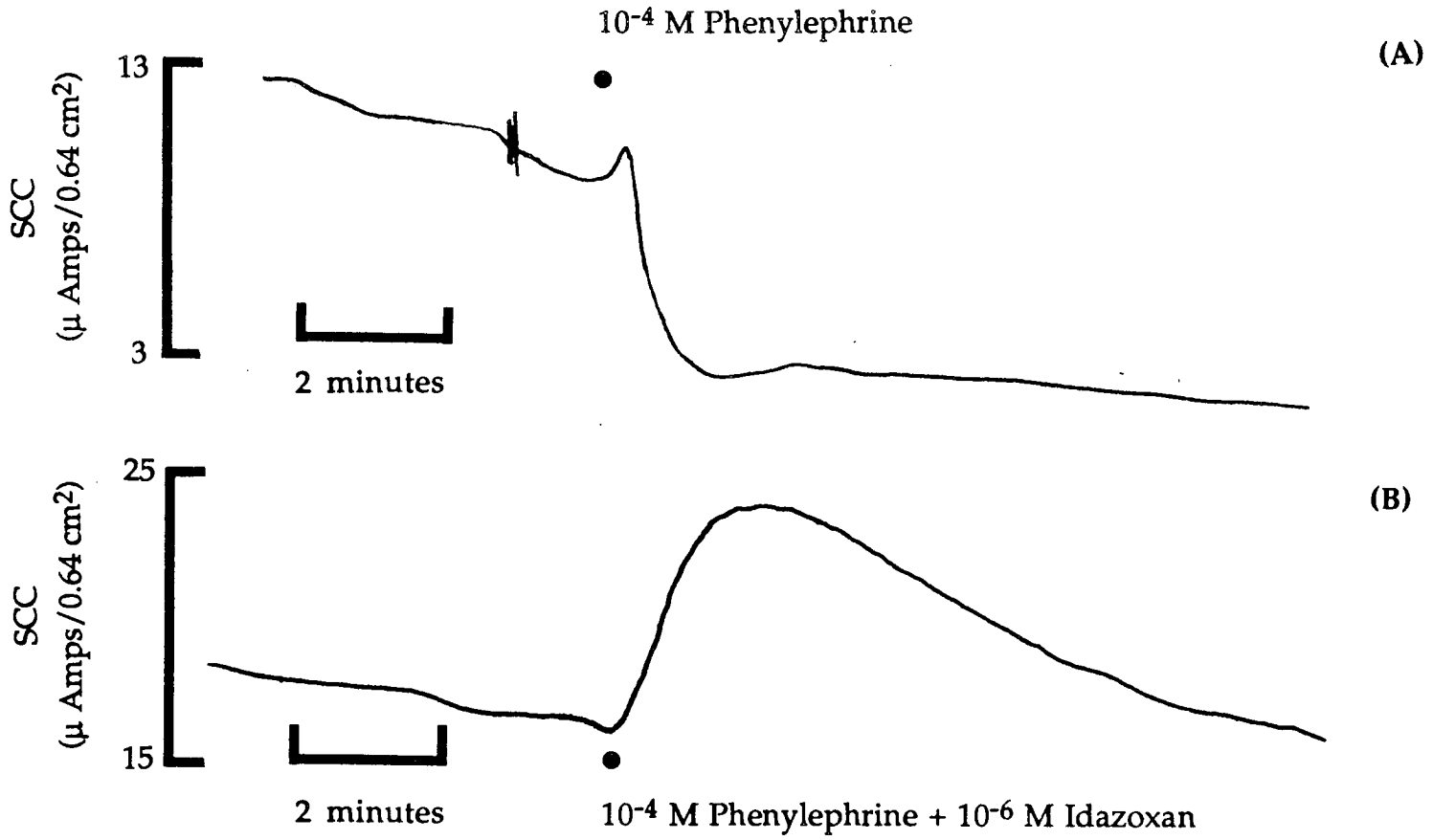

FIGURE 19: A trace of the basal SCC responses in the rat jejunum *in-vitro,* evoked by 10^{-4} M phenylephrine in the absence (A) and presence (B) of 10^{-6} M idazoxan.

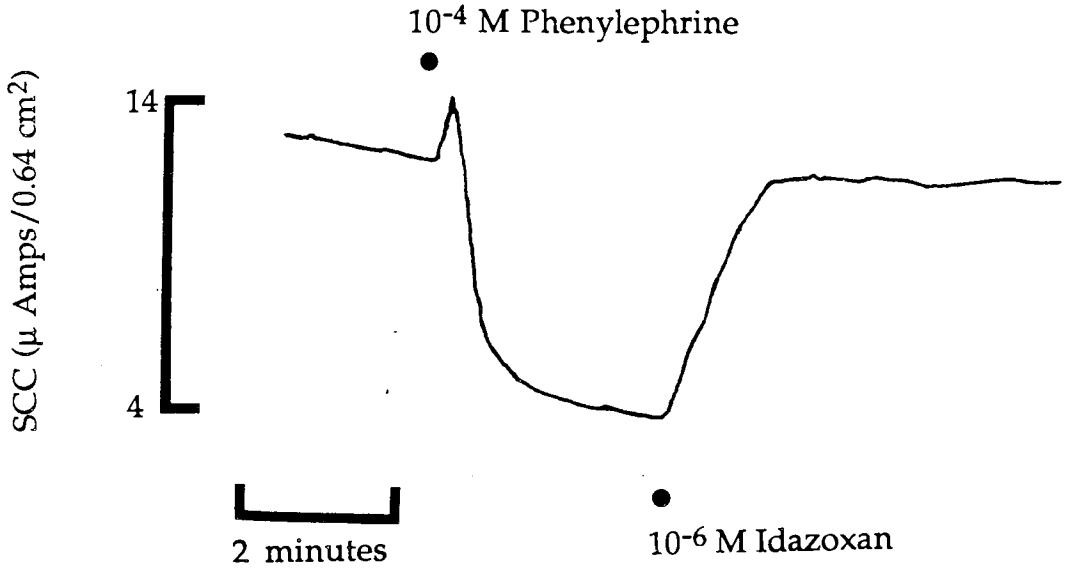

FIGURE 20: A trace of the basal SCC response of the rat jejunum *in-vitro* evoked by 10^{-4} M phenylephrine and the subsequent reversal of the response by 10^{-6} M idazoxan.

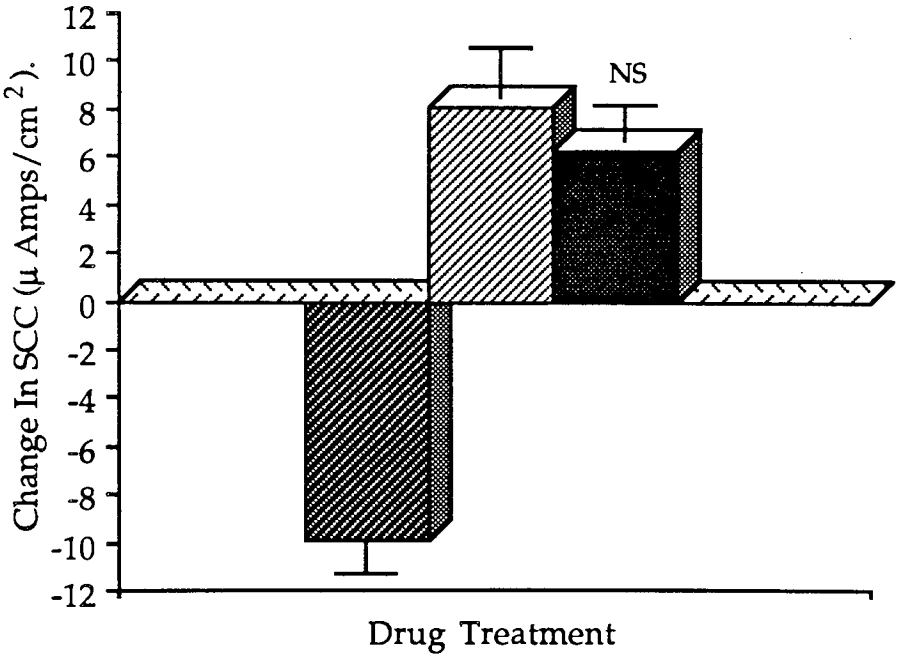

FIGURE 21: Changes in basal SCC in the rat jejunum *in-vitro* evoked by phenylephrine (10^{-4} M) alone (▨) and in the presence of 10^{-6} M idazoxan (▨), or 10^{-6} M idazoxan and 10^{-6} M propranolol (■). Results are mean values from six experiments and vertical lines indicate the SEM. NS = P>0.05.

B). The Involvement Of A Nervous Mechanism In The Transient Increase In Basal SCC Elicited By Phenylephrine After α_2-Adrenoceptor Antagonism.

It was considered worthwhile to indicate whether the phenylephrine induced increases in SCC were due to a direct or an indirect nerve mediated effect upon the enterocytes. The effect of the neurotoxin tetrodotoxin (Carey *et al,* 1985; Hubel, 1978) was therefore investigated upon the phenylephrine evoked secretory responses. Tetrodotoxin (10^{-6} M) alone produced a small decrease in basal SCC of 2.7 ± 1.4 μ Amps/cm^2, n=6. There was no significant difference (P>0.05) between the increases in SCC evoked by phenylephrine in the presence and absence of 10^{-6} M TTX (figure 22), which would suggest that the responses were due to a direct effect upon the enterocytes.

C). Possible Involvement Of Muscarinic-Cholinoceptor Activation In The Transient Increase In Basal SCC Elicited By Phenylephrine After α_2-Adrenoceptor Antagonism.

Since it has been reported that intestinal muscarinic-cholinceptor stimulation can evoke transient secretory increases in SCC (Hubel, 1976; Tapper *et al,* 1978), it was possible that phenylephrine might be acting indirectly via release of acetylcholine with stimulation of muscarinic-cholinoceptors. In the present investigation 5 X 10^{-7} M of the cholinoceptor agonist carbachol (Tapper, 1978), evoked a transient increase in SCC of 18.0 ± 4.5 μ Amps/cm^2 (n=6) in the rat jejunum *in-vitro* (Chapter 5, Section 5, figure 31). The transient increase in SCC evoked by carbachol peaked in 1.4 ± 0.1 minutes, and the half life for the decay of the peak was 1.7 ± 0.2 minutes (n=6). This increase in SCC elicited by carbachol was totally abolished by the selective muscarinic-cholinoceptor antagonist atropine (10^{-6} M) (Hubel, 1976). There was no significant difference (P>0.05) between the increases in SCC evoked by phenylephrine in the presence and absence of atropine (10^{-6} M)

(figure 22), suggesting that there was no involvement of muscarinic-cholinoceptors in this phenylephrine response.

D). Possible Involvement Of 5-HT-Receptor Activation In The Transient Increase In Basal SCC Elicited By Phenylephrine After α_2-Adrenoceptor Antagonism.

5-HT has also been reported to evoke secretory increases in SCC in the rat jejunum *in vitro* (Hardcastle *et al*, 1981). Furthermore in the present

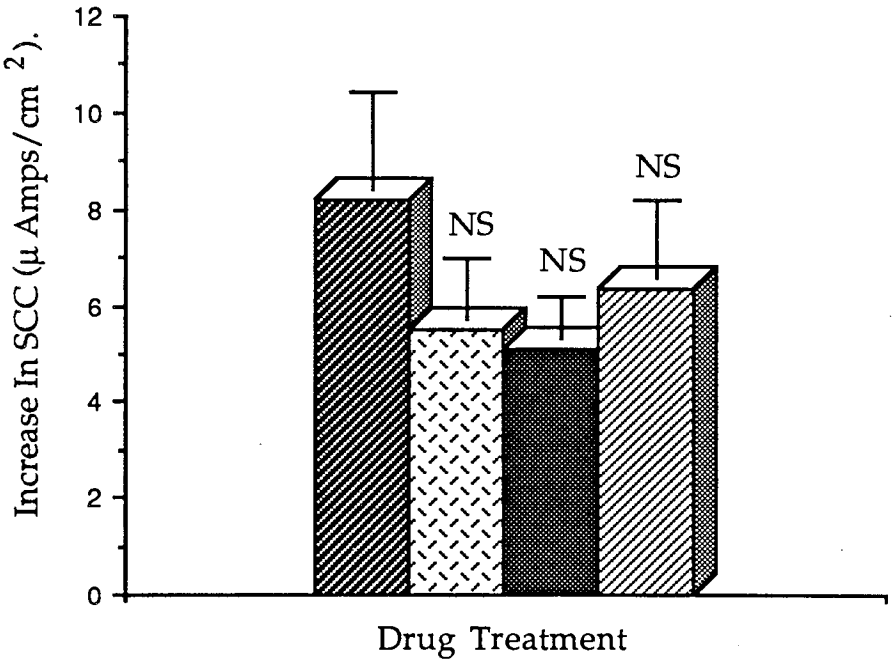

FIGURE 22: Increases in basal SCC of the rat jejunum *in-vitro* evoked by phenylephrine (10^{-4} M) in the presence of 10^{-6} M idazoxan (▨), 10^{-6} M idazoxan and 10^{-6} M tetrodotoxin (TTX) (⊡), 10^{-6} M idazoxan and 10^{-6} M atropine (■), or 10^{-6} M idazoxan and 10^{-5} M methysergide (▨). Results are means values from six experiments and vertical lines indicate the SEM. NS = P>0.05.

investigation 5-HT (10^{-6} M) was found to evoke a transient increase in basal SCC, and this response was antagonised by the 5-HT-receptor antagonist, methysergide (10^{-5} M) (Chapter 5, Section 4, figure 30). This concentration of

methysergide was also investigated upon the secretory response by phenylephrine, to see if there was any interaction with 5-hydroxytryptaminergic systems in evoking the response.

There was no significant difference ($P>0.05$) between the increases in SCC evoked by phenylephrine in the presence and absence of 10^{-5} M methysergide (figure 22), suggesting that there was in fact no involvement of 5-HT or 5-hydroxytryptaminergic systems in this phenylephrine response.

3. α_1-ADRENOCEPTORS AND THE INCREASE IN BASAL SCC EVOKED BY PHENYLEPHRINE.

An investigation was made into the possible involvement of α_1-adrenoceptors in the increase in SCC evoked by phenylephrine in the rat jejunum. Again antagonists were added serosally 15 minutes, and phenylephrine 30 minutes, after tissue mounting. Idazoxan (10^{-6} M) was again used to antagonise the α_2-adrenoceptors and "un-mask" the transient increase in SCC to 10^{-4} M phenylephrine. Again an equal volume of Krebs' Ringer solution was added mucosally with every drug addition. Tissue viability was tested as described previously.

The selective α_1-adrenoceptor antagonists prazosin (10^{-7} M) (Doxey *et al*, 1977) and corynanthine (10^{-4} M) (Shepperson *et al*, 1981) both caused a significant reduction ($P<0.01$) of the phenylephrine induced increase in SCC observed in idazoxan (10^{-6} M) pretreated preparations. Thus the phenylephrine induced increase in SCC in the absence of α_1-adrenoceptor antagonism (figure 23) was reduced to 0.6 ± 0.3 μ Amps/cm^2, n=6, after 10^{-7} M prazosin and to 0.6 ± 0.3 μ Amps/cm^2, n=6, after 10^{-4} M corynanthine. The selective α_1-adrenoceptor agonist cirazoline (Roach *et al*, 1978) alone evoked no SCC responses in this tissue with concentrations tested up to 10^{-4} M, and was therefore tested as an antagonist. Cirazoline (10^{-5} M) abolished the

increase in SCC evoked by phenylephrine and a decrease in SCC of 0.9 ± 0.4 μ Amps/cm2, n=6, was observed (figure 23). Thus the α_1-adrenoceptor agonist cirazoline appeared to act as an antagonist in this tissue. The results obtained here suggest that α_1-adrenoceptor stimulation mediated the increase in SCC evoked by phenylephrine in the presence of idazoxan.

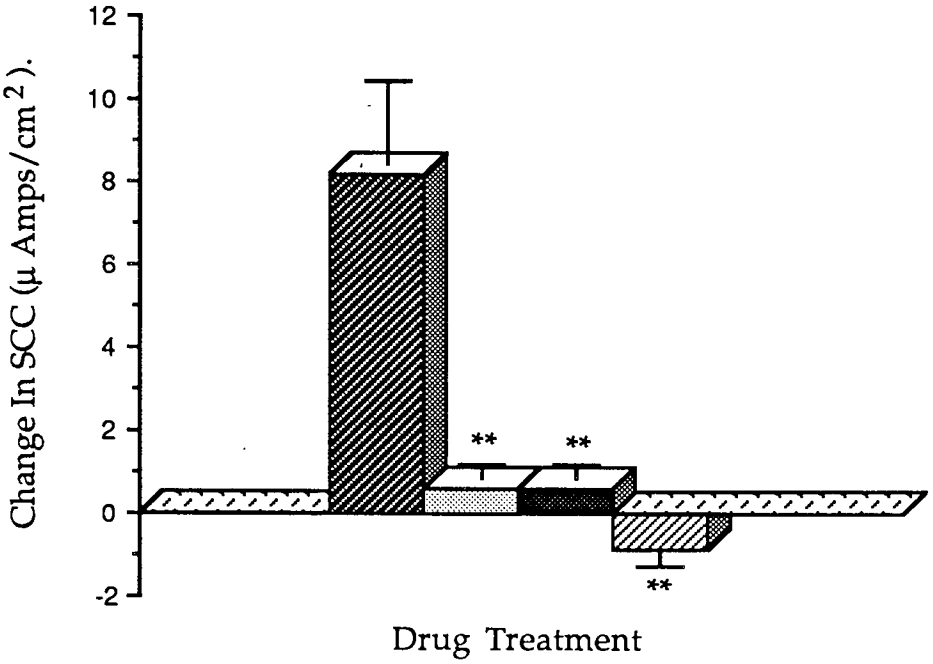

FIGURE 23: Changes in basal SCC of the rat jejunum *in-vitro* evoked by phenylephrine (10^{-4} M) in the presence of 10^{-6} M idazoxan (▨), 10^{-6} M idazoxan and 10^{-7} M prazosin (▩), 10^{-6} M idazoxan and 10^{-4} M corynanthine (▨), or 10^{-6} M idazoxan and 10^{-5} M cirazoline (■). Results are means values from six experiments and vertical lines indicate the SEM. NS = P>0.05. ** = P<0.01.

4. THE EFFECTS OF HIGH CONCENTRATIONS OF NORADRENALINE ON BASAL SCC.

The effect of a high concentration (10^{-4} M) of the sympathetic neurotransmitter noradrenaline, after α_2-adrenoceptor antagonism by idazoxan, was also investigated to see if a similar pharmacological profile to that of phenylephrine could be obtained.

Measurements were made of changes in SCC, evoked by 10^{-4} M noradrenaline in the presence and absence of various antagonists. The same

method of drug administration was employed as previously described for phenylephrine. Tissue viability was tested as described previously.

A). The Effect Of A High Concentration Of Noradrenaline And Antagonism of α_2- And β-Adrenoceptors Upon The Basal SCC Responses.

Noradrenaline (10^{-4} M) evoked a 16.8 ± 2.5 μ Amps/cm^2, n=6, sustained decrease in basal SCC (figures 24A and 25). Noradrenaline when added to tissues in the presence of 10^{-3} M idazoxan, the decrease in SCC was changed to a transient increase in SCC of 3.8 ± 0.3 μ Amps/cm^2, n=6 (figures 24B and 25). A higher concentration of idazoxan was used with noradrenaline because of the relatively greater α_2-adrenoceptor agonist activity of noradrenaline compared to phenylephrine. The transient increase in SCC obtained in the presence of idazoxan, peaked in 1.6 ± 0.2 minutes, and the half life for the decay of the peak was 2.1 ± 0.6 minutes (n=6). A second addition of noradrenaline (10^{-4} M) after the first response had subsided did not evoke any further SCC change. No consistent increases in SCC in response to noradrenaline could be produced with concentrations below 10^{-4} M. This smaller more residual increase in SCC observed with noradrenaline after α_2-adrenoceptor antagonism, when compared to that of phenylephrine, might be due to the ability of noradrenaline to surmount the α_2-adrenoceptor antagonism by idazoxan, whereas phenylephrine cannot (Chapter 3, Section 4).

There was no significant (P>0.05) difference between the increases in SCC evoked by 10^{-4} M noradrenaline in the presence and absence of 10^{-6} M propranolol (figure 25), which suggests that these increases in SCC evoked by noradrenaline were not mediated through β-adrenoceptor stimulation.

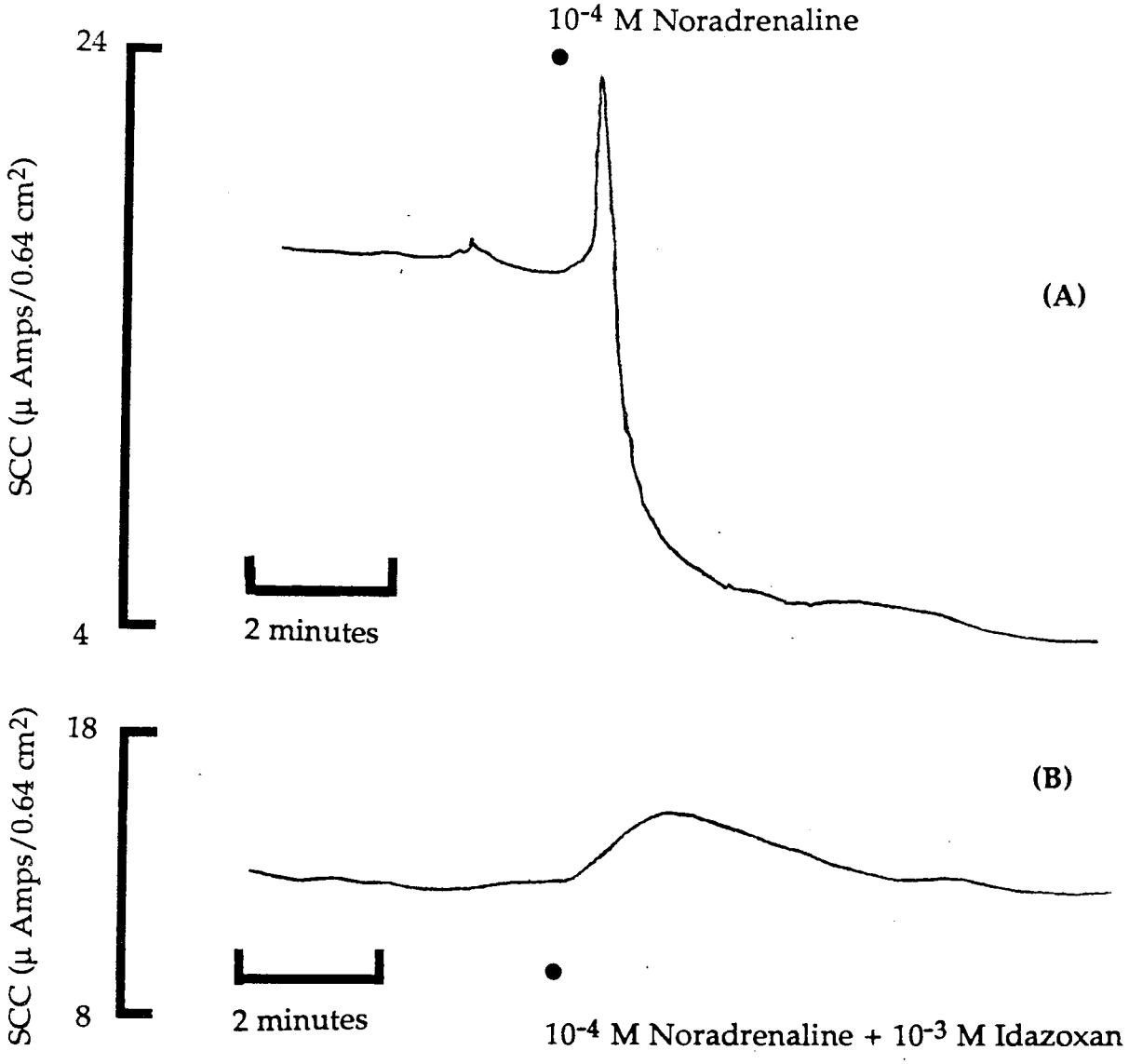

FIGURE 24: A trace of the basal SCC responses of the rat jejunum *in-vitro,* evoked by 10^{-4} M noradrenaline in the absence (**A**) and presence (**B**) of 10^{-3} M idazoxan.

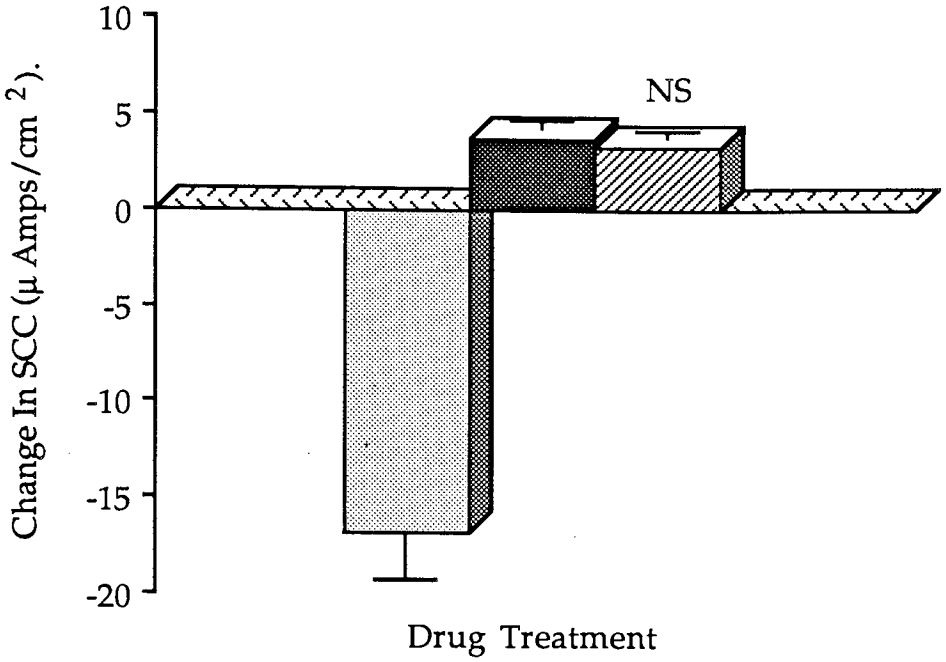

FIGURE 25: Changes in basal SCC of the rat jejunum *in-vitro* evoked by noradrenaline (10^{-4} M) alone (□) and in the presence of 10^{-3} M idazoxan (■), or 10^{-3} M idazoxan and 10^{-6} M propranolol (▨). Results are means values from six experiments and vertical lines indicate the SEM. NS = P>0.05.

B). α_1-Adrenoceptors And The Increase In Basal SCC Evoked By Noradrenaline After α_2-Adrenoceptor Antagonism.

A investigation was made into the possible involvement of α_1-adrenoceptors in the increase in SCC evoked by noradrenaline in the rat jejunum. Idazoxan (10^{-3} M) was again used to "un-mask" the transient increase in SCC to 10^{-4} M noradrenaline.

The selective α_1-adrenoceptor antagonists prazosin (10^{-7} M) and corynanthine (10^{-4} M) both caused a significant (P<0.01) reduction of the noradrenaline induced increase in SCC observed in idazoxan (10^{-3} M) pretreated preparations. Thus the noradrenaline induced increase in SCC in the absence of α_1-adrenoceptor antagonism (figure 26) was reduced to 1.3 ± 0.2 μ Amps/cm2, n=6, after 10^{-7} M prazosin and to 1.5 ± 0.2 μ Amps/cm2, n=6,

after 10^{-4} M corynanthine. The selective α_1-adrenoceptor agonist cirazoline (10^{-5} M) when tested as an antagonist, also caused a significant ($P<0.01$) reduction of the noradrenaline induced increase in SCC after idazoxan pretreatment (figure 26). Thus an increase in SCC of 1.6 ± 0.4 μ Amps/cm^2, n=6, was observed to noradrenaline, after idazoxan (10^{-3} M) and cirazoline (10^{-5} M) pretreatment. These results suggest that the increase in SCC evoked by noradrenaline after α_2-adrenoceptor antagonism in the rat jejunum was mediated through α_1-adrenoceptor stimulation.

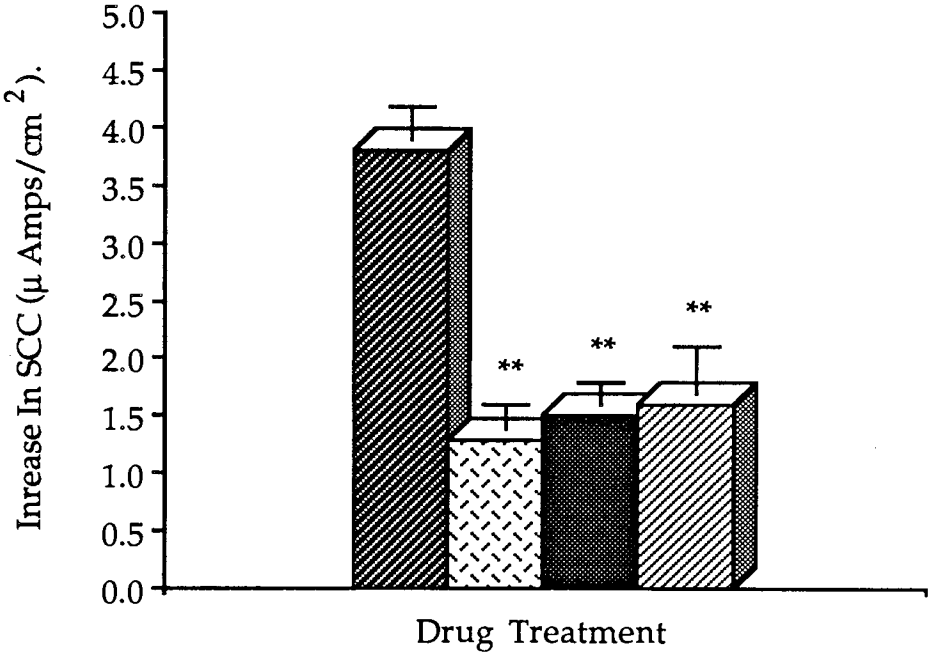

FIGURE 26: Increases in basal SCC of the rat jejunum *in-vitro* evoked by noradrenaline (10^{-4} M) in the presence of 10^{-3} M idazoxan (▨), 10^{-3} M idazoxan and 10^{-7} M prazosin (⊡), 10^{-3} M idazoxan and 10^{-4} M corynanthine (■), or 10^{-3} M idazoxan and 10^{-5} M cirazoline (▨). Results are means values from six experiments and vertical lines indicate the SEM. ** = $P<0.01$.

5. THE IONIC BASIS OF THE PHENYLEPHRINE EVOKED INCREASE IN BASAL SCC.

Increases in SCC across the mammalian small intestine *in-vitro*, in response to neuroendocrine agents or neurotransmitters, may represent a

secretory response with net stimulation of serosal to mucosal anion movement (Williams, 1986). It was considered worthwhile to investigate the ionic nature of the phenylephrine evoked increase in SCC after α_2-adrenoceptor antagonism, to determine whether this response was consistent with anion secretion.

It is difficult to use ion flux determinations for the evaluation of the ionic nature of a transient response like that seen with phenylephrine in the present investigation and attempts to do so have been inconclusive. Thus Hardcastle and Hardcastle (1987) reported that histamine applied to the rat jejunum *in-vitro*, evoked a transient increase in SCC but they concluded that, due to the transient nature of the response, the tissue was not in a steady-state condition following histamine application, when their flux determinations were performed, and in that respect it was not possible to conclude that a particular ion was fully responsible for the change in SCC based on ion flux determinations.

A pharmacological technique involving the selective inhibition of ion transport mechanisms (Baird and Margolius, 1987) was used in the present investigation. Piretanide was used to inhibit electrogenic Cl^- transport (Zeuthen *et al*, 1978), whilst amiloride was used to inhibit electrogenic Na^+ transport (Cuthbert *et al*, 1979). Acetazolamide, the carbonic anhydrase inhibitor (Waygood, 1955) was used to inhibit electrogenic bicarbonate transport. Piretanide (10^{-3} M) was administered serosally, amiloride (10^{-3} or 10^{-4} M) mucosally, and acetazolamide (10^{-3} M) both serosally and mucosally. The above drugs were co-administered along with idazoxan (10^{-6} M) 15 minutes after tissue mounting, whilst phenylephrine (10^{-4} M) was administered serosally 30 minutes after tissue mounting. Again an equal volume of Krebs' Ringer solution was added to the opposite side of the tissue when drug additions were made monolaterally. Tissue viability was tested as described previously by addition of glucose to the mucosal side.

There was no significant (P>0.05) difference between the increases in SCC evoked by 10^{-4} M phenylephrine in the presence and absence of 10^{-3} M acetazolamide or 10^{-4} M amiloride. However 10^{-3} M amiloride significantly (P<0.05) reduced the SCC response elicited by phenylephrine from an increase of 8.2 ± 2.0 μ Amps/cm^2, n=6, to an increase of 2.2 ± 0.6 μ Amps/cm^2, n=6, and 10^{-3} M piretanide completely abolished the response, n=6 (figure 27). This concentration of piretanide completely reversed the SCC response evoked by 4×10^{-3} M theophylline, a potent stimulant of electrogenic Cl^- secretion (Refer to Chapter 3, Section 2).

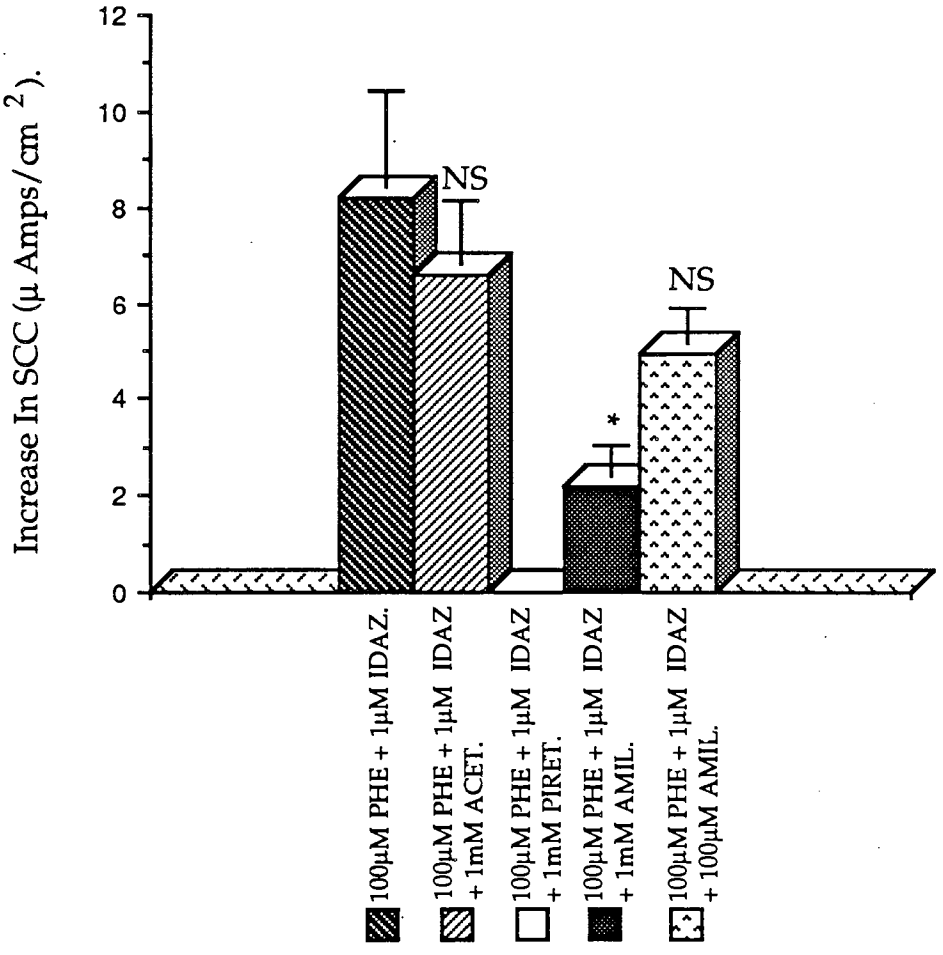

FIGURE 27: The basal SCC responses evoked by 10^{-4} M phenylephrine (PHE) after α_2-adrenoceptor antagonism by 10^{-6} M idazoxan (IDAZ) in the rat jejunum *in-vitro,* in the presence and absence of 10^{-3} M acetazolamide (ACET), 10^{-3} M amiloride (AMIL), 10^{-4} M AMIL, or 10^{-3} M piretanide (PIRET). Results are means values from six experiments and vertical lines indicate the SEM. NS = P>0.05. * = P<0.05.

The results obtained here suggest that the increase in SCC evoked by phenylephrine in the jejunum, was associated with Cl^- secretion, since piretanide totally abolished the response. The reduction in the SCC response to phenylephrine by 10^{-3} M amiloride may have been an indirect effect on Na^+/H^+ exchange, thus affecting HCO_3^-/Cl^- transport. The lower concentration of amiloride (10^{-4} M) however, did not produce a significant reduction of the secretory response to phenylephrine. Furthermore both 10^{-3} M and 10^{-4} M amiloride were without effect upon theophylline (4×10^{-3} M) stimulated increases in SCC in this tissue. Theophylline is a potent stimulant of electrogenic Cl^- secretion (Rao and Field, 1983).

It would therefore appear that the increases in SCC to phenylephrine after α_2-adrenoceptor antagonism are associated with stimulation of electrogenic chloride secretion, but it was not possible to exclude a stimulation of electrogenic Na^+ absorption.

6. DISCUSSION.

The present investigation has shown that after α_2-adrenoceptor antagonism by idazoxan, high concentrations of both noradrenaline and phenylephrine, evoke transient increases in SCC. This is consistent with the findings of Dettmar *et al*, (1986a), who also demonstrated a transient increase in SCC by high concentrations of phenylephrine, after α_2-adrenoceptor antagonism in the rat jejunum *in-vitro*. It has also been reported that β_2-adrenoceptor stimulation in the same tissue can evoke a similar transient increase in SCC (Dettmar *et al*, 1986b), and since high concentrations of phenylephrine can cause β-adrenoceptor activation (Lefevre, 1977), Williams (1986) suggested that the increase in SCC evoked by phenylephrine might be due to stimulation of β_2-adrenoceptors. The results of the present study are not consistent with this suggestion since the increase in SCC produced by both phenylephrine and noradrenaline after α_2-adrenoceptor antagonism by

idazoxan, were resistant to the non-selective β-adrenoceptor antagonist propranolol.

It has been reported that intestinal muscarinic-cholinoceptor stimulation can evoke secretory increases in SCC in the small intestine (Hubel, 1976; Tapper *et al*, 1978). Therefore the possibility existed that phenylephrine may have had an indirect action by stimulating an enteric nervous arc, which ultimately released acetylcholine which in turn activated muscarinic-cholinoceptors. The results of the present investigation suggest that there was no involvement of muscarinic-cholinoceptors in the increase in SCC to phenylephrine, because the response to phenylephrine was resistant to antagonism by the selective muscarinic-cholinoceptor antagonist atropine, at a concentration which completely abolished the SCC response to the cholinoceptor agonist carbachol.

5-HT has been reported to evoke transient increases in SCC in the rat ileum (Donowitz *et al*, 1979) and jejunum (Hardcastle *et al*, 1981) *in-vitro*. In addition the results presented in Chapter 5, of the present study are consistent with the findings of the latter, since 5-HT was found to evoke a transient increase in basal SCC, a response which was antagonised by the 5-HT-receptor antagonist, methysergide. It was considered pertinent to investigate whether there might be any inter-relationship between phenylephrine and 5-hydroxytryptaminergic systems, in evoking the increase in SCC. The response to phenylephrine was resistant to antagonism by methysergide, suggesting that there was in fact no involvement of 5-HT or 5-hydroxytryptaminergic systems in this phenylephrine response.

In evoking an increase in SCC, phenylephrine may have been stimulating the enterocytes either directly or indirectly through activation of an enteric neural network which eventually released a secretory neurotransmitter. The neurotoxin tetrodotoxin, which blocks neuronal sodium channels and so prevents the action potential-dependent release of neurotransmitters (Hubel, 1978), is reported to have a selective action upon enteric neurally mediated

intestinal transport, rather than affecting the transporting cells directly (Carey *et al*, 1985), and thus provides a useful pharmacological tool. In the present investigation tetrodotoxin alone evoked a small decrease in basal SCC, which would tend to suggest a very small level of basal jejunal neurogenic secretory tone. This is consistent with the findings of Cooke *et al* (1983a), who showed that tetrodotoxin decreased baseline ileal SCC and increased sodium and chloride transport. In the present study the increase in SCC evoked by phenylephrine was resistant to tetrodotoxin, suggesting that the response was actually due to a direct action of phenylephrine upon the enterocytes.

Although the increases in SCC were only observed with high concentrations of either noradrenaline or the selective α_1-adrenoceptor agonist phenylephrine, after α_2-adrenoceptor antagonism, the responses may still have been due to α_1-adrenoceptor activation on the enterocytes.

Such an α_1-adrenoceptor activation was supported by the observations that both the selective α_1-adrenoceptor antagonists prazosin and corynanthine antagonised the increase in SCC to phenylephrine. The concentration (10^{-7} M) of prazosin used in the present investigation compares favourably to that used to antagonise post-junctional α_1-adrenoceptors in the rat anococcygeus muscle *in-vitro* (Doxey *et al*, 1977), the maximum concentration for α_1-adrenoceptor selectivity was 2.38×10^{-7} M, above which pre-junctional α_2-adrenoceptors antagonism was observed. A higher concentration (10^{-4} M) of the selective α_1-adrenoceptor antagonist corynanthine was required to antagonise the secretory response to phenylephrine after α_2-adrenoceptor antagonism when compared to that of prazosin, which is consistent with documented evidence for a differential potency of these two α_1-adrenoceptor antagonists, the pA_2 values being 8.20 and 6.60 for prazosin in the rat anococcygeus muscle (Doxey *et al*, 1977) and corynanthine in the rabbit pulmonary artery (Weitzell *et al*, 1979)

respectively.

Dettmar *et al* (1986a), also reported that a high concentration of phenylephrine after α_2-adrenoceptor antagonism, induced an increase in SCC in the rat jejunum *in-vitro*, a response which was again inhibited by prazosin. Surprisingly in the present study, the selective α_1-adrenoceptor agonist cirazoline was without effect when tested as an agonist before and after α_2-adrenoceptor antagonism. Cirazoline has also been reported to be relatively inactive in affecting rabbit ileal electrolyte transport *in-vitro* (Dharmsathaphorn *et al*, 1984) and rat jejunal fluid transport *in-vitro* (Williams, 1986). When cirazoline was tested as an antagonist against phenylephrine in the present investigation, a significant inhibition of the phenylephrine response was observed. It therefore appears that cirazoline acts as an antagonist in this tissue, and that α_1-adrenoceptor stimulation mediates the increase in SCC evoked by phenylephrine, which is consistent with the observation in the rat jejunum *in-vivo*, that prazosin has a potent stimulatory effect upon net fluid absorption (Hemlin *et al*, 1987).

In contrast to the secretory response observed after rat jejunal α_1-adrenoceptor activation in the present study, Cotterell *et al* (1983) and Parsons *et al* (1983) have reported that α_1-adrenoceptor stimulation regulates an absorptive response in the rat jejunum based on *in-vitro* evidence using the everted intestinal sac technique, but such receptor activation may represent a stimulation of non-electrogenic absorption, as discussed later. However, one must be careful when comparing different techniques, since isolated intestinal sheets have secretory tone whereas everted intestinal sacs are strongly absorptive, therefore different techniques give different results.

It was considered important to see if the response to phenylephrine could be mimicked by noradrenaline since this agonist is the natural postganglionic sympathetic neurotransmitter. A small increase in SCC of approximately half

the magnitude of that seen with phenylephrine, was observed with a high concentration of noradrenaline (10^{-4} M) after α_2-adrenoceptor antagonism by idazoxan (10^{-3} M). A higher concentration of idazoxan was found to be necessary with noradrenaline, and it would seem likely that this was due to the relatively greater α_2-adrenoceptor stimulatory activity of noradrenaline compared to phenylephrine (Bunce and Spraggs, 1983b; Flavahan and McGrath, 1981; Williams, 1986). As was previously found for phenylephrine, this increase in SCC to noradrenaline was resistant to antagonism by propranolol, but was reduced by prazosin, corynanthine and cirazoline, again suggesting that the response was mediated through α_1- and not β-adrenoceptor activation.

The recorded SCC corresponds to the sum of the net fluxes of those ions which are electrogenically transported, but does not distinguish between the particular ions involved. The usual method for evaluating ionic movements across epithelial cells is to undertake ionic flux determinations, however it is difficult to use such determinations for investigating transient SCC responses of relatively short duration, since the tissue is not in a steady state condition (Hardcastle and Hardcastle, 1987). A selective pharmacological inhibition of electrogenic ion transport processes was thus employed to try and identify the ionic nature of the increase in SCC evoked by phenylephrine. Piretanide was used to inhibit chloride transport, amiloride to inhibit sodium transport and the carbonic anhydrase inhibitor acetazolamide, to inhibit bicarbonate transport.

It is reported that secreted HCO_3^- may be derived from cellular CO_2 metabolism rather than from the serosal solution (Carlinsky and Lew, 1970; Dietz and Field, 1973; Field, 1971; Field and McColl, 1973; Frizzell *et al*, 1976). Enteric cellular HCO_3^- production is consistent with the relatively high levels of intestinal carbonic anhydrase reported in the rat and guinea-pig (Carter and Parsons, 1971). Inhibition of intracellular carbonic anhydrase activity therefore would reduce the availability of intracellular HCO_3^- required for the secretory process, and such inhibition of HCO_3^- secretion by

acetazolamide has been observed in the rat colon by Parsons (1956) and Phillips and Schmalz (1970).

The diuretic agent amiloride, which in the mammalian kidney is reported to have a high affinity for the apical Na^+-channel protein found in cells of the distal tubule, and a lower affinity for the Na^+-H^+ exchange protein found in proximal tubule luminal membranes (Hendry and Ellory, 1988), also exhibits selective inhibition of apical sodium entry into enterocytes, and thus is a potent inhibitor of intestinal electrogenic Na^+ absorption (Frizzell *et al*, 1975 and 1976; Frizzell and Schultz, 1979).

The diuretic piretanide along with other 'loop acting' diuretics function by an inhibition of the $Na^+/K^+/Cl^-$ co-transport system in the loop of Henle of the kidney (Hendry and Ellory, 1988), in addition a similar inhibition of Cl^- transport has also been reported in intestinal enterocytes (Hardcastle and Hardcastle, 1987; Zeuthen *et al*, 1978). Intestinal electrogenic Cl^- secretion is therefore inhibited by basolateral attenuation of $Na^+/K^+/Cl^-$ co-transport, which results in reduced cellular accumulation of Cl^- required for apical exit through conductive channels in the secretory process.

Piretanide completely abolished the SCC response to phenylephrine, whilst acetazolamide was without effect. A high concentration of amiloride (10^{-3} M) also significantly reduced the SCC response, but a lower concentration (10^{-4} M) was with out effect. The transient increase in SCC evoked by phenylephrine therefore appears to be associated with a chloride secretion. The effect of the high concentration of amiloride may be due to an indirect effect on Na^+/H^+ exchange, thus affecting HCO_3^-/Cl^- transport. However both the high and lower concentrations of amiloride were without effect upon theophylline (4 mM) stimulated electrogenic chloride secretion. So an effect of phenylephrine upon electrogenic sodium transport cannot be ruled out as a component of the SCC response.

The results presented in this chapter suggest the presence of an α_1-adrenoceptor mediated control mechanism of basal electrogenic anion secretion in the rat jejunum *in-vitro*, although a stimulation of cationic absorption can not be totally excluded. An α_2-adrenoceptor antisecretory regulation appears to predominate, and has to be removed before the α_1-adrenoceptor control can be observed. The α_1-adrenoceptors in this tissue may be subtly different to the classical α_1-adrenoceptors classified in other tissues, since the selective α_1-adrenoceptor agonist phenylephrine was of only weak potency and showed a higher affinity for the α_2-adrenoceptors. In addition the selective α_1-adrenoceptor agonist cirazoline appeared to have an antagonistic action upon these α_1-adrenoceptors. Another possibility for the lack of potency of the agonists could be due to there only being a very small population of α_1-adrenoceptors compared to α_2-adrenoceptors in this tissue, but this is unlikely since Cotterell *et al* (1984), using radioligand binding studies, suggested that α_1-adrenoceptors predominate on rat jejunal epithelial cell membranes.

In contrast to the *in-vitro* evidence for an electrogenic secretory role of α_1-adrenoceptors reported in the present investigation, there is reasonable evidence to suggest that in the rat jejunum *in-vivo* an α_1-adrenoceptor mechanism mediates increases in basal fluid and electrolyte absorption (Levens *et al*, 1981a; Levens, 1983), and reflex increases in fluid absorption in response to haemorrhage (Levens, 1984a) or dehydration (Levens, 1984b). Increases in fluid absorption in response to dehydration or haemorrhage are thought to be mediated through the release of angiotensin, which in turn enhances noradrenaline release from sympathetic nerve endings synapsing on α_1-adrenoceptors (Levens, 1984a and 1984b). An absorptive response to α_1-adrenoceptor activation contrasts the findings of Hemlin *et al* (1987), who observed a stimulation of absorption by prazosin in the rat jejunum *in-vivo*. Williams (1986), suggested that the pro-absorptive effects of α_1-adrenoceptor activation in the jejunal mucosa, probably reflects upon stimulation of

electrically silent sodium chloride absorption. In addition Levens *et al* (1979), have shown that the effects of angiotensin *in-vivo* occur in the absence of any changes in transmural potential difference.

RESULTS CHAPTER 5.

THE ACTION OF 5-HYDROXYTRYPTAMINE UPON BASAL SCC IN THE RAT JEJUNUM *IN-VITRO*.

1. INTRODUCTION.

Evidence has been presented for a rat jejunal α_2-adrenoceptor antisecretory and α_1-adrenoceptor secretory regulation, in Results Chapters 3 and 4 respectively.

5-HT is reported to evoke transient increases in SCC in the rat ileum (Donowitz *et al*, 1979) and jejunum (Hardcastle *et al*, 1981) *in-vitro*. Furthermore it has been suggested that cholera toxin induced intestinal secretion *in-vivo*, is mediated via the release of 5-HT from mucosal endocrine cells, resulting in the depolarization of afferent nerves of a reflex arc and the eventual release of a secretory neurotransmitter (Cassuto *et al*, 1981a, 1981b, 1982a, 1982b and 1982c). This cholera toxin evoked response is reported to involve nicotinic, but not muscarinic-cholinoceptor stimulation. In contrast, *in-vitro* evidence from studies on the rat jejunum, suggest that the 5-HT evoked secretory responses are mediated through neither muscarinic- or nicotinic-cholinoceptor stimulation (Hardcastle *et al*, 1981). Furthermore Keast *et al* (1985), have shown that in the guinea-pig small intestine, 5-HT at high concentrations stimulates both cholinergic and non-cholinergic nerves, whilst lower concentrations preferentially stimulate non-cholinergic nerves.

The particular 5-HT-receptor sub-types involved in the secretory responses to 5-HT are unclear. Ball *et al* (1988b), have suggested that in the rat ileum, high concentrations of 5-HT stimulate neuronal 5-HT_3 receptors, whereas low concentrations stimulate non-neuronal benzamide-sensitive 5-HT-receptors, which are not 5-HT_1, 5-HT_2 or 5-HT_3-like in nature. In contrast Beubler and

Burg (1989), have reported that 5-HT released by cholera toxin in the rat jejunum, stimulates both 5-HT_2 and 5-HT_3 receptors.

The true nature of 5-HT evoked intestinal secretion is thus unclear. In the present study the increases in SCC evoked by 5-HT were investigated to determine whether the effects were neurally mediated and/or associated with either 5-HT receptor, α_1-adrenoceptor or muscarinic cholinoceptor activation. Possible sympathetic nervous activation was examined by pre-treatment with reserpine, and the effects of α_2- and β-adrenoceptor antagonism were also investigated. The ionic nature of the 5-HT evoked response was studied by a pharmacological inhibition of electrogenic ion transport processes, whilst the effect of ganglionic stimulation was examined to see if a similar pharmacological profile to that of 5-HT could be obtained.

2. THE EFFECT OF 5-HYDROXYTRYPTAMINE ON BASAL SCC.

5-HT was added serosally 30 minutes after tissue mounting when resting SCC was stable. An equal volume of Krebs' Ringer solution was simultaneously added to the opposite side of the Ussing chamber with every monolateral drug addition. Measurements were made of changes in basal SCC evoked by 10^{-6} M 5-HT. Preliminary studies showed that this concentration of 5-HT produced a 75.9 ± 8.4 % increase in SCC of 21.7 ± 3.8 μ Amps/cm^2, n=6, whilst 10^{-5} M evoked the maximum increase in SCC of 28.6 ± 2.4 μ Amps/cm^2, n=6. Tissue viability was checked at the end of each experiment by a bilateral addition of glucose (10^{-2} M). 5-HT (10^{-6} M) evoked a transient increase in basal SCC of 21.7 ± 3.8 μ Amps/cm^2, n=6, (figure 28). This transient increase in SCC peaked in 1.2 ± 0.1 minutes, and the half life for the decay of the peak was 0.8 ± 0.1 minutes (n=6). A second addition of 5-HT (10^{-6} M), after the first response had subsided produced either no further SCC response, or an inconsistent smaller transient increase in SCC. This suggests that the tissue was showing acute tachyphylaxisis.

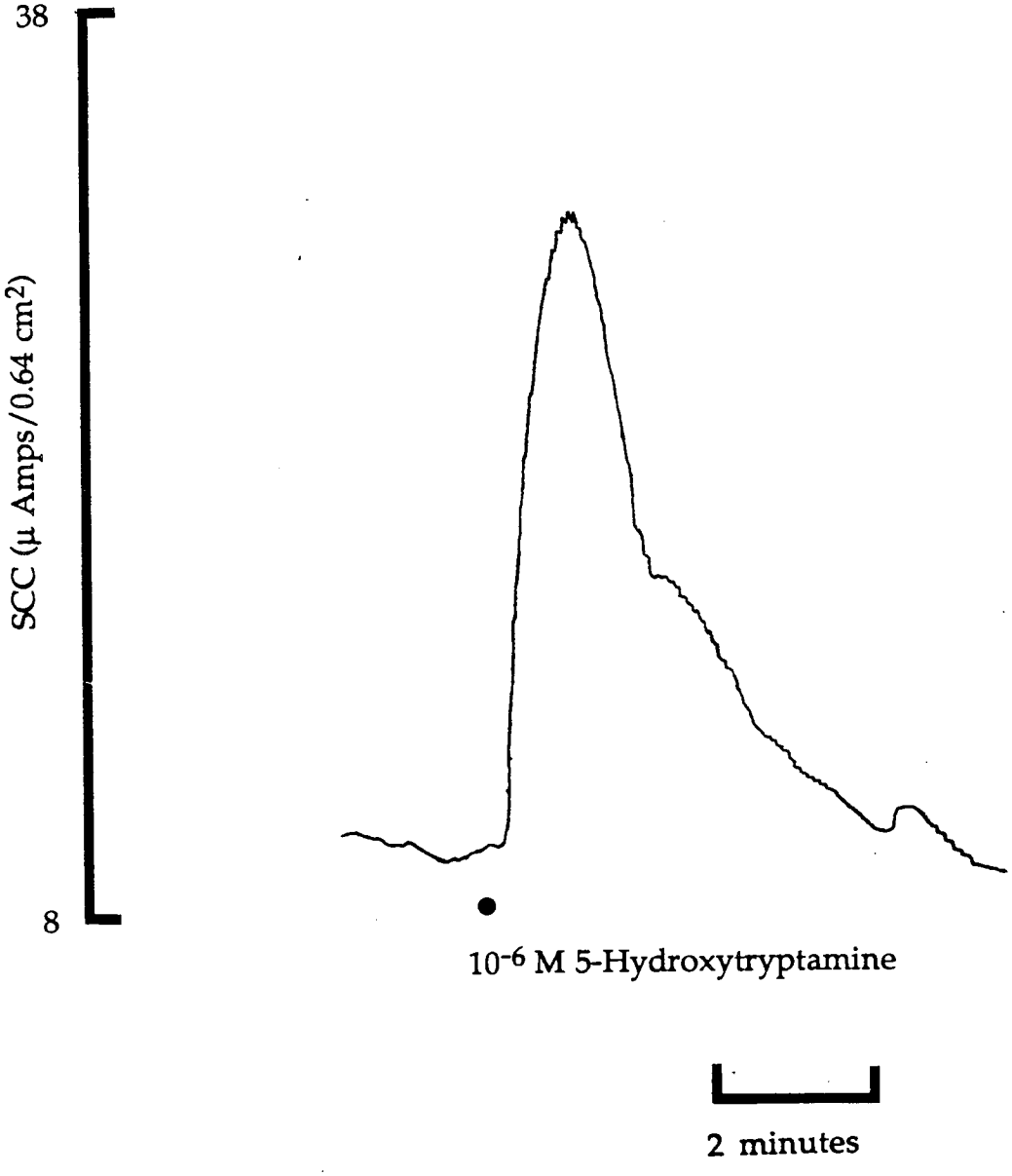

FIGURE 28: A trace of the increase in basal SCC evoked by 10^{-6} M 5-HT in the rat jejunum *in-vitro*.

3. THE EFFECT OF 5-HT-RECEPTOR ANTAGONISM UPON THE INCREASES IN BASAL SCC EVOKED BY 5-HT.

The influence of a maximal effective concentration of methysergide, a weak antagonist at 5-HT_1-receptors, but a potent antagonist at 5-HT_2-receptors (Baird and Cuthbert, 1987), and the influences of maximal effective

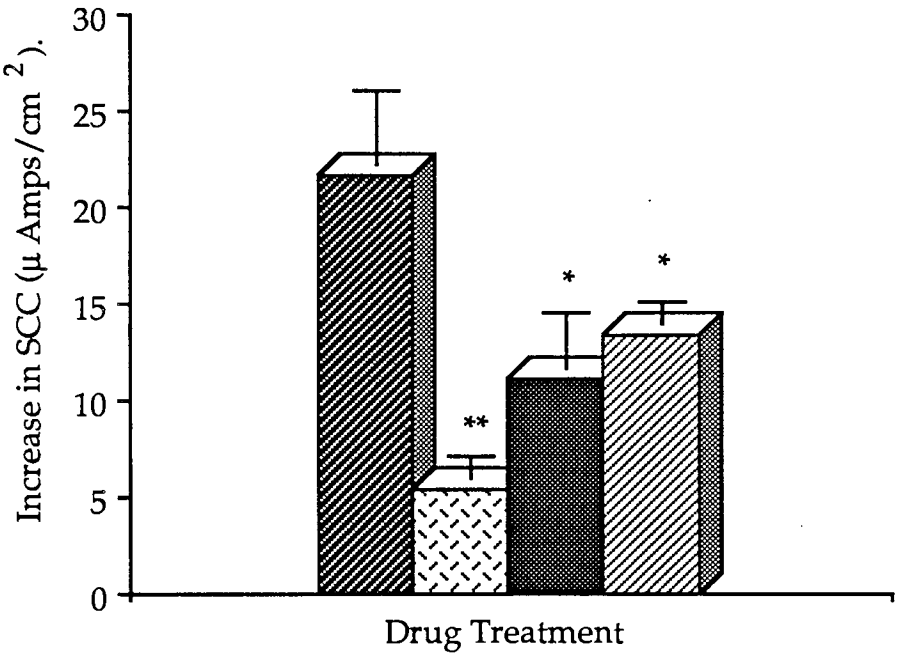

FIGURE 29: The increases in SCC evoked by 10^{-6} M 5-HT in the rat jejunum *in-vitro*, in the absence (▨) and presence of either 10^{-5} M methysergide (⊡), 10^{-6} M ICS 205930 (■), or 10^{-6} M BRL 24924 (▨). Results are means values from six experiments and vertical lines indicate the SEM. * = $P<0.05$ and ** = $P<0.01$, when compared to the responses by 5-HT alone.

concentrations of the neuronal 5-HT_3-receptor (M receptor) antagonists, ICS-205930 (Donatsch *et al*, 1984; Round and Wallis, 1986) and BRL 24924 (Dunbar *et al*, 1986), were investigated upon these 5-HT evoked responses. However no attempt was made to fully classify particular regulatory 5-HT sub-receptors due to the lack of documented evidence for the selectivity of the above antagonists in this jejunal electrolyte transport model, although an

interaction with a 5-HT receptor was anticipated. Antagonists were added serosally 15 minutes after tissue mounting. Tissue viability was tested as described previously.

Methysergide (10^{-5} M) significantly ($P<0.01$) reduced the increase in SCC evoked by 5-HT, to 5.5 + 1.1 μ Amps/cm^2, n=6, (figure 29). The increase in SCC to 5-HT was also significantly ($P<0.05$) reduced in the presence of the neuronal 5-HT_3-receptor antagonists, ICS 205930 and BRL 24924. ICS 205930 (10^{-6} M) reduced the increase in SCC to 11.1 ± 2.9 μ Amps/cm^2, n=6, whilst BRL 24924 (10^{-6} M) reduced the increase to 13.5 ± 1.1 μ Amps/cm^2, n=6 (figure 29).

Methysergide which was used as a general 5-HT receptor antagonist and the 5-HT_3 antagonists receptors ICS 205930 and BRL 24924 significantly reduced the 5-HT evoked increase in SCC, suggesting that 5-HT was activating enteric 5-HT receptors in evoking this response.

4. THE EFFECTS OF THE NEUROTOXIN, TETRODOTOXIN UPON THE INCREASE IN BASAL SCC EVOKED BY 5-HT.

It was considered to be important to determine whether the 5-HT induced responses were due totally to a direct effect on the transporting cells or whether there was some indirect neural component. The effect of the neurotoxin tetrodotoxin was therefore investigated upon the SCC response to 5-HT. Tetrodotoxin (10^{-6} M) was added serosally 15 minutes before the addition of 5-HT (10^{-6} M). As described previously (Chapter 4, Section 2), tetrodotoxin alone, evoked a small decrease in basal SCC. Tetrodotoxin significantly ($P<0.001$) reduced the increase in SCC evoked by 5-HT, to a residual increase in SCC of 2.0 ± 0.5 μ Amps/cm^2, n=6 (figure 30). Since tetrodotoxin competitively antagonises neuronal sodium channels (Hubel, 1978), the residual response evoked by 5-HT in the presence of tetrodotoxin might have been due to incomplete sodium channel antagonism, however a

non-neuronal or a non-specific action of 5-HT cannot be totally excluded. In addition this residual response was resistant to antagonism by methysergide (10^{-5} M) (figure 30). Ball *et al* (1988b) have reported that both neuronal and non-neuronal 5-HT-receptors control electrolyte secretion in the rat ileal mucosa.

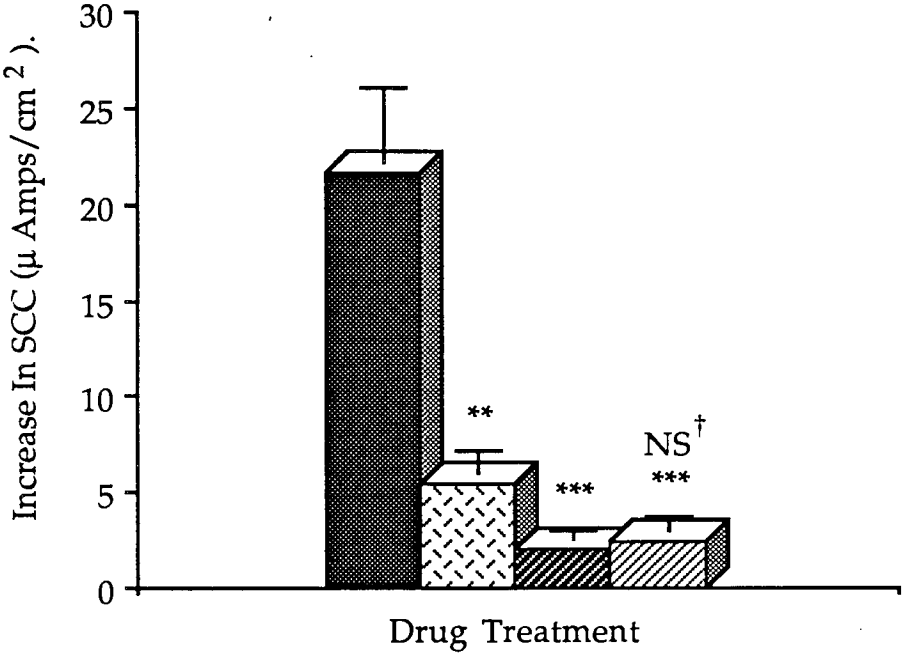

FIGURE 30: The Increases in SCC evoked by 10^{-6} M 5-HT in the rat jejunum *in-vitro*, in the absence (■) and presence of either 10^{-5} M methysergide (▣), 10^{-6} M tetrodotoxin (TTX) (▨), or 10^{-5} M methysergide and 10^{-6} M TTX (▨). Results are means values from six experiments and vertical lines indicate the SEM. (** = $P<0.01$ and *** = $P<0.001$, when compared to the responses by 5-HT alone. NS† = $P>0.05$, when compared with the responses by 5-HT + TTX).

5. THE EFFECTS OF MUSCARINIC-CHOLINOCEPTOR ANTAGONISM UPON THE INCREASE IN BASAL SCC EVOKED BY 5-HT.

Since the previous investigation in Section 4, has shown that most of the response to 5-HT is mediated through an indirect neural link, there was the possibility that 5-HT may be stimulating a cholinergic system which

ultimately releases acetylcholine resulting in stimulation of muscarinic-cholinoceptors. Muscarinic-cholinoceptor stimulation is reported to evoke an increase in electrogenic secretory processes in the mammalian small intestine (Hubel, 1976; Tapper *et al*, 1978). It was therefore decided to investigate the effects of the muscarinic-cholinoceptor antagonist, atropine upon the increase in SCC to 5-HT. Atropine was added serosally 15 minutes

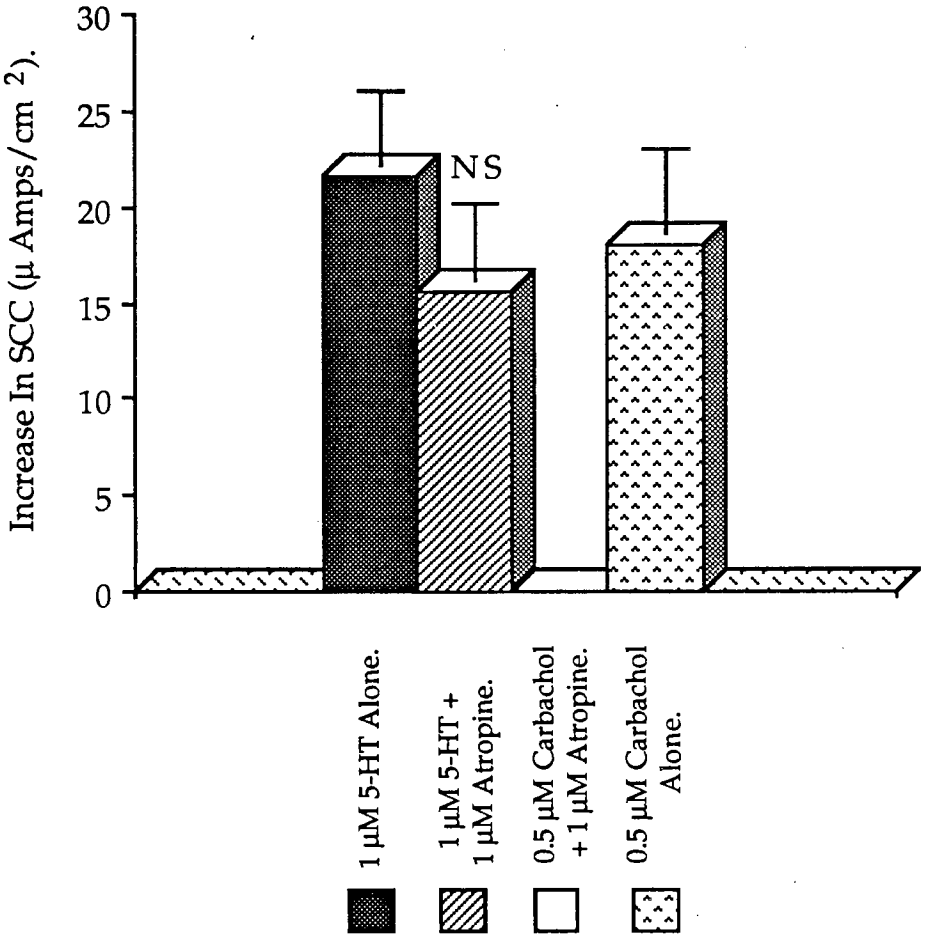

FIGURE 31: The SCC responses evoked by carbachol (5 X 10^{-7} M) and 5-HT (10^{-6} M) in the rat jejunum *in-vitro*, both in the presence and absence of atropine (10^{-6} M). Results are means values from six experiments and vertical lines indicate the SEM (NS = P>0.05 when compared to the responses by 5-HT alone).

before the addition of 5-HT, and tissue viability was tested as described previously. There was no significant difference (P>0.05) between the increases in SCC evoked by 10^{-6} M 5-HT in the presence and absence of 10^{-6} M atropine (figure 31), suggesting that there was no involvement of muscarinic-cholinoceptors in the 5-HT evoked response. This concentration of atropine

completely abolished the SCC response evoked by 5 X 10^{-7} M carbachol.

6. α_1-ADRENOCEPTORS AND THE INCREASE IN BASAL SCC EVOKED BY 5-HT.

Since the results in Chapter 4 have shown the presence of an α_1-adrenoceptor mediated secretory process in the rat jejunum, it was considered relevant to see whether there was any inter-relationship between

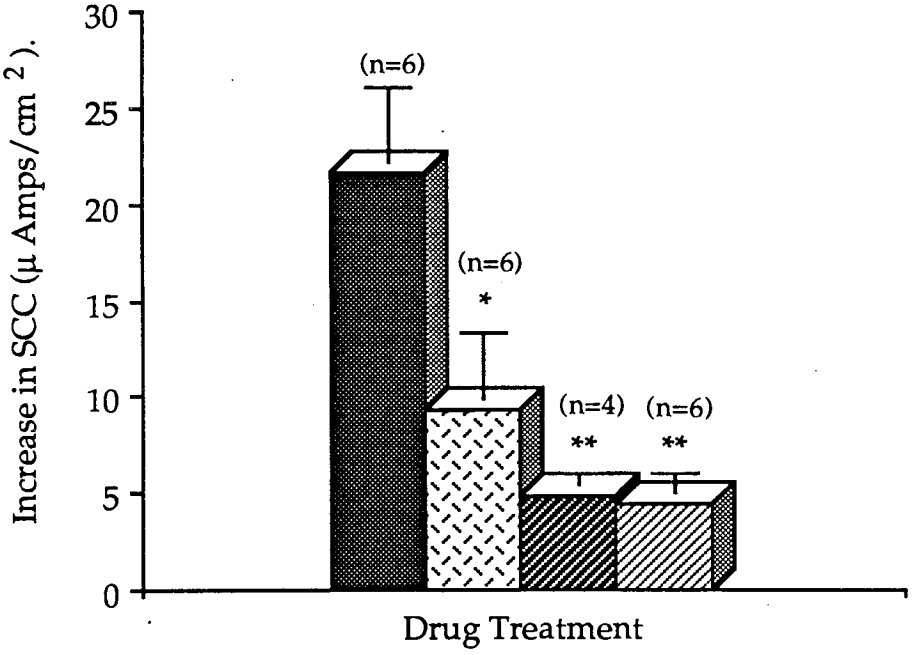

FIGURE 32: The increases in SCC responses evoked by 10^{-6} M 5-HT in the rat jejunum *in-vitro* in the absence (■) and presence of either 10^{-7} M prazosin (▣), 10^{-5} M corynanthine (▨), or 10^{-5} M cirazoline (▨). Results are means values and vertical lines indicate the SEM (* = P<0.05 and ** = P<0.01, when compared to the responses by 5-HT alone).

adrenoceptor processes and 5-hydroxtryptaminergic systems, in the control of jejunal electrolyte transport. To this end the effects of the selective α_1-adrenoceptor antagonists, prazosin and corynanthine, and the α_1-

adrenoceptor agonist cirazoline (tested as an antagonist), were investigated upon the increases in SCC to 5-HT. Antagonists were added serosally 15 minutes after tissue mounting, and 5-HT added a further 15 minutes later. Tissue viability was tested as described previously.

In the presence of 10^{-7} M prazosin, the increase in SCC evoked by 10^{-6} M 5-HT was significantly ($P<0.05$) reduced (9.3 + 3.5 μ Amps/cm^2, n=6) when compared with that seen in the absence of prazosin. Similarly, both 10^{-5} M corynanthine and 10^{-5} M cirazoline caused a significant ($P<0.01$) reduction of the SCC response to 5-HT, to 4.4 ± 1.1 μ Amps/cm^2, n=4 and 4.9 ± 0.6 μ Amps/cm^2, n=6 respectively (figure 32).

There thus appears to be some interaction between 5-hydroxtryptaminergic systems and α_1-adrenoceptors in evoking the increases in SCC by 5-HT.

7. THE EFFECT OF α_2- AND β-ADRENOCEPTOR ANTAGONISM UPON THE INCREASE IN BASAL SCC EVOKED BY 5-HT.

Since the results of Section 6 above, suggest an interaction between α_1-adrenoceptors and 5-hydroxtryptaminergic systems, in evoking the increase in SCC to 5-HT, it was considered worthwhile to investigate whether there was any other interaction with adrenoceptor mechanisms in this response.

β_2-Adrenoceptor stimulation is also reported to evoke transient increases in SCC in the rat jejunum (Dettmar *et al*, 1986b), and thus there was the possibility that there may be an interaction between β-adrenoceptors and 5-hydroxtryptaminergic systems in evoking the 5-HT responses. So the effects of the non-selective β-adrenoceptor antagonist propranolol were investigated upon these responses to 5-HT.

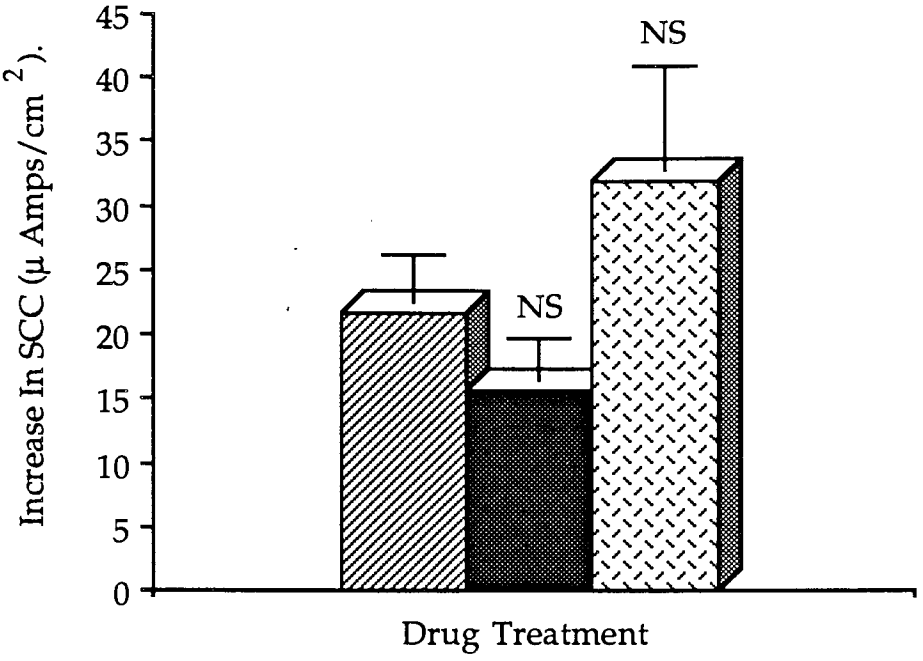

FIGURE 33: The increases in SCC evoked by 10^{-6} M 5-HT in the rat jejunum *in-vitro* in the absence (▨) and presence of either 10^{-6} M idazoxan (⊡) or 10^{-6} M propranolol (■). Results are means values from six experiments and vertical lines indicate the SEM (NS = P>0.05, when compared to the responses by 5-HT alone).

The effects of selective α_2-adrenoceptor antagonism by idazoxan was investigated upon the 5-HT evoked secretory responses.

Antagonists were added serosally 15 minutes after tissue mounting, and 5-HT added a further 15 minutes later. Tissue viability was tested as described previously. There was no significant difference (P>0.05) between the increases in SCC evoked by 10^{-6} M 5-HT in the presence and absence of 10^{-6} M propranolol (figure 33), suggesting that there was no involvement of β-adrenoceptors in the 5-HT evoked response.

Although there was a slight enhancement of the increase in SCC evoked by 10^{-6} M 5-HT, in the presence of 10^{-6} M idazoxan (figure 33), significance was not achieved (P>0.05), suggesting in fact that there was no α_2-adrenergic down-regulation of the 5-HT secretory response.

8. THE EFFECT OF RESERPINIZATION UPON THE INCREASE IN BASAL SCC EVOKED BY 5-HT.

The results of the present study have shown that the increases in SCC evoked by 5-HT in the rat jejunum, are in part neurally mediated and also associated with α_1-adrenoceptor stimulation. The question therefore arises,

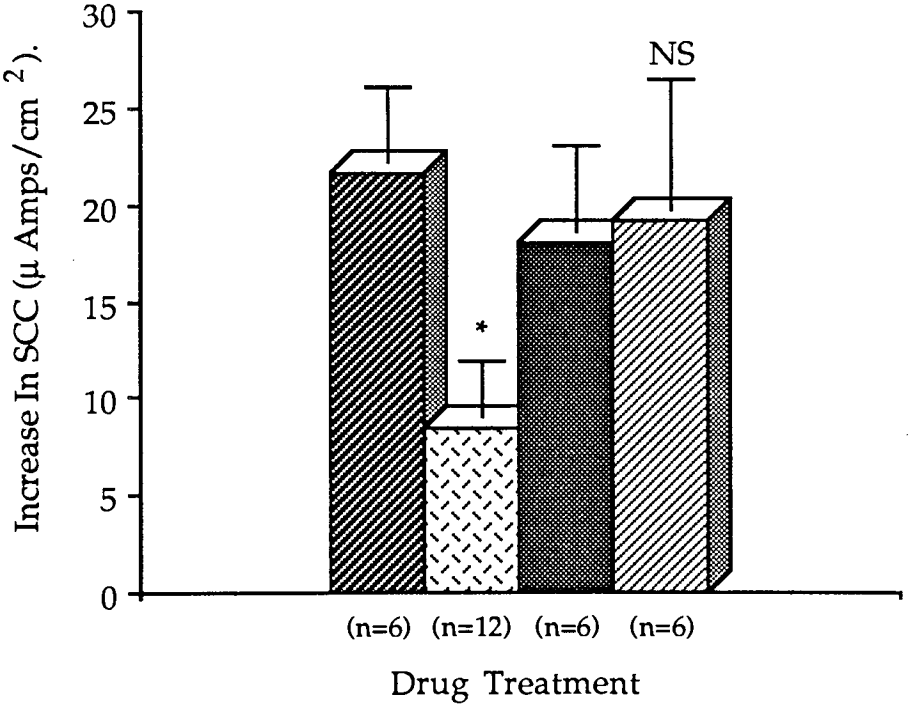

FIGURE 34: Increases in SCC of the rat jejunum *in-vitro* evoked by 10^{-6} M 5-HT (▨), 5 X 10^{-7} M carbachol (■), 10^{-6} M 5-HT after 48 hour pretreatment with reserpine (5 mg/Kg, i.p.) (▨), or 5 X 10^{-7} M carbachol also after 48 hour pretreatment with reserpine (5 mg/Kg, i.p.) (▨). Results are means values and vertical lines indicate the SEM (* = P<0.05, when compared to the responses by 5-HT in untreated tissues. NS = P>0.05 when compared to the responses by carbachol in untreated tissues).

as to whether the 5-HT evoked responses are associated with sympathetic nerve stimulation. In an attempt to examine this possibility, the effects of animal pre-treatment with reserpine were investigated upon the 5-HT evoked responses. Reserpine depletes the sympathetic nervous system of the neurotransmitter noradrenaline, and results in a pharmacological denervation (Juorio and Gabella, 1974). A similar treatment regime was used to that described by Ainsworth *et al* (1982), which involved the pre-treatment of animals with reserpine (5 mg/Kg, i.p.) 48 hours prior to being prepared for experimentation. 5-HT was again added 30 minutes after tissue mounting and tissue viability was tested as described previously. The effects of reserpine pre-treatment on the secretory responses to carbachol were also investigated.

There was no significant difference ($P>0.05$) between the basal levels of SCC recorded 30 minutes after mounting in tissues prepared from rats pretreated with reserpine (29.3 ± 5.0 μ Amps/cm^2, n=6) and those from untreated animals (26.6 ± 2.7 μ Amps/cm^2, n=6).

The increase in SCC evoked by 10^{-6} M 5-HT after reserpine pretreatment was significantly ($P<0.05$) reduced to 8.4 ± 3.0 μ Amps/cm^2, n=12, whereas the response to 5×10^{-6} M carbachol was not significantly effected ($P>0.05$) (figure 34). This would suggest that part of the 5-HT evoked response was in fact mediated via the stimulation of sympathetic nerves.

9. THE IONIC NATURE OF THE INCREASE IN BASAL SCC EVOKED BY 5-HT.

To determine the ionic nature of the increases in SCC to 5-HT, a similar pharmacological technique to that used in Chapter 4, Section 8, was employed, which involved the selective inhibition of ion transport mechanisms. Electrogenic Na^+ and Cl^- transport was attenuated by the Na^+ and Cl^- transport inhibitors amiloride and piretanide, respectively, whereas

electrogenic HCO_3^- secretion was attenuated by the carbonic anhydrase inhibitor acetazolamide. Piretanide (10^{-3} M) was administered serosally, amiloride (10^{-3} M) mucosally, and acetazolamide (10^{-3} M) both serosally and mucosally. 5-HT was added 30 minutes after tissue mounting and tissue viability was tested as described previously.

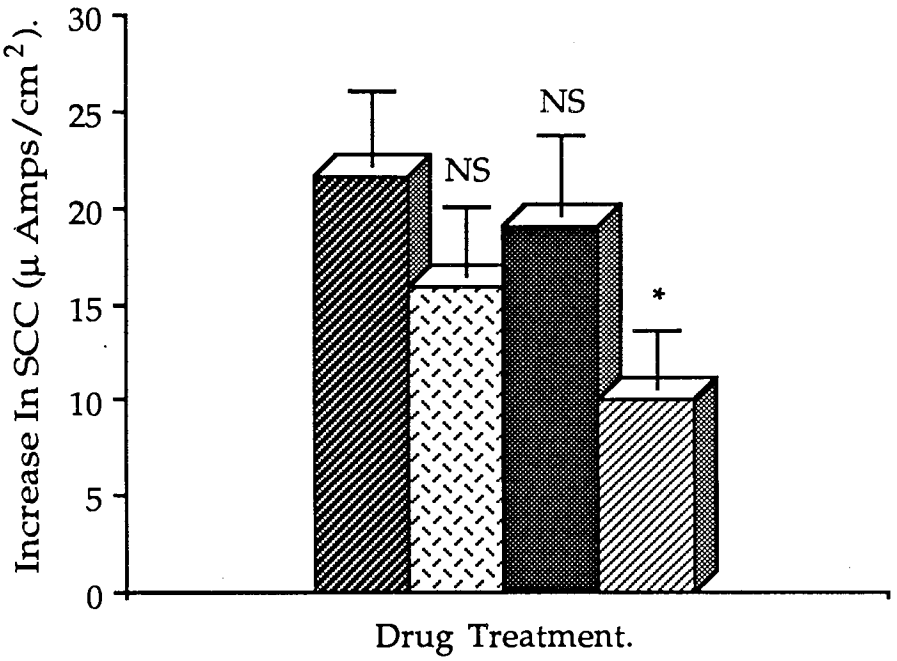

FIGURE 35: The increases in SCC evoked by 10^{-6} M 5-HT in the rat jejunum *in-vitro* in the absence (▨) and presence of either 10^{-3} M acetazolamide (▨), 10^{-3} M amiloride (⊡), or 10^{-3} M piretanide (■). Results are means values from six experiments and vertical lines indicate the SEM (* = $P<0.05$ and NS = $P>0.05$).

There was no significant ($P>0.05$) difference between the increases in SCC evoked by 5-HT (10^{-6} M) in the presence and absence of amiloride (10^{-3} M) or piretanide (10^{-3} M). However acetazolamide (10^{-3} M) significantly ($P<0.05$) reduced the 5-HT induced response, to an increase in SCC of 10.1 ± 3.0 μ Amps/cm2, n=6 (figure 35). These concentrations of acetazolamide and

amiloride were without effect upon theophylline stimulated SCC, whereas piretanide completely reversed the theophylline evoked response. Part of the 5-HT response therefore appeared to be associated with HCO_3^- transport, possibly electrogenic secretion.

10. GANGLIONIC STIMULATION BY 1,1-DIMETHYL-4-PHENYL-PIPERAZINIUM, AND THE EFFECT ON BASAL SCC.

Since a major component of the secretory response to 5-HT appears to be associated with neural stimulation, it was considered of interest to investigate whether the response could be mimicked by ganglionic

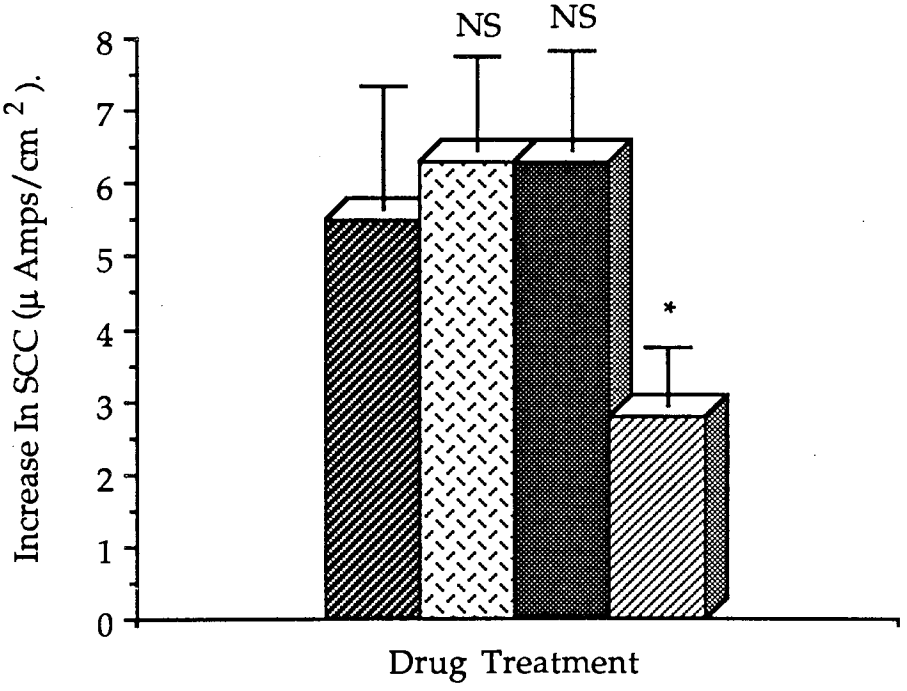

FIGURE 36: Increases in SCC of the rat jejunum *in-vitro* evoked by 10^{-6} M 1,1-dimethyl-4-phenyl-piperazinium (DMPP), in the absence (▨) or presence of either 10^{-7} M prazosin (◹) or 10^{-6} M atropine (▨), or after 48 hour pretreatment with reserpine (5 mg/Kg, i.p.) (■). Results are means values from six experiments and vertical lines indicate the SEM. NS = $P>0.05$ and * = $P<0.05$, when compared to the responses by DMPP in untreated tissues.

stimulation. The nicotinic receptor agonist, 1,1-dimethyl-4-phenyl-piperazinium (DMPP) was used to stimulate the ganglia. DMPP (10^{-6} M) was added serosally 30 minutes after tissue mounting, and tissue viability was tested as described previously. DMPP evoked a small transient increase in SCC of 5.5 ± 1.7 μ Amps/cm^2, n=6. This transient increase in SCC peaked in

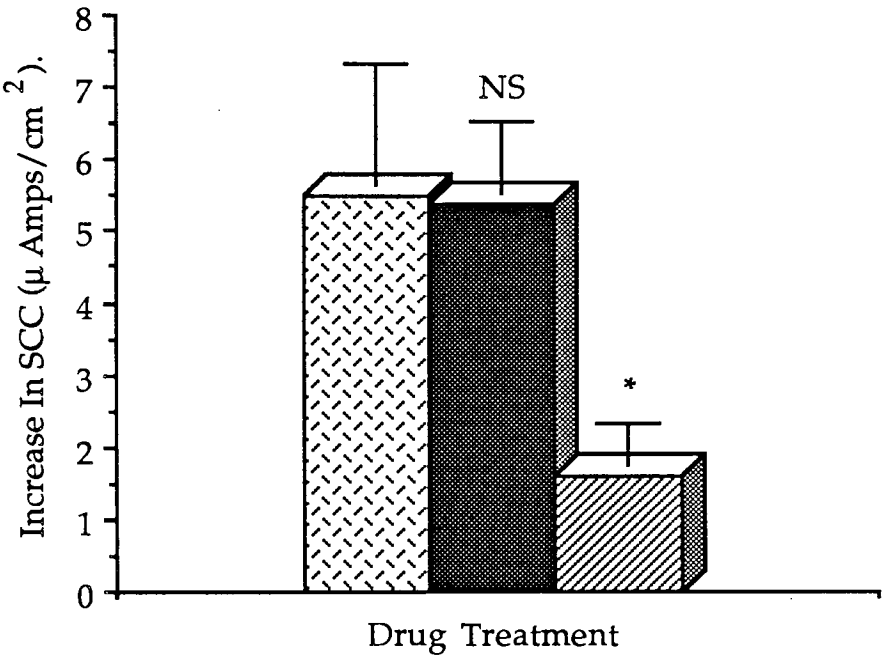

FIGURE 37: Increases in SCC of the rat jejunum *in-vitro* evoked by 10^{-6} M DMPP in the absence (▨) and presence of either 10^{-3} M acetazolamide (■) or 10^{-3} M piretanide (▨). Results are means values from six experiments and vertical lines indicate the SEM. NS = P>0.05 and * = P<0.05.

0.8 ± 0.1 minutes, and the half life for the decay of the peak was 0.7 ± 0.1 minutes (n=6). The effects of various antagonists were investigated upon this DMPP response, to see if a similar pharmacological profile to that observed for the 5-HT could be obtained. The effects of the antagonists prazosin and atropine, and the effects of reserpine pretreatment, were investigated upon the DMPP evoked response. In addition the ionic nature of the response was determined, again using the selective pharmacological inhibition of ion transport processes. Antagonists were administered again serosally 15

minutes after tissue mounting.

The response to DMPP was not significantly ($P>0.05$) changed either in the presence of prazosin, or in tissues from rats which had undergone a 48 hour pretreatment with reserpine (5 mg/Kg, i.p.). There was therefore no evidence to suggest involvement of either α_1-adrenoceptors or the sympathetic nervous system in the response to DMPP. However the DMPP evoked response was significantly antagonised by 10^{-6} M atropine, to 2.8 ± 0.8 μ Amps/cm^2, n=6, suggesting that muscarinic-cholinoceptor stimulation might be involved in the response (figure 36).

There was no significant ($P>0.05$) difference between the increases in SCC evoked by 10^{-6} M DMPP in the presence and absence of 10^{-3} M acetazolamide, whereas 10^{-3} M piretanide significantly ($P<0.05$) reduced the DMPP induced response, to an increase in SCC of 1.6 ± 0.6 μ Amps/cm^2, n=6 (figure 37). This suggests that the response to DMPP was at least in part mediated through electrogenic Cl^- secretion and not HCO_3^- secretion.

11. DISCUSSION.

The present investigation has shown that 5-HT administered to the rat jejunum *in-vitro*, evoked transient increases in SCC. Similar findings have also been reported in the rat ileum (Donowitz *et al*, 1979) and jejunum (Hardcastle *et al*, 1981) *in-vitro*.

Peripheral neural activation by 5-HT has been reported by Round and Wallis (1986), who showed that 5-HT depolarizes both the afferent vagal neurones in the nodose ganglia and sympathetic neurones in the superior cervical ganglia of the rabbit, both effects were specifically and selectively antagonised by ICS 205930. In addition Branchek *et al* (1984) described high affinity, saturable, reversible binding sites for [^{3}H] 5-HT in enteric neural membranes. These sites are different from either the 5-HT$_1$ or the 5-HT$_2$ class

of central nervous system 5-HT receptor, and the structure-activity requirements of indoles for the enteric binding sites parallel their requirements for the pharmacological activity at M receptors. Furthermore Mawe *et al* (1986) have suggested that there are two classes of enteric neural 5-HT receptors located on myenteric neurones, i.e., 5-HT_{1P} and 5-HT_{2P} receptors, which are selectively antagonised by 5-hydroxytryptophenyl-5-hydroxytryptophan amide and ICS 205930 respectively. ICS 205930 has also been reported to reduce by approximately 50%, cholera toxin induced intestinal secretion in the mouse (Buchheit, 1989), however the doses required were about 1000 times higher than those which inhibit the Bezold Jarisch reflex. It was suggested that this differential potency might have as its basis 5-HT_3 receptor heterogeneity. The affinity of ICS 205930 for 5-HT_3 receptors has been shown to be 200 times higher in the isolated rabbit vagus nerve than in the isolated guinea-pig ileum (Richardson and Engel, 1986). Buchheit (1989) has suggested that different 5-HT_3 receptor subtypes might account for the different potencies in the Bezold-Jarisch experiment and on intestinal secretion. Ball *et al* (1988b), have suggested that in the rat ileum, high concentrations of 5-HT stimulate neuronal 5-HT_3 receptors, whereas low concentrations stimulate non-neuronal benzamide-sensitive 5-HT-receptors, which are not 5-HT_1, 5-HT_2 or 5-HT_3-like in nature. In contrast Beubler and Burg (1989), have reported that 5-HT released by cholera toxin in the rat jejunum, stimulates both 5-HT_2 and 5-HT_3 receptors.

Although in the present investigation no attempt was made to fully classify 5-HT sub-receptors involved in the increase in jejunal SCC evoked 5-HT, the general 5-HT receptor antagonist, methysergide did significantly reduce the secretory response elicited by 5-HT. Since evidence of a neurogenic component in the 5-HT evoked secretory response was obtained (as discussed later) in the present study, the effects of 5-HT_3 receptor antagonism were investigated using ICS 205930 and BRL 24924. However no conclusion about

5-HT_3 receptor involvement in the secretory response to 5-HT can be made, since both of these antagonists produced a smaller level of inhibition of the 5-HT evoked response, when compared to that of methysergide.

The neurotoxin tetrodotoxin, which has a selective blocking action upon enteric neurally mediated intestinal transport rather than affecting the transporting cells directly (Carey *et al*, 1985), was found to inhibit the 5-HT evoked response but not totally abolish it. This suggests that the major part of the 5-HT response was mediated through nervous stimulation, and is consistent with the finding of Ball *et al* (1988b), who showed that 5-HT evoked secretory responses in the rat ileum, were mediated via both neuronal and non-neuronal mechanisms. In the present study the residual response to 5-HT after neuronal blockade by tetrodotoxin, was resistant to antagonism by the general 5-HT-receptor antagonist methysergide. The residual response evoked by 5-HT in the presence of tetrodotoxin might have been due to incomplete sodium channel antagonism, however a non-neuronal or a non-specific action of 5-HT cannot be totally excluded.

There is also considerable circumstantial evidence supporting a 5-hydroxtryptaminergic neural mechanism. The sparse distribution of 5-hydroxtryptaminergic fibers in the mucosa reduces the possibility that these neurones influence mucosal function directly by releasing neurotransmitters at the neuroenterocyte junction (Keast *et al*, 1984). The morphology of 5-hydroxtryptaminergic neurones with their single long axons that project over relatively long distances down the intestine, suggests that they are involved in transmitting information down the gut to the musculature and the submucosal plexus. Wood (1984) has hypothesised that activation of 5-hydroxtryptaminergic neurones excites a network of synaptically coupled neurones, that function to ensure the simultaneous excitation or inhibition of the musculature and secretory epithelium around the circumference of the intestine. Furthermore Gaginella *et al* (1983), using a receptor ligand binding

technique were unable to detect any 5-HT-receptors on rat intestinal epithelial cell membranes. It is possible that 5-hydroxtryptaminergic neurones are involved in synaptic interactions with interneurones in the submucosal ganglia and with motor neurones that innervate the mucosal effectors. In addition it has been suggested that the neural effect to cholera toxin in cats and rats is mediated by the release of 5-HT from mucosal endocrine cells, which then depolarizes afferent nerves of a reflex circuit that eventually release secretory neurotransmitters (Cassuto *et al*, 1981a, 1981b, 1982a, 1982b, 1982c and 1983).

The increase in SCC evoked by 5-HT was resistant to antagonism by the selective muscarinic-cholinoceptor antagonist atropine, at a concentration which completely abolished the secretory response to the muscarinic-cholinoceptor agonist carbachol, thus suggesting that there was no indirect involvement of muscarinic-cholinoceptor stimulation in the 5-HT evoked response, and this is in agreement with a similar *in-vivo* observation reported by Hardcastle *et al* (1981).

Surprisingly the 5-HT evoked response was antagonised by the selective α_1-adrenoceptor antagonists, prazosin and corynanthine, and by the selective α_1-adrenoceptor agonist cirazoline when tested as an antagonist. This would suggest that the 5-HT induced response was indirectly associated with α_1-adrenoceptor stimulation. It was previously demonstrated in Chapter 4, that α_1-adrenoceptor stimulation can evoke a secretory response in the rat jejunum. Furthermore Hardcastle *et al* (1981), reported that 5-HT elicited an increase in transepithelial potential difference in the rat jejunum *in-vivo*, and that the α-adrenergic blocking agent phenoxybenzamine did not alter the affinity of 5-HT for its receptor, as reflected in the concentration required to elicit a 50% maximum response, but it did reduce the maximum effect of 5-HT. The possibility therefore exists that 5-HT stimulates a neurally located 5-HT-receptor in the jejunum, which then results in depolarization and

release of a neurotransmitter that activates an α_1-adrenoceptor, leading to an increase in SCC. If the neurotransmitter released was to be noradrenaline, then the possibility of interactions with other adrenoceptors might exist.

β_2-Adrenoceptor stimulation by β-adrenoceptor agonists has been reported to elicit transient increases in SCC in the rat jejunum *in-vitro* (Dettmar *et al*, 1986b). However in the present investigation the response induced by 5-HT was resistant to β-adrenoceptor antagonism by propranolol, suggesting no involvement of β-adrenoceptors. Noradrenaline can also stimulate α_2-adrenoceptors in this tissue, which have been shown to be antisecretory (Chapter 3), and thus such activation would tend to downgrade any α_1-adrenoceptor mediated secretory responses. If this is the case then α_2-adrenoceptor antagonism should potentiate the response to 5-HT. Although the response elicited by 5-HT was slightly larger after α_2-adrenoceptor antagonism by idazoxan, the increase was not significant, and so if noradrenaline is the neurotransmitter involved, then α_1-adrenoceptor activation would appear to predominate in the region of the neuroenterocyte junction innervated by the nerves stimulated by 5-HT.

If 5-HT is stimulating sympathetic nerves to release noradrenaline at the neuroenterocyte junction, then depletion of the noradrenaline stores in the nerve terminals by reserpine pretreatment should reduce the 5-HT evoked response. This was found to be the case, reserpine pretreatment did in fact reduce the 5-HT evoked response, although it did not abolish it. The residual response may have been due to incomplete depletion of the noradrenaline stores, the release of another unknown neurotransmitter and/or the non-neuronal component of the 5-HT response. The 5-HT evoked increase in SCC does therefore appear at least in part, to be associated with the activation of sympathetic nerves resulting in noradrenaline release and α_1-adrenoceptor stimulation.

In an attempt to determine the ionic nature of the 5-HT response, selective

pharmacological inhibition of ion transport processes was undertaken. The 5-HT induced response was resistant to the chloride and basolateral $Na^+/K^+/Cl^-$ co-transport inhibitor, piretanide, at a concentration which completely reversed theophylline stimulated SCC. In addition the sodium transport inhibitor amiloride was also without effect. This is consistent with the work of Sheerin (1979), who could not demonstrate any changes in the movement of sodium and chloride across sheets of rabbit ileal mucosa, although a transient increase in SCC was obtained when 5-HT was added. In contrast Hardcastle *et al* (1981), suggested that the 5-HT evoked SCC responses in the rat jejunum were associated with chloride secretion, based on a sodium and chloride flux study. However there must be some doubt as to the interpretation of this study since in 1987 Hardcastle and Hardcastle, when investigating transient responses to histamine in the rat proximal colon, suggested that it is not acceptable to use ion flux determinations for investigating transient SCC responses of relatively short duration, since the tissue is not in a steady state condition.

In the present investigation the carbonic anhydrase inhibitor acetazolamide, at a concentration which was without effect on theophylline stimulated SCC, significantly reduced the 5-HT evoked response but did not abolish it, suggesting an association with electrogenic HCO_3^- secretion. The involvement of other ionic species apart from HCO_3^- in the 5-HT response cannot be discounted, since acetazolamide only partly reduced the response. In addition, caution must be exercised in interpreting the lack of effect by piretanide, since very recent evidence has suggested that although a loop diuretic-sensitive co-transport mechanism may be present in the small intestine, it may not contribute significantly to basolateral Cl^- uptake under secretory conditions (Stewart and Turnberg, 1989). Selective ion replacement studies would be required to fully elucidate the complete ionic nature of the 5-HT evoked secretory response.

The association of the 5-HT response with HCO_3^- transport, sets up a pharmacological paradox since the response was also associated with indirect α_1-adrenoceptor activation, and such activation was found previously to be associated with chloride secretion and not HCO_3^- transport (Chapter 4, Section 8). One possibility which was not tested, is that the effect of 5-HT upon HCO_3^- transport may not have been associated with the indirect neuronal activation of α_1-adrenoceptors, but due to an association with the non-neuronal component of the response. Another possibility is that the α_1-adrenoceptors activated by the sympathetic nerves stimulated by 5-HT, were located on epithelial cells which were predominately involved with HCO_3^- transport, whereas exogenous α_1-adrenoceptor agonists would activate α_1-adrenoceptors which are not innervated and linked to Cl^- secretion.

Since a major component of the secretory response to 5-HT appears to be associated with neural stimulation, it was considered of interest to see if the response could be mimicked by ganglionic stimulation and a similar pharmacological profile obtained. Although the nicotinic receptor agonist DMPP did evoke a small transient increase in SCC in the rat jejunum, the response appeared to have a different pharmacological profile to that of 5-HT. The response to DMPP was resistant to antagonism by prazosin or by animal pretreatment with reserpine, but was significantly antagonised by atropine. In addition the DMPP induced response appeared to be associated with electrogenic chloride and not bicarbonate transport, as a reflection of the inhibitory effect piretanide and lack of effect of acetazolamide. This data suggests that the DMPP induced response was not associated with sympathetic nerve stimulation or activation of α_1-adrenoceptors, but due to muscarinic-cholinoceptor stimulation. The above findings are consistent with the report of Hardcastle *et al* (1981), who were unable to antagonise the 5-HT responses in the rat jejunum *in-vivo* with the nicotinic receptor antagonist hexamethonium, and the report of Keast *et al* (1985), who suggested that the

ganglionic stimulant DMPP preferentially stimulated cholinergic nerves, in eliciting increases in SCC in the guinea-pig small intestine.

The results of the present study have shown that 5-HT evokes a transient increase in SCC in the rat jejunum *in-vitro*. This response appears to be partly mediated via sympathetic nerve stimulation, resulting in activation of α_1-adrenoceptors. The neural activation by 5-HT may possibly be post-ganglionic, and there appears to be an association of electrogenic HCO_3^- transport in the total SCC response. A cholinergic mechanism does not appear to be associated with the 5-HT evoked response, although there is a small non-neural component for which the mechanism is unknown.

RESULTS CHAPTER 6.

THE ACTION OF β-ADRENOCEPTOR STIMULATION UPON BASAL SCC IN THE RAT DISTAL COLON *IN-VITRO*.

1. INTRODUCTION.

In the previous Chapters evidence has been presented for a jejunal α_2-adrenoceptor antisecretory and α_1-adrenoceptor secretory regulation in the rat, the latter also being associated with sympathetic nervous activation during 5-HT evoked secretory responses. In addition Dettmar *et al* (1986b) have reported that electrogenic secretory responses in the rat jejunum *in-vitro*, may be mediated via β_2-adrenoceptor activation.

Numerous studies have also supported a physiological role for adrenergic regulation of large intestinal electrolyte transport, although there is some uncertainty about the particular adrenoceptor sub-types involved, which probably arises from species variation and the heterogeneity of this transporting epithelium. Adrenaline administered to the rabbit colon *in-vitro* (Sellin and DeSoignie, 1984), and to the rat colon *in-vitro* (Racusen and Binder, 1979), stimulates coupled sodium chloride absorption. In addition Racusen and Binder (1979) have attributed the antisecretory decreases in SCC in response to adrenaline, to both α and β-adrenoceptor activation, the latter regulation contrasting the findings of Dettmar *et al* (1986b) in the rat jejunum.

In-vitro, studies in rat colon suggest that catecholamines affect electrolyte transport directly, in that their effects are not inhibited by tetrodotoxin, reserpine, naloxone, or atropine (Racusen and Binder, 1979). It therefore appears that no known neurotransmitter, opioid, or cholinergic intermediate are involved.

There is considerable *in-vitro* evidence that the α_2-adrenoceptor sub-type has an antisecretory regulation over water and electrolyte transport in the mammalian colon, similar to that observed in the rat jejunum in the present study. Dharmsathaphorn *et al* (1984), found that decreases in SCC and secretory processes were induced by α_2-adrenoceptor agonists in the rat colon *in-vitro*. Albin and Gutman (1980) found that α-adrenoceptor stimulation in the rabbit distal colon *in-vitro*, enhanced net sodium absorption through the mucosa, whilst Boige *et al* (1984) have shown that stimulation of α_2-adrenoceptors in human colonic crypts inhibited VIP-induced cAMP accumulation.

There is less information regarding the role of β-adrenoceptors in the regulation of colonic intestinal water and electrolyte transport. Coyne *et al* (1974 and 1976), reported that propranolol inhibited bile acid stimulation of rabbit colonic adenylate cyclase *in-vitro*, and furthermore Conley *et al* (1976), have reported that propranolol inhibited adenylate cyclase and secretion by deoxycholic acid in the rabbit colon *in-vivo*. In contrast Hall *et al* (1981) found that propranolol was ineffective in the treatment of bile acid induced diarrhoea in humans. β-Adrenoceptor activation in the rabbit distal colon has been shown to stimulate active potassium secretion (Halm *et al*, 1983; Halm and Frizzell, 1986), a response which was later attributed to β_1-adrenoceptors (Smith and McCabe, 1986). Furthermore cAMP has been reported to elicit a similar secretory response in the rat proximal colon (Foster *et al*, 1983). A stimulation of electrogenic cationic secretion such as K^+, would manifest as a decrease in SCC. When Racusen and Binder (1979) observed the β-adrenoceptor mediated decreases in SCC in the rat colon, the region of the colon used for the investigation was not specified. It is now known that there are distinct differences in electrolyte transport between the proximal and distal colonic regions and between species (Binder and Sandle, 1987). In the present study an attempt was made to distinguish the particular β-adrenoceptor sub-type involved in the regulation of rat colonic basal

electrogenic electrolyte transport in a specified region of the colon, i.e., the distal colon *in-vitro*.

2. THE EFFECTS OF ADRENALINE UPON BASAL SCC IN THE RAT DISTAL COLON *IN-VITRO*.

Tissues were prepared and SCC recorded as described in Chapter 2, Section 1. Control tissues showed that basal SCC (and PD) initially gradually declined with time, but levelled off 30 minutes after tissue mounting. Tissue resistance was calculated according to Ohm's law, and was found to be 101 ± 5 $\Omega.cm^{-2}$, n=6. Antagonists were added 15 minutes and agonists 30 minutes after tissue mounting. With every monolateral drug addition an equal volume of Krebs' bicarbonate Ringer solution was added to the opposite side of the tissue. Control tissues received a bilateral addition of Krebs' bicarbonate Ringer solution.

The effects of the non-selective adrenoceptor agonist adrenaline were investigated upon basal SCC. No attempt was made to construct log. concentration-response curves with respect to decreases in basal SCC evoked by adrenaline, because of the variability in the basal SCC recorded. Adrenaline (10^{-6} M) evoked a sustained decrease in basal SCC of 43 ± 4 μ Amps/cm^2, n=6 (figure 38). Racusen and Binder (1979) have reported that 5 X 10^{-5} M adrenaline evoked a maximal reduction in SCC in the rat colon *in-vitro*, whilst the EC_{50} was 1.8 X 10^{-7} M.

Propranolol (10^{-6} M), the non-selective β-adrenoceptor antagonist, used alone caused a decrease basal SCC of 26 ± 7 μ Amps/cm^2, n=4, whereas 5 X 10^{-6} M of the non-selective β-adrenoceptor antagonist timolol (Scriabine *et al*, 1973) was without effect upon basal SCC. Unlike propranolol, timolol is reported to have little membrane stabilizing activity (Brogden *et al*, 1979), and was therefore used in the following investigation.

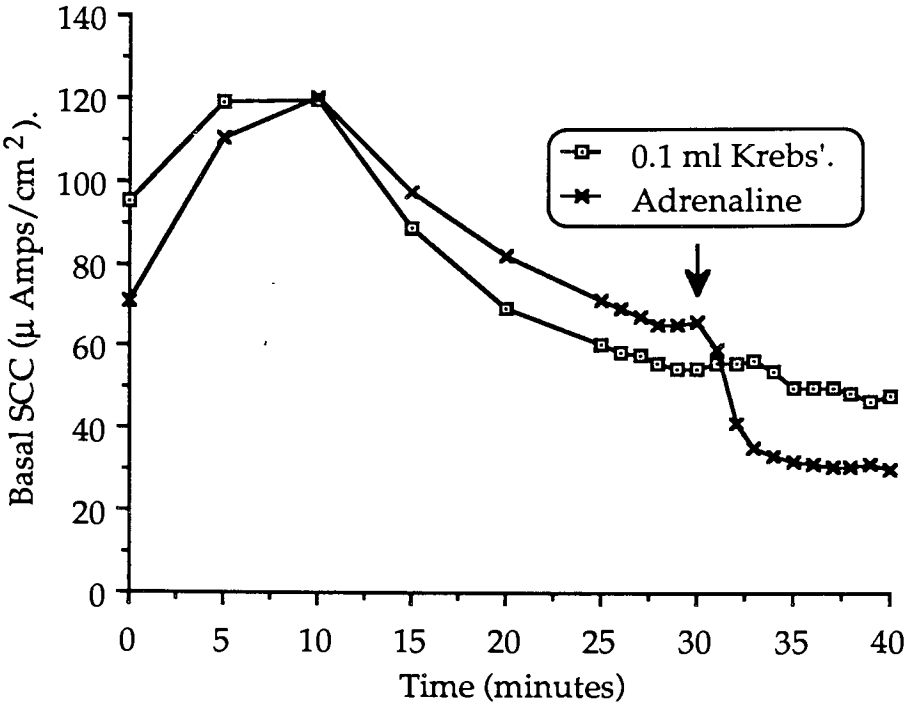

FIGURE 38: The basal SCC of the rat distal colon *in-vitro,* expressed as a function of time. The effect of a serosal addition of 10^{-6} M adrenaline is also illustrated. Control tissues received a bilateral addition of Krebs' bicarbonate Ringer solution. Each point represents the mean of six experiments.

Timolol significantly ($P<0.05$) reduced the SCC response to adrenaline to a decrease in SCC of 15 ± 2 μ Amps/cm^2, n=6 (figure 39), suggesting that β-adrenoceptor stimulation was involved. The selective α_2-adrenoceptor antagonist idazoxan (5 X 10^{-6} M) used alone had no effect upon basal SCC, but caused a non-significant ($P>0.05$) reduction of the SCC response to adrenaline. This concentration of idazoxan did however significantly ($P<0.001$) antagonise the decrease in SCC (17 ± 2 μ Amps/cm^2, n=6) evoked by the selective α_2-adrenoceptor agonist UK-14,304 (10^{-6} M), to a decrease of 2 ± 2 μ Amps/cm^2, n=6 (figure 39). Although the decrease in SCC evoked by adrenaline in the presence of idazoxan was not significantly ($p=0.08$) smaller than the response in the absence of idazoxan, an α_2-adrenoceptor component of the response to adrenaline cannot be excluded, since a functional role for

α_2-adrenoceptor activation has been demonstrated in this tissue, as reflected by the idazoxan sensitive response induced by UK-14,304. In addition, from the present study it is not known if the concentration of adrenaline used (10^{-6} M) was a supra-maximal concentration at the α_2-adrenoceptors. Furthermore β-adrenoceptor antagonism only reduced the adrenaline evoked response by approximately half (figure 39).

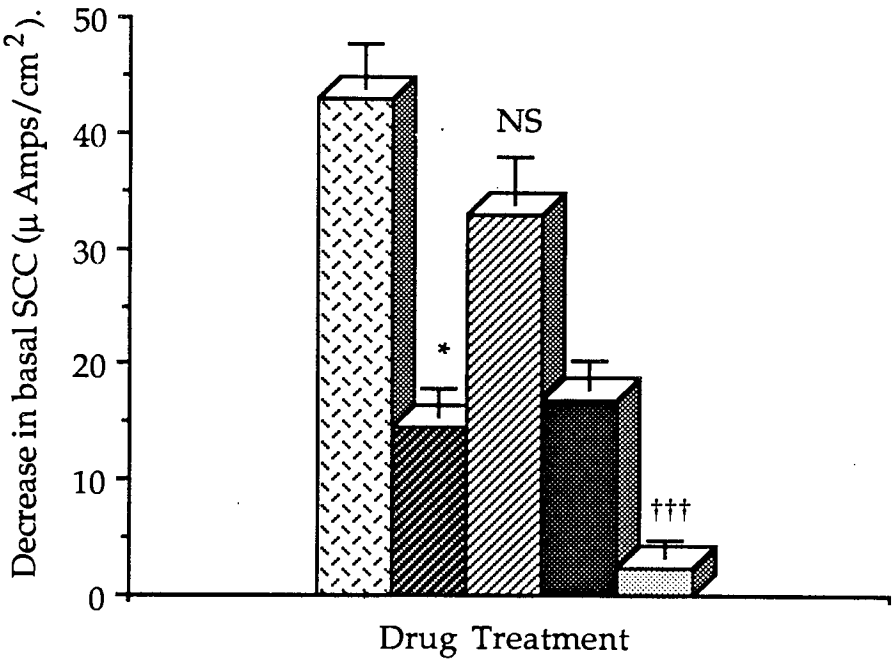

FIGURE 39: The decreases in basal SCC in the rat distal colon *in-vitro* evoked by adrenaline (10^{-6} M) in the absence (▭) and presence of either timolol (5 X 10^{-6} M) (▨) or idazoxan (5 X 10^{-6} M) (▨), and the decreases in SCC evoked by UK-14,304 (10^{-6} M), also in the absence (■) and presence (▩) of idazoxan (5 X 10^{-6} M). Results are means values from six experiments and vertical lines indicate the SEM. * = $P<0.05$, and NS = $P>0.05$ when compared to the responses by adrenaline alone. ††† = $P<0.001$, when compared with the responses by UK-14,304 alone.

3. THE EFFECTS OF β-ADRENOCEPTOR STIMULATION UPON BASAL SCC IN THE RAT DISTAL COLON *IN-VITRO*.

A). The Effects of Isoprenaline, Prenalterol and Salbutamol On Basal SCC.

The effects of the non-selective β-adrenoceptor agonist isoprenaline, the selective β_1-adrenoceptor agonist prenalterol (Carlsson *et al*, 1977), and the selective β_2-adrenoceptor agonist salbutamol (Farmer *et al*, 1970), were investigated upon colonic basal SCC. Drug addition was undertaken as described previously.

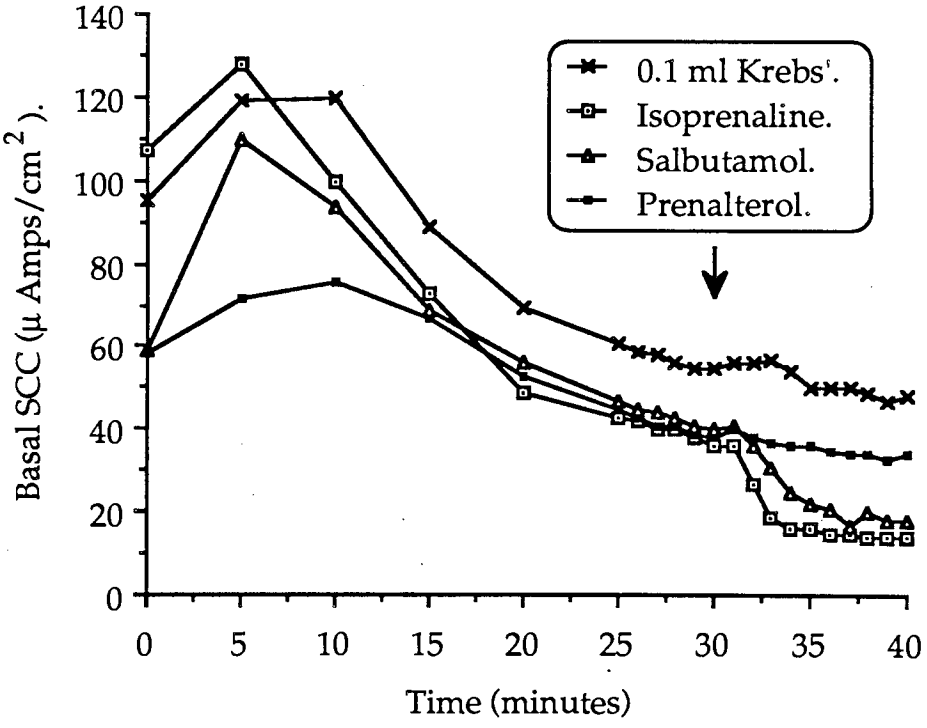

FIGURE 40: The basal SCC of the rat distal colon *in-vitro*, expressed as a function of time. Each point is the mean value of six experiments. The effects of serosal additions of either 10^{-6} M isoprenaline, prenalterol, or salbutamol are also illustrated. Control tissues received a bilateral addition of Krebs' bicarbonate Ringer solution.

Isoprenaline (10^{-6} M) caused a decrease in SCC of 22 ± 2 μ Amps/cm^2, n=6, and a similar effect was observed with salbutamol which decreased basal SCC by 21 ± 2 μ Amps/cm^2, n=6. In contrast, 10^{-6} M prenalterol was without effect upon SCC (figures 40 and 41). This would tend to suggest that a β_2-adrenoceptor mediates these decreases in SCC.

B). The Effects Of β-Adrenoceptor Antagonism On The Isoprenaline Evoked Responses.

The effects of selective adrenoceptor antagonism were investigated upon the SCC responses to isoprenaline. Drug addition was undertaken as

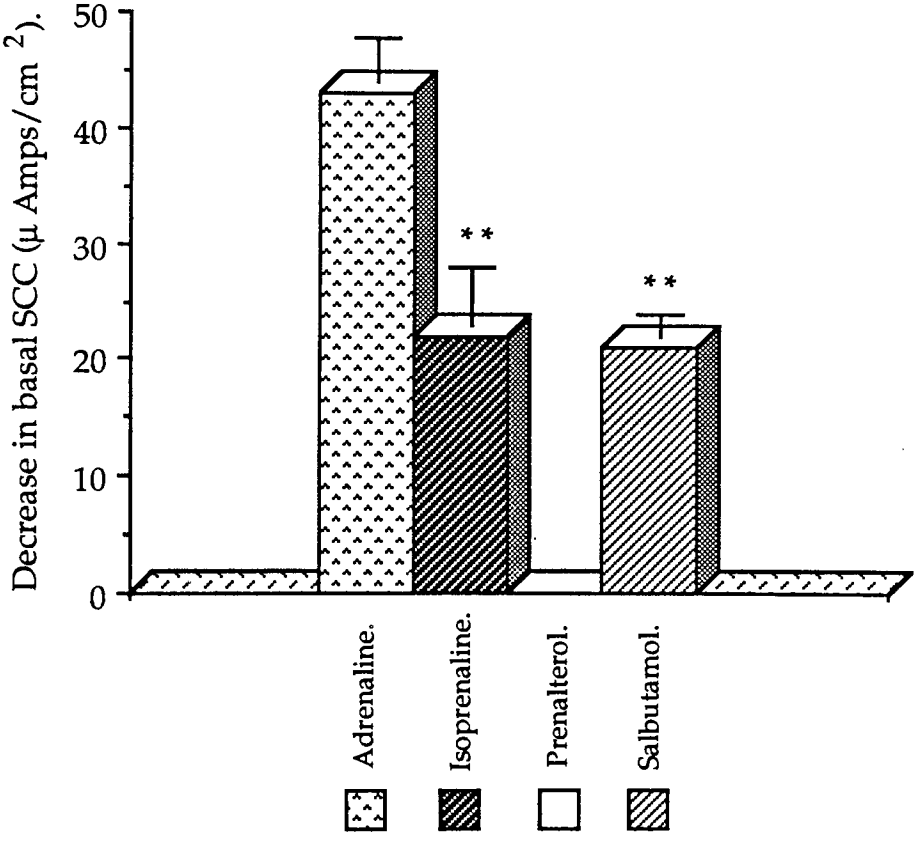

FIGURE 41: The effects of either 10^{-6} M adrenaline, isoprenaline, prenalterol, or salbutamol, on basal SCC in the rat distal colon *in-vitro*. Results are means values from six experiments and vertical lines indicate the SEM.

described previously. Unfortunately 10^{-6} M of the selective β_2-adrenoceptor antagonist ICI 118,551 (Bilski *et al*, 1980) added alone caused a decrease in SCC of 26 ± 10 μ Amps/cm^2, n=3. Bilski *et al* (1980) have reported that ICI 118,551 has membrane stabilizing action equivalent to that of propranolol, and therefore ICI 118,551 was not used for these experiments. The selective β_1-adrenoceptor antagonist metoprolol (Brogden *et al*, 1979) alone, at a concentration of 5 X 10^{-6} M was without effect upon basal SCC, and in addition the SCC response to isoprenaline was not significantly (P>0.05) affected following incubation with this concentration of metoprolol. The response to isoprenaline was also unaffected by 5 X 10^{-6} M idazoxan, whereas 5 X 10^{-6} M timolol significantly (P<0.05) reduced the response to an increase in SCC of 7 ± 4 μ Amps/cm^2, n=6 (figure 42).

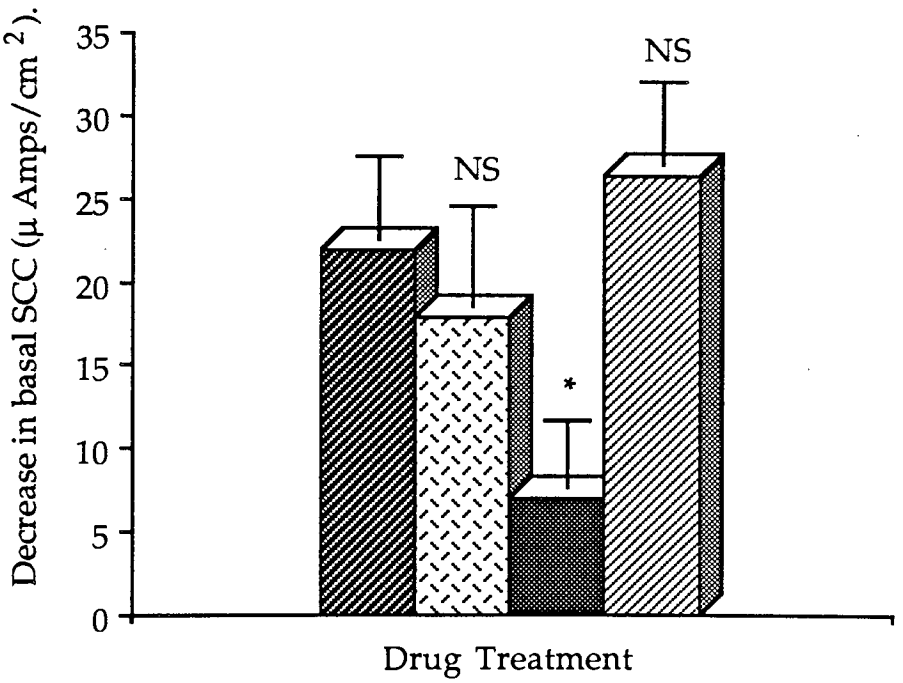

FIGURE 42: The decreases in basal SCC evoked by isoprenaline (10^{-6} M) in the rat distal colon *in-vitro*, in the absence (▨) and presence of either idazoxan (5 X 10^{-6} M) (▨), metoprolol (5 X 10^{-6} M) (▨), or timolol (5 X 10^{-6}M) (■). Vertical lines indicate SEM, and n=6 in all cases. NS = P>0.05 and *=P<0.05 when compared to the responses by isoprenaline alone.

C). The Effects Of β-Adrenoceptor Antagonism On The Salbutamol Evoked Responses.

The effects of β-adrenoceptor antagonism were investigated upon the SCC responses to the selective β_2-adrenoceptor agonist salbutamol. Drug addition was undertaken as described previously. The SCC response to salbutamol was significantly ($P<0.001$) reduced in the presence of the non-selective β-adrenoceptor antagonist timolol (5×10^{-6} M) to a decrease in SCC of 5 ± 2 μ Amps/cm^2, n=6, whereas the selective β_1-adrenoceptor antagonist metoprolol (5×10^{-6} M) was without significant ($P>0.05$) effect (figure 43).

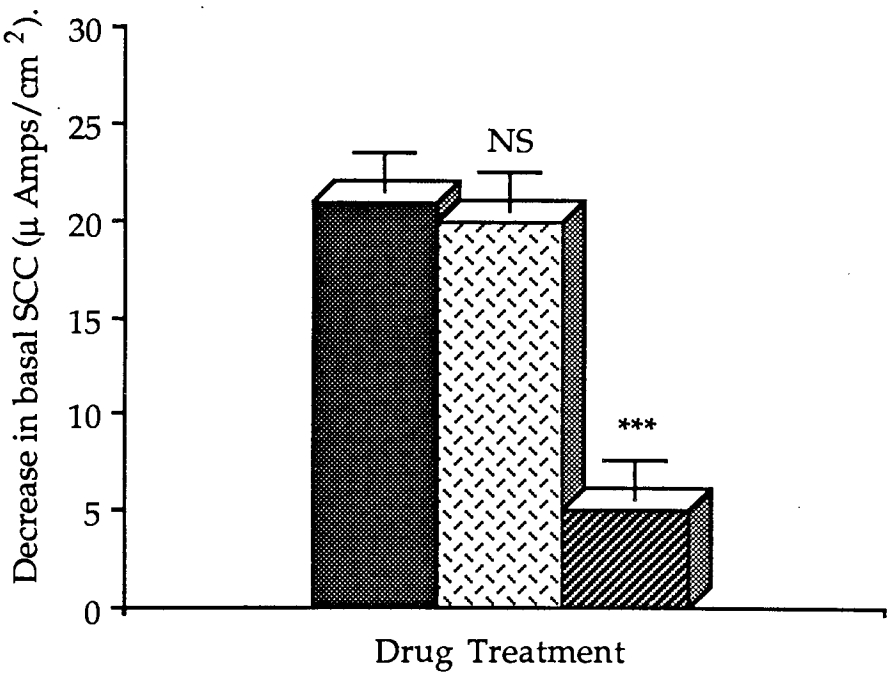

FIGURE 43: The decreases in basal SCC evoked by 10^{-6} M salbutamol in the rat distal colon *in-vitro,* in the absence (■) and presence of either 5×10^{-6} M metoprolol (▨) or 5×10^{-6} M timolol (▨). Results are means values from six experiments and vertical lines indicate the SEM. *** = $P<0.001$ and NS=$P>0.05$, when compared to the responses by salbutamol alone.

4. DISCUSSION.

The present investigation has shown that the adrenoceptor agonist adrenaline, the non-selective β-adrenoceptor agonist isoprenaline, and the selective β_2-adrenoceptor agonist salbutamol, can evoke decreases in basal SCC in the rat distal colon *in-vitro*, whereas the selective β_1-adrenoceptor agonist prenalterol is without effect. These β-adrenoceptor mediated decreases in rat colonic SCC are consistent with the findings of Racusen and Binder (1979), who also observed a similar β-adrenoceptor regulation, along with an α-adrenoceptor control.

In the present investigation the selective α_2-adrenoceptor agonist UK-14,304 elicited a decrease in basal SCC, a response which was antagonised by the selective α_2-adrenoceptor antagonist idazoxan. Such an α_2-adrenoceptor regulation has been suggested by Dharmsathaphorn *et al* (1984), who found that decreases in SCC and secretory processes were induced by α_2-adrenoceptor agonists in the rat colon *in-vitro*.

Since α_2-adrenoceptor activation appears to have a functional antisecretory role in the regulation of distal colonic electrolyte transport, and β-adrenoceptor antagonism only reduced the adrenaline evoked response by approximately half, an α_2-adrenoceptor component cannot be totally excluded from the adrenaline evoked response, even though the reduction of the response by idazoxan was just outside significance. However it is not known from the present investigation if the concentration of adrenline used is supra-maximal at the α_2-adrenoceptors in this tissue.

Not only was the selective β_1-adrenoceptor agonist prenalterol without effect upon basal SCC in the present investigation, but the β_1-adrenoceptor antagonist metoprolol also failed to antagonise the decrease SCC elicited by either isoprenaline or the selective β_2-adrenoceptor agonist salbutamol. This

suggests that it is β_2-adrenoceptors and not β_1-adrenoceptors which have the regulatory role over colonic electrogenic electrolyte transport. Unfortunately further evidence of a β_2-adrenoceptor regulation could not be obtained using the selective β_2-adrenoceptor antagonist ICI 118,551, since this drug also decreased basal SCC when used alone. Propranolol alone also produced a similar response to that of ICI 118,551, whereas timolol was without effect. Timolol is reported to have little membrane stabilizing activity (Brogden *et al*, 1979), unlike propranolol or ICI 118,551 which are reported to show equivalent membrane stabilization (Bilski *et al*, 1980). So these effects of propranolol and ICI 118,551 alone, on SCC might be due to their membrane stabilizing activity. Further investigation of this effect is necessary, in particular it would be interesting to know why there is no reduction of SCC by propranolol alone in the small intestine.

The ionic nature of the β_2-adrenoceptor mediated decreases in SCC was not addressed in the present investigation. Racusen and Binder (1979) have reported that adrenaline causes a stimulation of NaCl absorption in the rat colon *in-vitro*, therefore both pro-absorptive and/or antisecretory effects may be associated with the β_2-adrenoceptor mediated decreases SCC. Further investigations are required. In addition β_1-adrenoceptor activation in the rabbit distal colon (Smith and McCabe, 1986) and raised cAMP in the rat proximal colon (Foster *et al*, 1983) have been shown to stimulate active electrogenic potassium secretion, which manifests as a decrease in SCC, and so the possibility also exists that in the rat distal colon, the observed decreases in SCC mediated through β_2-adrenoceptor stimulation may also be associated with K^+ transport.

In contrast to the present evidence in the colon, Dettmar *et al* (1986b) have reported that in the rat jejunum *in-vitro*, β_2-adrenoceptor stimulation elicits a transient increase in SCC. In addition Coyne *et al* (1974 and 1976), reported that propranolol inhibited bile acid stimulation of rabbit colonic

adenylate cyclase *in-vitro*, and furthermore Conley *et al* (1976), have reported that propranolol inhibited adenylate cyclase and secretion induced by deoxycholic acid in the rabbit colon *in-vivo*. These colonic effects of propranolol may be due to β-adrenoceptor antagonism, however in view of the effects of propranolol alone in the present study, other mechanisms cannot be excluded, e.g., non-selective β-adrenoceptor mediated effects, or membrane stabilization. Interestingly, Hall *et al* (1981) found that propranolol was ineffective in the treatment of bile acid induced diarrhoea in humans.

RESULTS CHAPTER 7.

AN INVESTIGATION INTO POSSIBLE ADRENERGIC REGULATION OF WATER AND ELECTROLYTE TRANSPORT IN THE RAT DISTAL COLON *IN-VIVO*.

1. INTRODUCTION.

The work presented in the previous Chapters has concentrated primarily upon *in-vitro* evidence for adrenergic regulation of intestinal electrogenic ion transport in the rat. The intention of the study in this Chapter was to try and determine whether basal and/or secretagogue stimulated fluid and electrolyte transport in the rat distal colon is under adrenergic control in the *in-vivo* situation. The work reported in this Chapter was performed in the Department of Pharmacology, Reckitt and Colman Pharmaceuticals, Hull.

Based on circumstantial evidence, Chang *et al* (1983a) have suggested the concept of an adrenergic tonic regulation of fluid and electrolyte transport. They observed that a destruction of sympathetic nerve endings supplying the ileum and colon in diabetic rats, was accompanied by a malabsorption of ions. Clonidine is reported to be of use in treatment of patients with diabetic diarrhoea and autonomic neuropathy. In addition clonidine has been shown to inhibit caster oil induced diarrhoea (Lal and Shearman, 1981; Spraggs and Bunce, 1983) and to prevent naloxone-precipitated morphine withdrawal diarrhoea in rats (Nakaki *et al*, 1981; Schreier and Burks, 1980), and acute opiate withdrawal diarrhoea in humans (Gold *et al*, 1978). It is likely that some side effects of drugs may associated with their effects on intestinal electrolyte and water transport (Schmitt, 1977), e.g., clonidine is also known to cause constipation, and drug induced diarrhoea can be produced with reserpine or adrenergic blocking drugs such as guanethidine (Bowman and

Rand, 1980).

The technique employed in this study was an isolated loop technique of Bunce and Spraggs (1982). This technique was chosen in preference to the single lumen perfusion technique of Bright-Asare and Binder (1973), since Williams in 1986, when attempting to investigate adrenoceptor regulation in the colon of the rat using the single lumen perfusion technique, reported that the method lacked the necessary resolution.

In the following study, distal colonic α-adrenergic regulation of basal and secretagogue stimulated water and electrolyte transport was investigated.

2. THE EFFECT OF NORADRENALINE UPON BASAL ELECTROLYTE AND FLUID TRANSPORT IN THE RAT DISTAL COLON *IN-VIVO*.

The colonic loop was prepared as described in Chapter 2, Section 2A. At time zero a 0.25 ml bolus of known standard isotonic electrolyte solution (145 mM NaCl and 5 mM KCl) was introduced into the lumen of the colonic loop (2.5 cm in length), and the loop subsequently sealed. Anaesthesia was maintained by a slow infusion (1.5 ml/hour) of pentobarbitone sodium (6 mg/Kg/hour) via the femoral vein. The loop was then incubated for a 1 hour period, after which the contents were collected and analysed as described previously in Chapter 2, Sections 2B and 2C. Electrolyte was initially determined as a concentration and then multiplied by the volume measured at the end of the incubation period, as to yield a total amount for a particular ion. The change in the amount of a particular electrolyte in the colonic lumenal loop could then be calculated, since the original amount of each ion and initial volume introduced into the loop was known.

In experiments where the effects of noradrenaline were investigated upon

basal colonic electrolyte and fluid transport, noradrenaline was infused along with the anaesthetic, for 30 minutes starting from time zero during the experimental incubation period. Two doses of noradrenaline were investigated, i.e., 3 X 10^{-8} mol./Kg/minute and 10^{-7} mol./Kg/minute. Negligible colonic mucus was observed to be present in the lumenal samples collected under basal conditions (no secretagogue present).

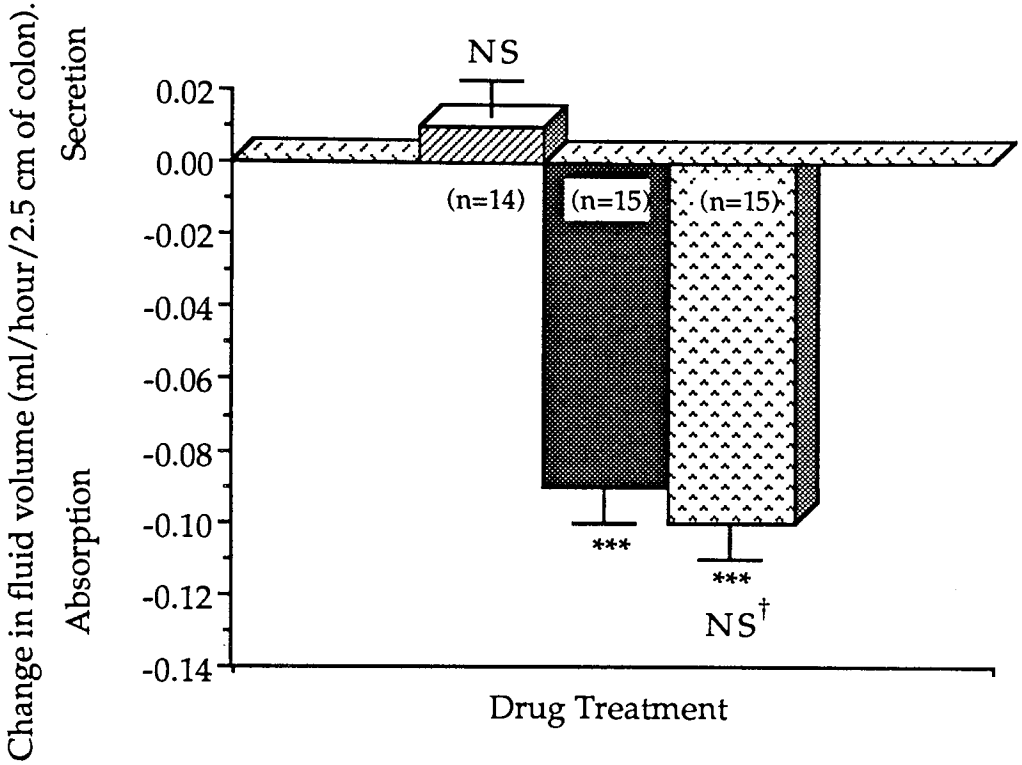

FIGURE 44: The change in lumenal fluid volume in a 2.5 cm distal colonic loop of the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of 30 minute i.v. infusions of 3 X 10^{-8} mol./Kg/minute noradrenaline (■), or 10^{-7} mol./Kg/minute noradrenaline (⊡). Results are mean values and vertical lines indicate the SEM. *** $P<0.001$, when compared to control. NS = $P>0.05$, when compared to zero. NS† = $P>0.05$, when compared to the samples after the 3 X 10^{-8} mol./Kg/minute noradrenaline infusion.

After the 1 hour incubation period a slight increase in volume (0.01 ± 0.01 ml/hour/2.5 cm of colon, n=14) of the fluid sample was observed (figure 44),

as reflected by an increase in weight of the lumenal contents, however this increase was not significantly different (P>0.05) from zero.

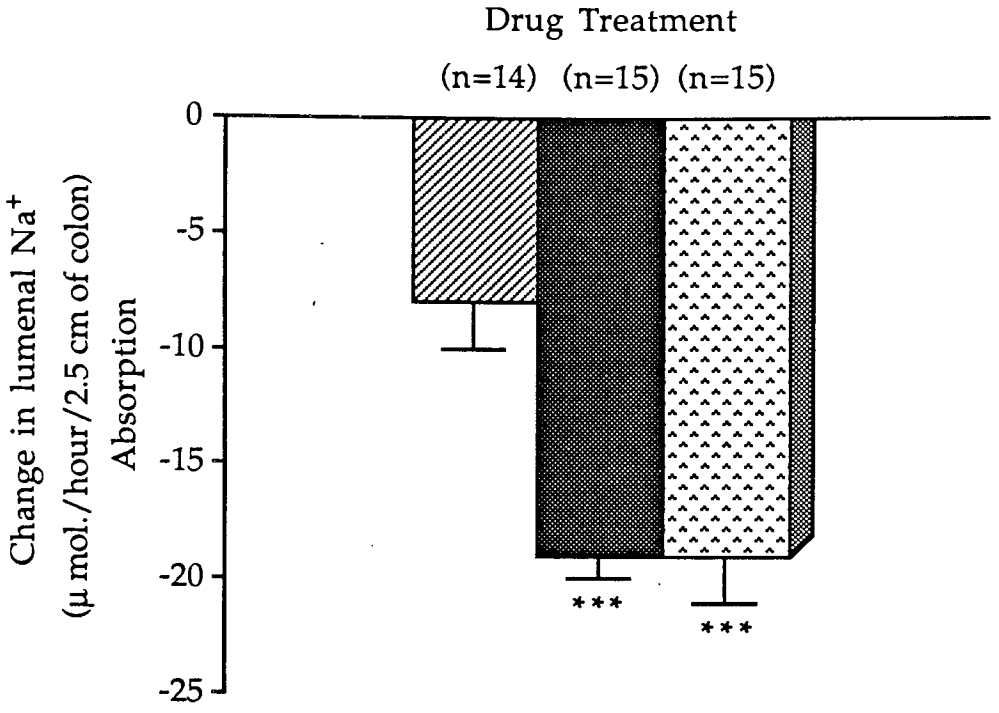

FIGURE 45: Absorption of lumenal Na^+ by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of 30 minute i.v. infusions of either 3 X 10^{-8} mol./Kg/minute noradrenaline (■), or 10^{-7} mol./Kg/minute noradrenaline (⊡). Results are mean values and vertical lines indicate the SEM. *** = P<0.001, when compared to control basal transport.

Infusion of both concentrations of noradrenaline caused a statistically significant (P<0.001) stimulation of net fluid absorption in the colonic loop. $3X10^{-8}$ mol./Kg/minute noradrenaline evoked a fluid absorption of 0.09 ± 0.01 ml/hour/2.5 cm of colon, n=15, whereas 10^{-7} mol./Kg/minute noradrenaline, stimulated fluid absorption by 0.10 ± 0.01 ml/hour/2.5 cm of colon, n=15 (figure 44). However there was no significant (P>0.05) difference between the stimulation of fluid absorption by the two doses of noradrenaline tested.

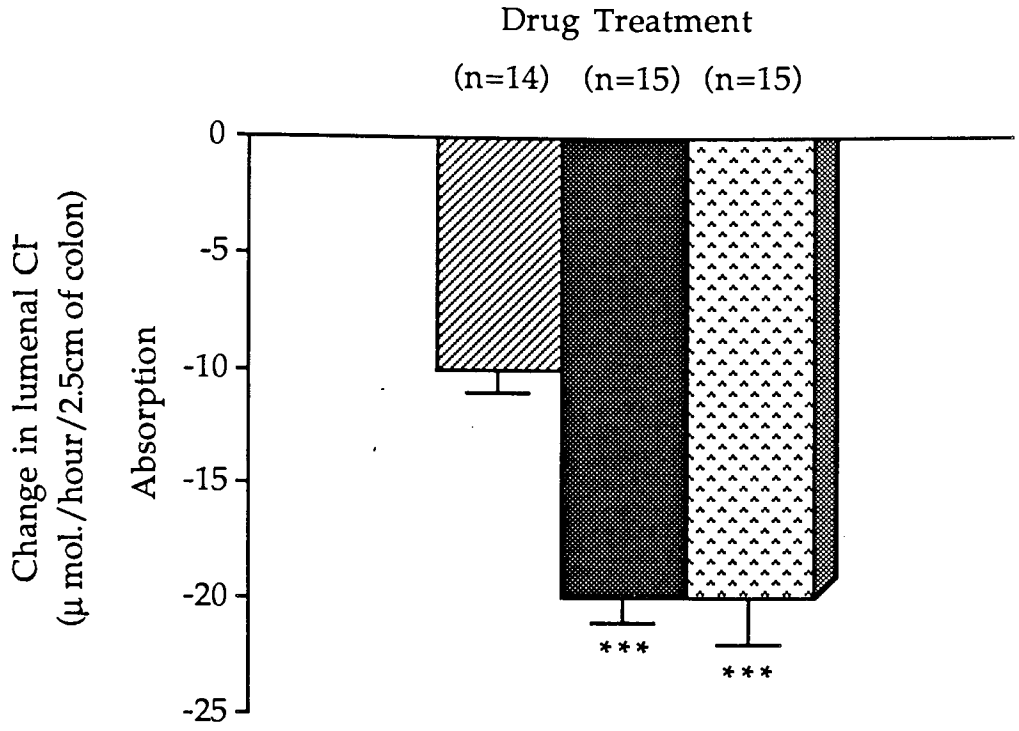

FIGURE 46: Absorption of lumenal Cl^- by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of 30 minute i.v. infusions of either 3 X 10^{-8} mol./Kg/minute noradrenaline (■), or 10^{-7} mol./Kg/minute noradrenaline (▣). Results are mean values and vertical lines indicate the SEM. *** = $P<0.001$, when compared to the control level of basal transport.

A net absorption of both Na^+ (8 ± 2 μ mol./hour/2.5 cm of colon, n=14) (figure 45) and Cl^- (10 ± 1 μ mol./hour/2.5 cm of colon, n=14) (figure 46) was observed under basal conditions. This basal transport of both Na^+ and Cl^- was significantly ($P<0.001$) stimulated by noradrenaline i.v. infusion. Net Na^+ absorption of 19 ± 1 and 19 ± 2 μ mol./hour/2.5 cm of colon, n=15, was observed after infusions of 3 X 10^{-8} and 10^{-7} mol./Kg/minute noradrenaline respectively, whilst net Cl^- absorption of 20 ± 1 and 20 ± 2 μ mol./hour/2.5 cm of colon, n=15, was also observed after noradrenaline infusion respectively (figures 45 and 46). Although there was a reduction in the lumenal concentration of both Na^+ (34 ± 2 m mol./l/hour/2.5 cm of colon, n=14) and

Cl^- (42 ± 2 m mol./l/hour/2.5 cm of colon, n=14) under basal conditions (with no noradrenaline infusion), no significant (P>0.05) further reductions were observed after noradrenaline infusion. The net reductions in the Na^+ concentration were 36 ± 3 and 35 ± 4 m mol./l/hour/2.5 cm of colon, n=15, following infusions of 3 X 10^{-8} and 10^{-7} mol./Kg/minute noradrenaline respectively, whereas the net reductions in Cl^- concentration were 50 ± 3 and 44 ± 4 m mol./l/hour/2.5 cm of colon, n=15, respectively. This would suggest that the stimulatory effects of noradrenaline on Na^+ and Cl^- transport were upon isotonic absorption.

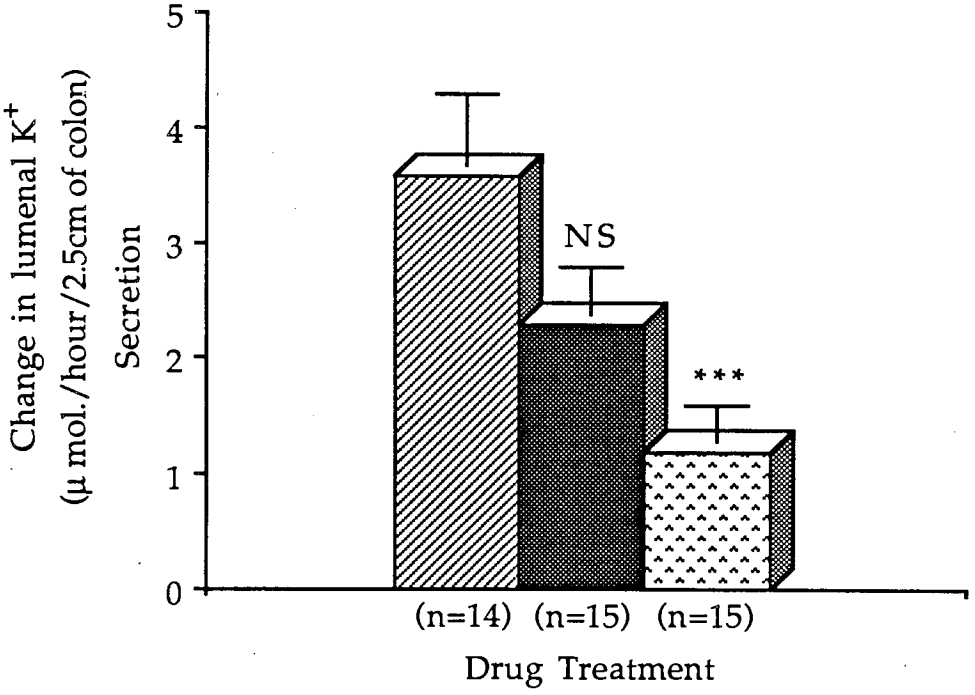

FIGURE 47: Secretion of lumenal K^+ by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of 30 minute i.v. infusions of either 3 X 10^{-8} mol./Kg/minute noradrenaline (■), or 10^{-7} mol./Kg/minute noradrenaline (▣). Results are mean values and vertical lines indicate the SEM. NS = P>0.05) and *** = P<0.001, when compared to the control level of basal transport.

Net K^+ secretion of 3.6 ± 0.6 μ mol./hour/2.5 cm of distal colon, n=14, was recorded under basal conditions (figure 47). Although an inhibition of this

basal secretion was observed after a 30 minute infusion of 3 X 10^{-8} mol./Kg/minute noradrenaline, significance was not reached ($P>0.05$), however a significant ($P<0.001$) inhibition of this basal K^+ secretion was recorded after a 30 minute infusion of 10^{-7} mol./Kg/minute noradrenaline. Net K^+ secretion of 2.3 ± 0.4 and 1.2 ± 0.3 μ mol./hour/2.5 cm of colon, n=15, were observed after infusions of 3 X 10^{-8} and 10^{-7} mol./Kg/minute noradrenaline respectively (figure 47).

Although there was an increase in the lumenal concentration of K^+ (14.1 ± 1.8 m mol./l/hour/2.5 cm of colon, n=14) under basal conditions (with no noradrenaline infusion), no significant ($P>0.05$) effect was observed after noradrenaline infusion, the net increases in concentration being 15.3 ± 2.1 and 10.3 ± 2.0 m mol./l/hour/2.5 cm of colon, n=15, following infusions of 3 X 10^{-8} and 10^{-7} mol./Kg/minute noradrenaline respectively. This would suggest that the inhibitory effect of noradrenaline on K^+ transport were upon isotonic secretion.

These results suggest that noradrenaline stimulates rat distal colonic absorption of Na^+, Cl^- and fluid, whilst also inhibiting K^+ secretion. The stimulation of Na^+ and Cl^- absorption was closely matched and thus might represent a stimulation of coupled NaCl absorption.

3. AN ATTEMPT TO DEMONSTRATE α-ADRENERGIC TONIC REGULATION OF BASAL WATER AND ELECTROLYTE TRANSPORT IN THE RAT DISTAL COLON *IN-VIVO*.

Since the rat distal colon exhibited a net absorption of Na^+ and Cl^-, and a net secretion of K^+, it was considered important to determine whether there was any ongoing α-adrenergic tonic regulation of these transport processes. In this respect the effects of selective α-adrenoceptor antagonists were investigated upon the ongoing water and electrolyte transport processes.

Antagonists were administered by an i.v. bolus injection 15 minutes before the 1 hour incubation recording period. The effects of the selective α_1-adrenoceptor antagonist prazosin and the effects of the selective α_2-adrenoceptor antagonists, idazoxan and CH38083 were investigated.

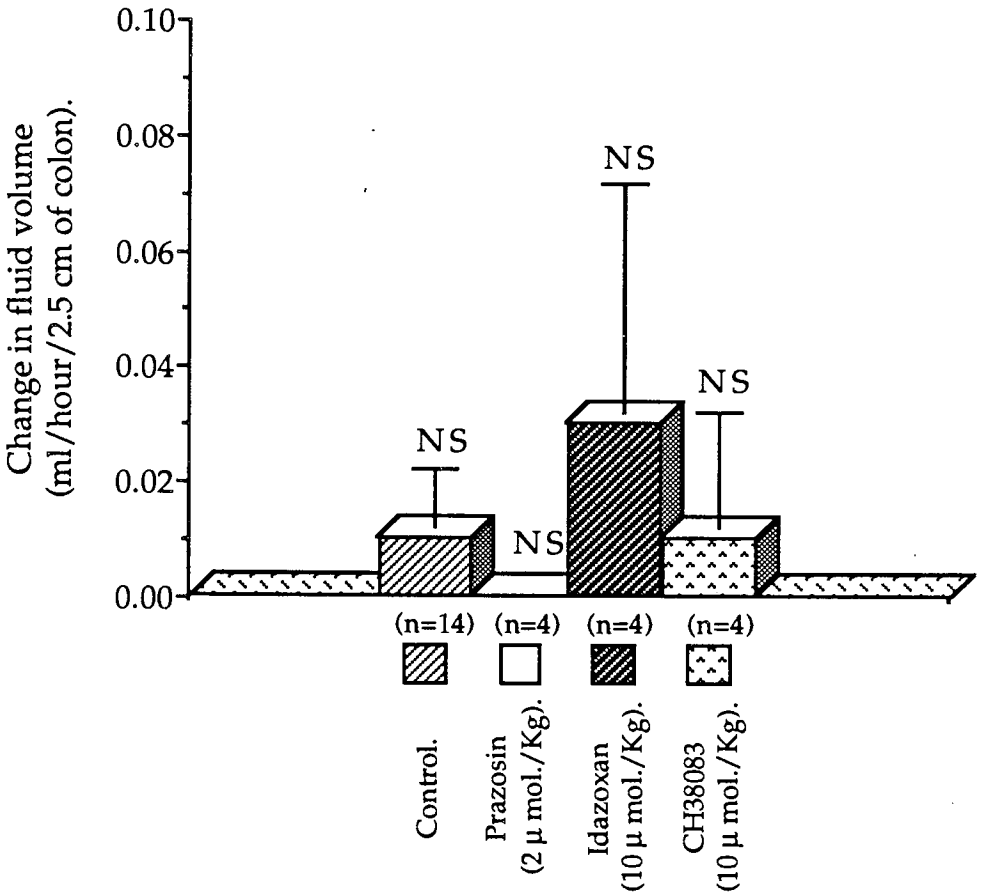

FIGURE 48: The change in lumenal fluid volume in a 2.5 cm distal colonic loop of the rat *in-vivo*, after a 1 hour incubation period (control), and the effects of a bolus i.v. injection 15 minutes prior to the incubation period, of either 2 X 10^{-6} mol./Kg prazosin, 10^{-5} mol./Kg idazoxan or 10^{-5} mol./Kg CH38083. NS = P>0.05, when compared to zero and control level of basal transport.

There was no significant difference (P>0.05) between the control level of fluid transport or a zero level of transport, and the net fluid transport level after pretreatment with either 2 X 10^{-6} mol./Kg prazosin, 10^{-5} mol./Kg idazoxan or 10^{-5} mol./Kg CH38083 (figure 48). This tends to suggest that any

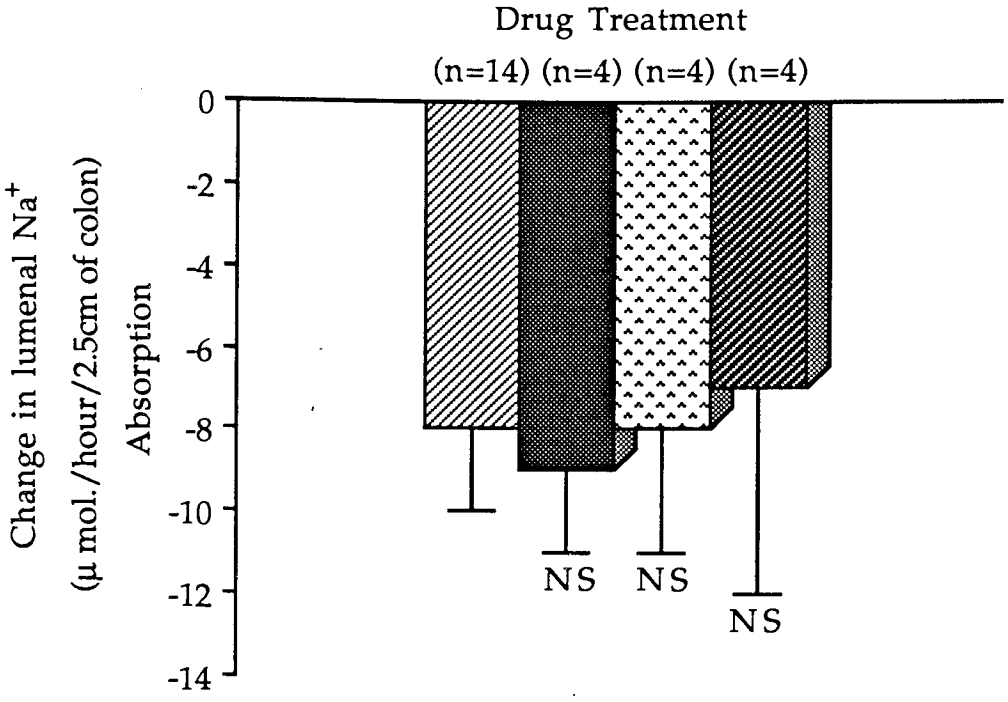

FIGURE 49: Absorption of lumenal Na^+ by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of a bolus i.v. injection 15 minutes prior to the incubation period, of either 10^{-5} mol./Kg idazoxan (▨), 2 X 10^{-6} mol./Kg prazosin (■), or 10^{-5} mol./Kg CH38083 (⊡). NS = P>0.05, when compared to the control level of basal transport.

tonic regulation of distal colonic basal fluid transport is not under α_1- or α_2-adrenergic control. Similarly there was no significant difference (P>0.05) between the basal levels of Na^+ absorption in the presence or absence of either prazosin, idazoxan or CH38083 (figure 49), at the doses stated above. In addition a parallel observation was made with these α_1- and α_2-adrenoceptor antagonists upon basal Cl^- absorption, i.e. there was no significant (P>0.05) effects of them on the net absorptive level (figure 50). Furthermore no significant (P>0.05) effects of α_1- or α_2-adrenoceptor antagonism were observed upon net basal K^+ secretion (figure 51).

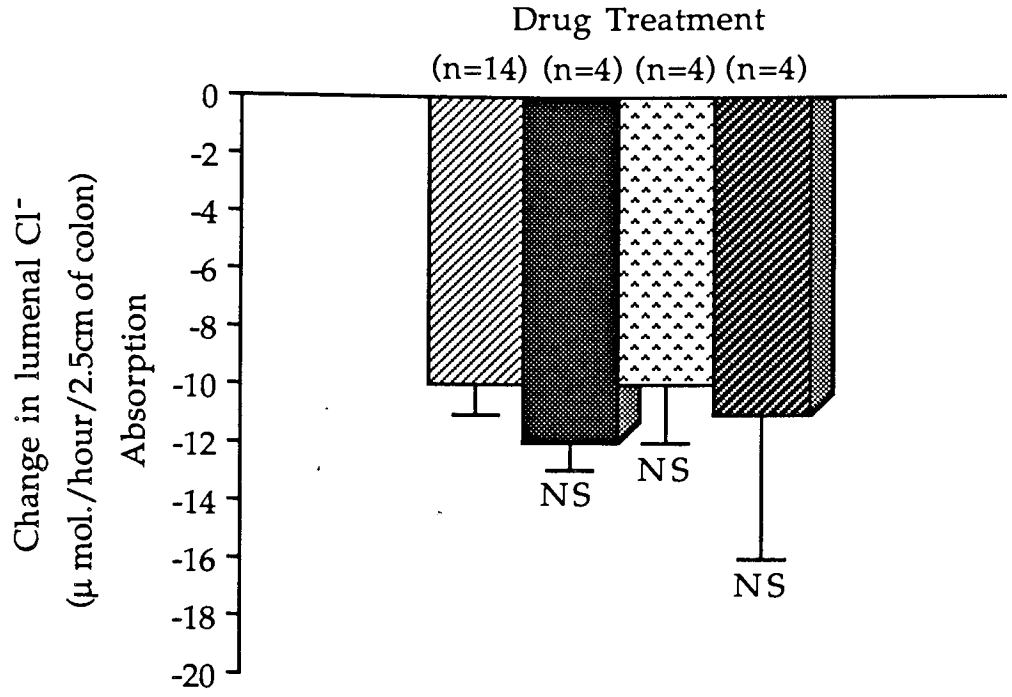

FIGURE 50: Absorption of lumenal Cl^- by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of a bolus i.v. injection 15 minutes prior to the incubation period, of either 10^{-5} mol./Kg idazoxan (▨), 2 X 10^{-6} mol./Kg prazosin (■), or 10^{-5} mol./Kg CH38083 (⊡). NS = P>0.05, when compared to the control level of basal transport.

The results presented here suggest that the ongoing transport Na^+, Cl^- and K^+, under basal conditions in the rat distal colon *in-vivo*, is not under α_1- nor α_2-adrenergic tonic control.

4. THE EFFECT OF α_2-ADRENOCEPTOR ACTIVATION UPON SECRETAGOGUE STIMULATED WATER AND ELECTROLYTE TRANSPORT IN THE RAT DISTAL COLON *IN-VIVO*.

The effects of secretagogue stimulation were investigated upon water and electrolyte transport in the distal colonic loop. The initial investigation looked at the effects of both theophylline and PGE_2, whilst the later study concentrated upon the effects of PGE_2.

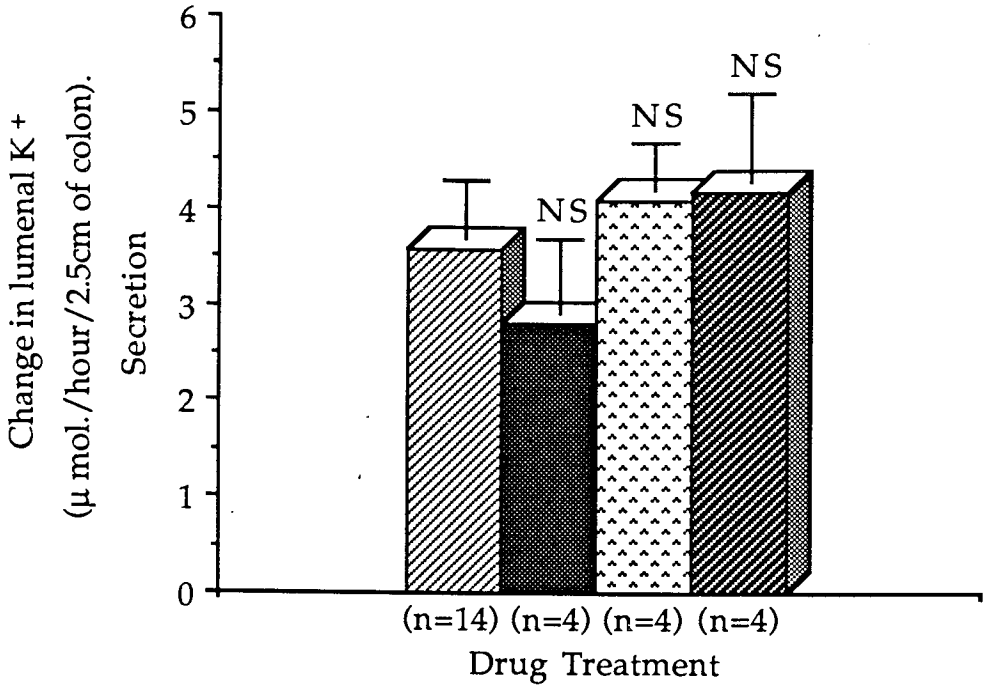

FIGURE 51: Secretion of lumenal K^+ by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of a bolus i.v. injection 15 minutes prior to the incubation period, of either 10^{-5} mol./Kg idazoxan (▨), 2 X 10^{-6} mol./Kg prazosin (■), or 10^{-5} mol./Kg CH38083 (⊡). NS = P>0.05, when compared to the control level of basal transport.

A). The Effects Of The Secretagogues, Theophylline And PGE_2 On Colonic Water And Electrolyte Transport.

Lumenal application of both theophylline or PGE_2 (10^{-6} mol./Kg) evoked significant (P<0.05) increases in fluid secretion, the increases being 0.09 ± 0.01 (n=4), and 0.08 ± 0.01 ml/hour/2.5 cm of colon (n=6), respectively. However noticeable colonic mucus secretion was observed after secretagogue challenge, and therefore 1% w/v N-acetyl-L-cysteine was added to the samples after collection to degrade the mucus matrix (Norris *et al*, 1983) and so account for any error in the gravimetric calculation. A significant (P<0.05) stimulation of fluid secretion (0.10 ± 0.01 ml/hour/2.5 cm of colon, n=11) was also evoked by PGE_2 when the weight of colonic mucus was accounted for (figure 52).

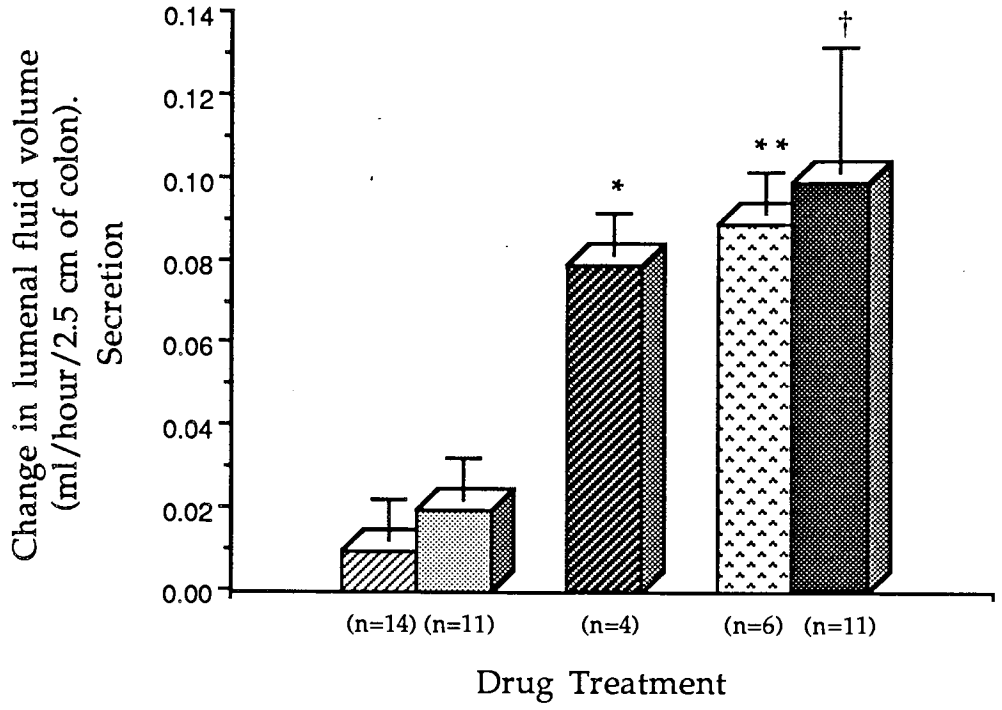

FIGURE 52: The change in lumenal fluid volume in a 2.5 cm distal colonic loop of the rat *in-vivo*, after a 1 hour incubation period (control, ▨), and the effects of lumenal theophylline (10^{-6} mol./Kg) (▨), or PGE_2 (10^{-6} mol./Kg) (⊡). Noticeable colonic mucus secretion was observed after secretagogue challenge, and therefore 1% w/v N-acetyl-L-cysteine was added to the samples after collection to degrade the mucus and account for any error in the gravimetric calculation. PGE_2 (10^{-6} mol./Kg) after N-acetyl-L-cysteine treatment (■). Control after N-acetyl-L-cysteine treatment (□). * $P<0.05$ and ** $P<0.01$, when compared to the control level of basal transport in samples not treated with N-acetyl-L-cysteine. † $P<0.05$, when compared to the control level of basal transport in samples treated with N-acetyl-L-cysteine.

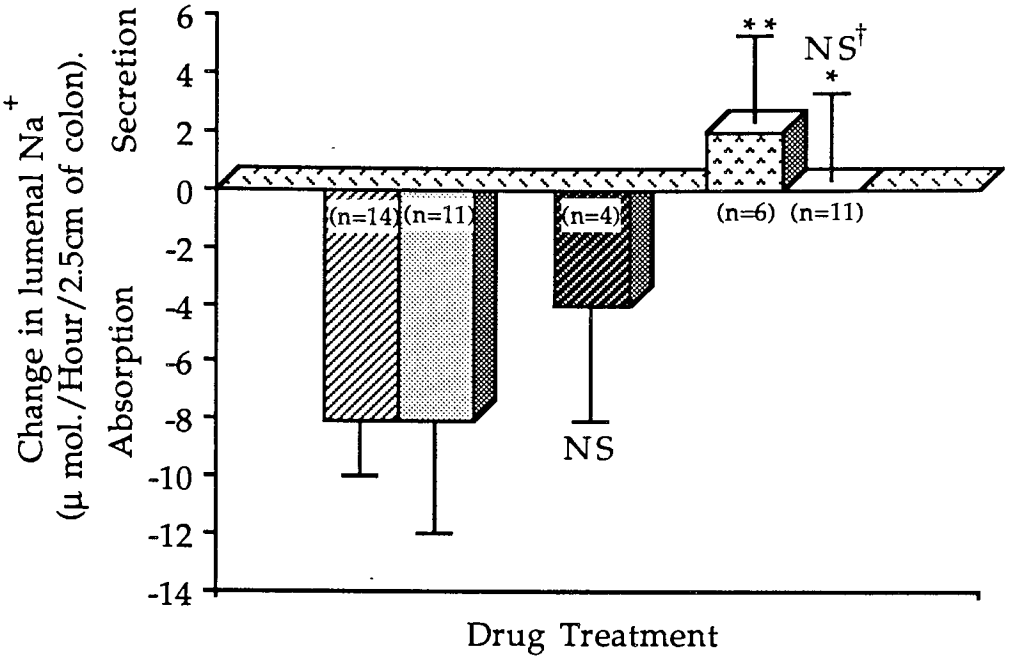

FIGURE 53: Absorption of lumenal Na^+ by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of lumenal theophylline (10^{-6} mol./Kg) (▩), or PGE_2 (10^{-6} mol./Kg) (⊡). Noticeable colonic mucus secretion was observed after secretagogue challenge, and therefore 1% w/v N-acetyl-L-cysteine was added to the samples after collection to degrade the mucus. PGE_2 (10^{-6} mol./Kg) after N-acetyl-L-cysteine treatment (□). Control after N-acetyl-L-cysteine treatment (▩). NS = $P>0.05$, * $P<0.05$ and ** $P<0.01$, when compared to the control level of basal transport in samples not treated with N-acetyl-L-cysteine. NS† = $P>0.05$, when compared to the control level of basal transport in samples treated with N-acetyl-L-cysteine.

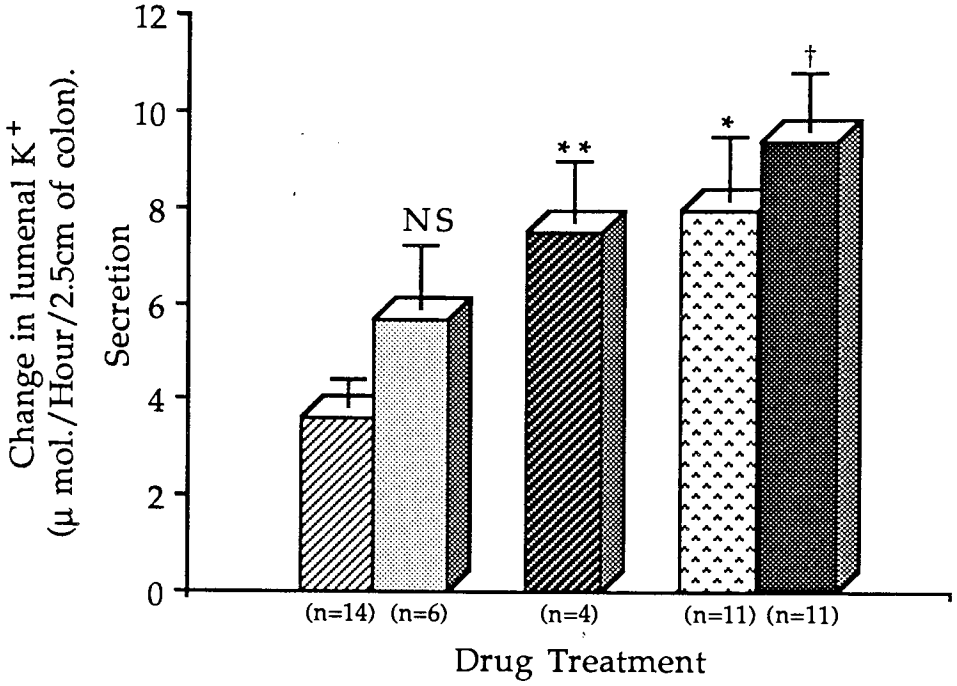

FIGURE 54: Secretion of lumenal K^+ by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of lumenal theophylline (10^{-6} mol./Kg) (▨), or PGE_2 (10^{-6} mol./Kg) (▨). Noticeable colonic mucus secretion was observed after secretagogue challenge, and therefore 1% w/v N-acetyl-L-cysteine was added to the samples after collection to degrade the mucus. PGE_2 (10^{-6} mol./Kg) after N-acetyl-L-cysteine treatment (■). Control after N-acetyl-L-cysteine treatment (▨). NS = $P>0.05$, * $P<0.05$ and ** $P<0.01$, when compared to the control level of basal transport in samples not treated with N-acetyl-L-cysteine. † $P<0.05$, when compared to the control level of basal transport in samples treated with N-acetyl-L-cysteine.

Theophylline (10^{-6} mol./Kg) reduced the ongoing Na^+ absorption to a level of 4 ± 4 μ mol./hour/2.5 cm of colon (n=4), in samples not treated with N-acetyl-L-cysteine, however statistical significance was not reached ($P>0.05$). In contrast PGE_2 (10^{-6} mol./Kg) significantly ($P<0.001$) reduced Na^+ absorption in samples not treated with the mucolytic (figure 53). Although in samples treated with N-acetyl-L-cysteine, a reduction in Na^+ absorption to a level of 0 ± 3 μ mol./hour/2.5 cm of colon (n=11) was induced by PGE_2 (10^{-6} mol./Kg), statistical significance was not achieved ($P>0.05$) (figure 53).

Theophylline (10^{-6} mol./Kg) significantly ($P<0.01$) stimulated K^+ secretion to a level of 7.5 ± 1.3 μ mol./hour/2.5 cm of colon (n=4), in samples not treated with N-acetyl-L-cysteine (figure 54). PGE_2 (10^{-6} mol./Kg) significantly ($P<0.05$) stimulated K^+ secretion in both N-acetyl-L-cysteine treated and untreated samples, the observed levels were 9.4 ± 1.2 (n=11) and 8.0 ± 1.3 (n=6) μ mol./hour/2.5 cm of colon, respectively (figure 54).

A problem was encountered when the samples treated with N-acetyl-L-cysteine, were analysed for Cl^-, the chloride analyzer (Corning Chloride Analyzer 925) showed a lack of specificity and titrated the N-acetyl-L-cysteine along with Cl^- ions, thus leading to an error in the determination. Cl^- measurements therefore had to be taken from samples which were not treated with the mucolytic, and the assumption that there was a equal distribution of Cl^- ions both in 'free fluid' and fluid trapped in the mucus matrix had to be made.

Surprisingly the level of Cl^- absorption (9 ± 4 μ mol./hour/2.5 cm of colon, n=4) in the presence of 10^{-6} M lumenal theophylline was not significantly ($P>0.05$) different from the level in the absence of this secretagogue. Theophylline would be expected to inhibit Cl^- absorption and/or stimulate electrogenic Cl^- secretion (Frizzell and Schultz, 1979; Rao and Field, 1983). Cl^-

absorption was however significantly ($P<0.05$) reduced to a level of 3 ± 3 μ mol./hour/2.5 cm of colon (n=6), by PGE_2 (10^{-6} mol./Kg) (figure 55).

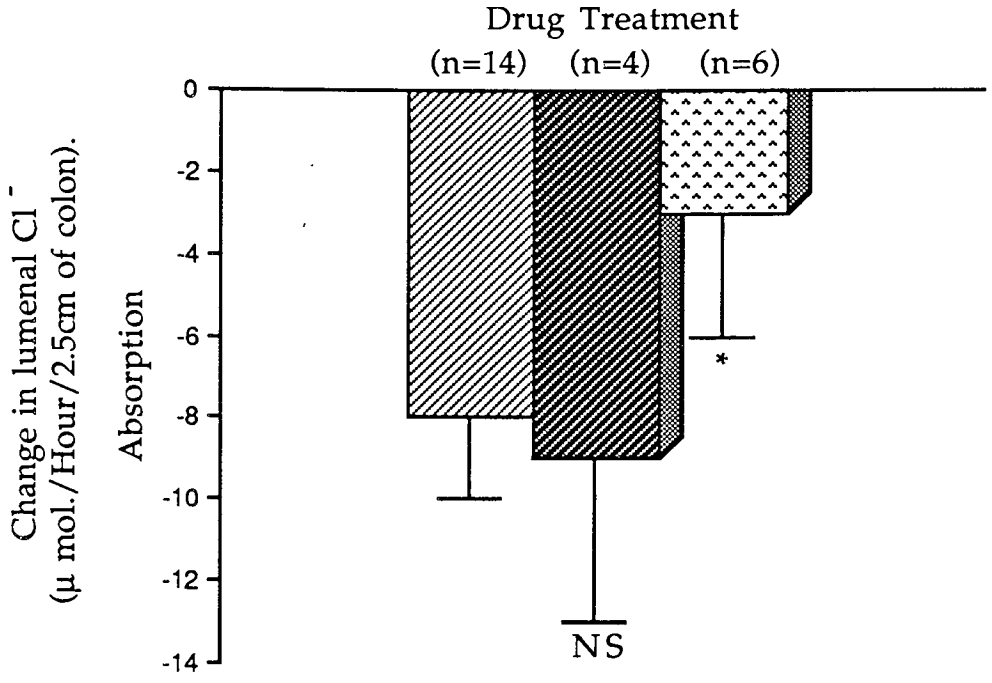

FIGURE 55: Absorption of lumenal Cl^- by a 2.5 cm distal colonic loop in the rat *in-vivo*,after a 1 hour incubation period (control, ▨), and the effects of lumenal theophylline (10^{-6} mol./Kg) (▩) or PGE_2 (10^{-6} mol./Kg) (⊡). NS = $P>0.05$ and * = $P<0.05$, when compared to the control level of basal transport.

There was no significant ($P>0.05$) effect of both theophylline and PGE_2 upon the luminal concentration of all of the ions investigated (data not shown), suggesting that any changes in electrolyte transport evoked by of these drugs were upon isotonic rather than non-isotonic transport.

These results suggest that the stimulation of fluid secretion evoked by PGE_2 in the rat distal colon *in-vivo*, might be associated with a stimulation of isotonic K^+ secretion and an inhibition of isotonic Cl^- absorption. There is also some evidence for an inhibition of Na^+ absorption although the evidence is less clear since statistical significance was only achieved in samples not treated with the mucolytic, N-acetyl-L-cysteine.

B). The Effects Of α_2-Adrenoceptor Activation By UK-14,304, Upon Colonic Water And Electrolyte Transport In The Presence Of Lumenal PGE_2.

The effect of a 30 minute i.v. infusion of the α_2-adrenoceptor agonist UK-14,304 (10^{-9} mol./Kg/minute) was investigated upon colonic fluid and electrolyte transport in the absence and presence of lumenal PGE_2 (10^{-6} mol./Kg). UK-14,304 was administered by a similar method to that described for noradrenaline infusions in Section 2, whereas PGE_2 was administered as describe in Section 4A. Experiments were only investigated with samples treated with the mucolytic N-acetyl-L-cysteine (1% w/v), whilst all the other experimental conditions were as described previously in this Chapter.

Although UK-14,304 (10^{-9} mol./Kg/minute) had no significant ($P>0.05$) effect upon basal fluid transport in the rat distal colon, a significant ($P<0.05$) inhibition of secretion evoked by lumenal PGE_2 (10^{-6} mol./Kg) was observed, the secretion being reduced from 0.10 ± 0.01 (n=11) to 0.01 ± 0.03 (n=12) ml/hour/2.5 cm of colon (figure 56). This might suggest that α_2-adrenoceptor regulation of colonic fluid transport is of greater importance after secretagogue challenge, or that this effect simply reflects the fact that there is only a minimal level of basal secretion and so it is difficult to observe any antisecretory effect.

A 30 minute infusion of the selective α_2-adrenoceptor agonist, UK-14,304 (10^{-9} mol./Kg/minute) produced no significant ($P>0.05$) effect upon ongoing basal distal colonic Na^+ absorption (figure 57). This would tend to suggest that basal Na^+ absorption is not under α_2-adrenoceptor regulation, especially since the selective α_2-adrenoceptor antagonist, idazoxan alone was previously found to be without effect (figure 49).

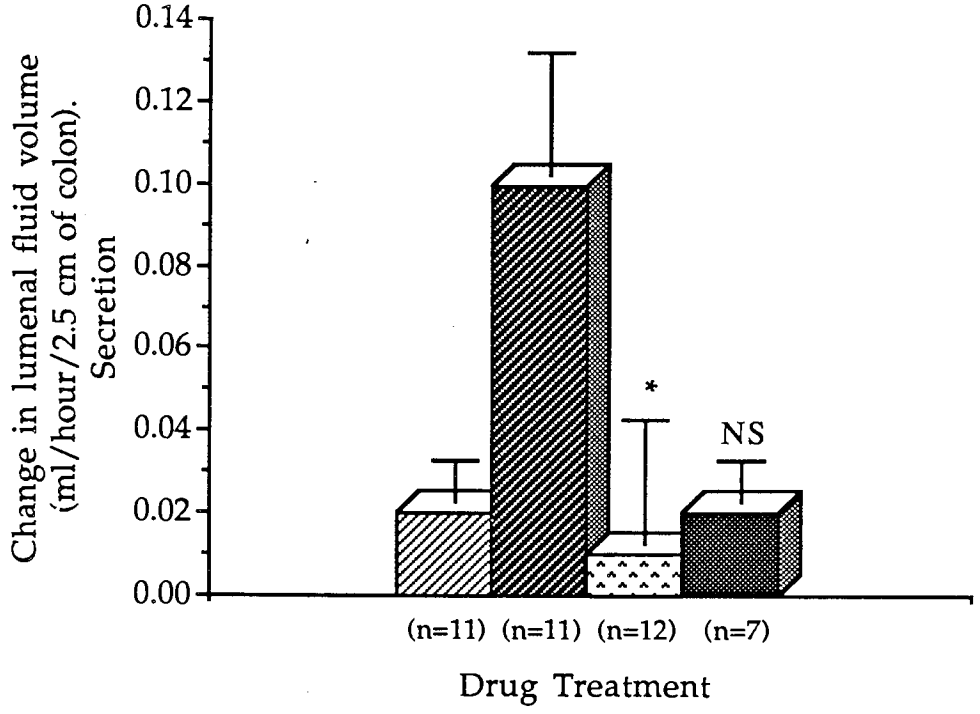

FIGURE 56: The change in lumenal fluid volume in a 2.5 cm distal colonic loop of the rat *in-vivo*, after a 1 hour incubation period (control, ▨). 1% w/v N-acetyl-L-cysteine was added to all the samples after collection to degrade any colonic mucus and account for any error in the gravimetric calculation. The effect of 10^{-6} mol./Kg PGE_2 alone (▨) and with a 30 minute i.v. infusion of 10^{-9} mol./Kg/minute UK-14,304 (⊡). This infusion of UK-14,304 was also investigated upon basal fluid transport (■). NS = $P>0.05$ when compared to basal transport and * = $P<0.05$, when compared to PGE_2 stimulated transport.

No conclusion can be made about the effects of UK-14,304 upon Na^+ transport in preparations treated with 10^{-6} mol./Kg PGE_2 since none of the effects were significant (figure 57).

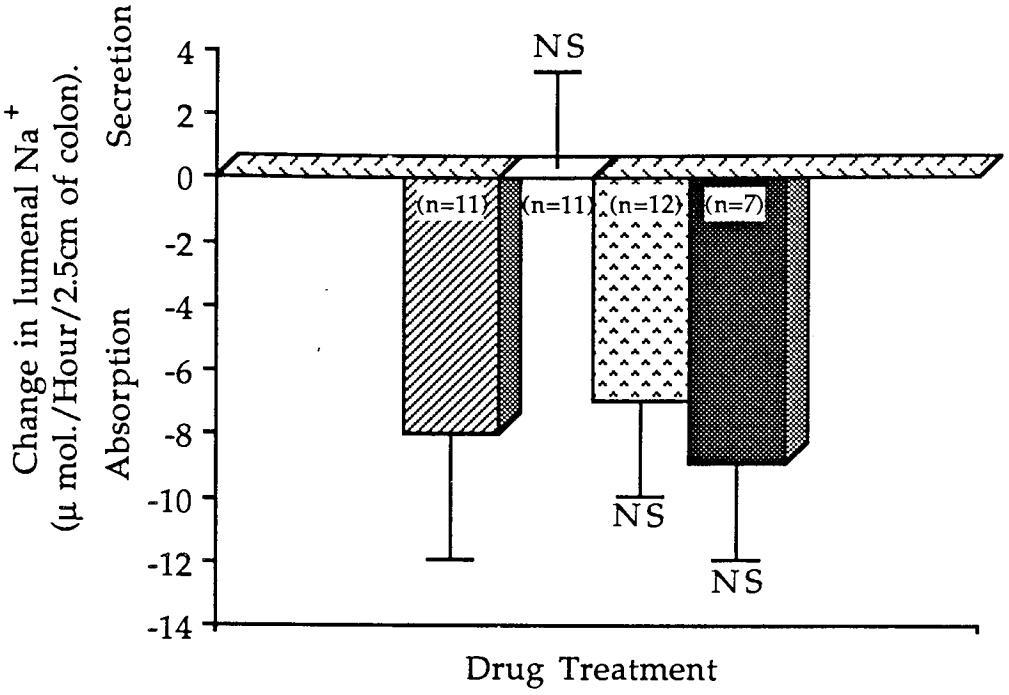

FIGURE 57: The absorption of Na^+ by a 2.5 cm distal colonic loop of the rat *in-vivo*, after a 1 hour incubation period (control, ▨). 1% w/v N-acetyl-L-cysteine was added to all the samples after collection to degrade any colonic mucus. The effect of 10^{-6} mol./Kg PGE_2 alone (□) and with a 30 minute i.v. infusion of 10^{-9} mol./Kg/minute UK-14,304 (⊡). This infusion of UK-14,304 was also investigated upon basal Na^+ absorption (■). NS = $P>0.05$, when compared to basal transport and transport in the presence of PGE_2.

UK-14,304 (10^{-9} mol./Kg/minute) had no significant ($P>0.05$) effect upon basal distal colonic K^+ secretion. However infusion of UK-14,304 (10^{-9} mol./Kg/minute) significantly reduced the secretory effect of PGE_2 (10^{-6} mol./Kg) to a level of 4.5 ± 0.7 μ mol./hour/2.5 cm of colon (n=12) (figure 58). These results suggest that basal K^+ secretion is not influenced by α_2-adrenoceptor activation, however PGE_2 stimulated K^+ secretion is inhibited by such α_2-adrenoceptor stimulation.

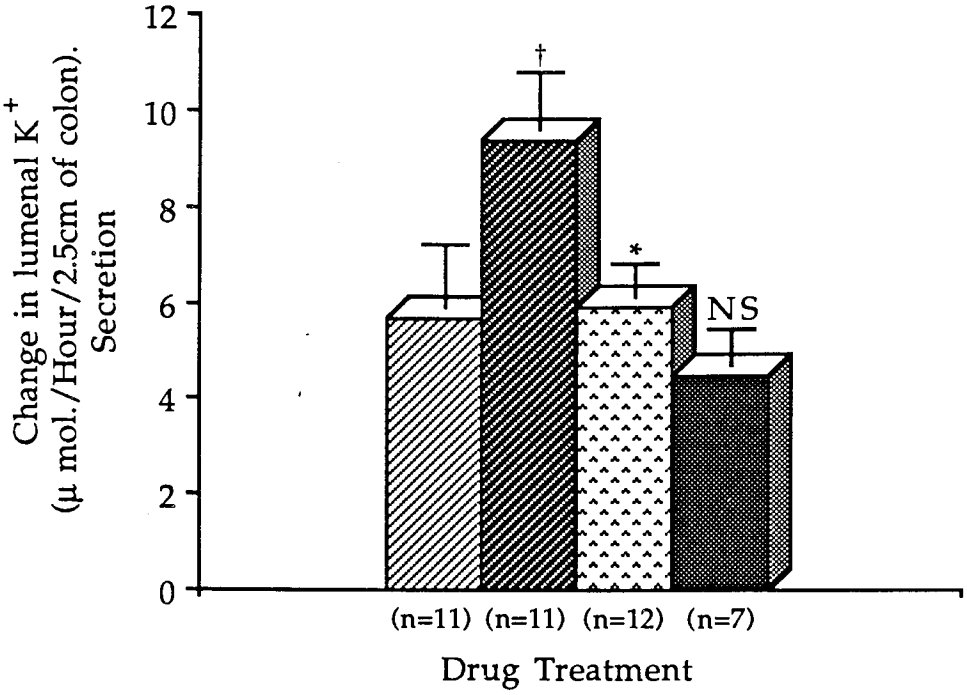

FIGURE 58: The secretion of K^+ by a 2.5 cm distal colonic loop of the rat *in-vivo*, after a 1 hour incubation period (control, ▨). 1% w/v N-acetyl-L-cysteine was added to all the samples after collection to degrade any colonic mucus. The effect of 10^{-6} mol./Kg PGE_2 alone (▨) and with a 30 minute i.v. infusion of 10^{-9} mol./Kg/minute UK-14,304 (▣). This infusion of UK-14,304 was also investigated upon basal K^+ secretion (■). NS = $P>0.05$ and † = $P<0.05$, when compared to basal transport. * = $P<0.05$, when compared to PGE_2 stimulated transport in the absence of UK-14,304..

The effects of UK-14,304 infusion was not investigated upon colonic Cl^- transport because of the lack of specificity of the chloride analyzer which titrated N-acetyl-L-cysteine along with Cl^- ions.

5. DISCUSSION.

As described previously in the introduction to this chapter and in Chapter 1, Section 3, there is considerable circumstantial evidence for the presence of adrenergic tonic regulation of colonic water and electrolyte transport.

Although in the present study using the rat isolated colonic loop to investigate such transport, an ongoing absorption of Na^+ and Cl^-, and a net secretion of K^+ was observed, this basal ionic transport does not appear to be under α-adrenergic tonic regulation since the addition of the α_1-adrenoceptor antagonist, prazosin or the α_2-adrenoceptor antagonists CH38083 and idazoxan (with no agonist present) were without effect. In contrast, noradrenaline infusion did evoke a considerable effect upon basal fluid transport, converting a non-significant secretion into a highly significant absorption. In addition a simultaneous highly significant stimulation of Na^+ and Cl^- absorption was also observed. The levels of stimulated Na^+ and Cl^- absorption were closely matched, suggesting a possible stimulation of coupled NaCl transport, although radio labelled ionic flux studies would be required to confirm this. This suggestion is consistent with *in-vitro* evidence in the rabbit (Sellin and DeSoignie, 1984) and rat (Racusen and Binder, 1979) colon, where noradrenaline is reported to stimulate coupled sodium and chloride absorption. As well as an effect of noradrenaline upon NaCl absorption, a significant inhibition of K^+ secretion was also observed with the high dose (10^{-7} mol./Kg/minute) of noradrenaline. These effects of noradrenaline on NaCl absorption and K^+ secretion occured without any concomitant changes in lumenal concentration, suggesting that the effects were upon isotonic transport.

Other ionic species not investigated in the present study might also be associated with the absorptive effect of noradrenaline, such as an effect upon HCO_3^- transport. Racusen and Binder (1979) have reported that α-adrenoceptor activation in the rat colon decreases HCO_3^- secretion concomitantly with stimulation of NaCl absorption, whilst Smith *et al* (1985) have reported that adrenaline stimulates sodium-dependent HCO_3^- absorption in the rabbit ileum.

Although selective α_2-adrenoceptor antagonism was not investigated upon the responses to noradrenaline, it is unlikely that the absorptive effect

evoked by noradrenaline was associated with α_2-adrenoceptor activation, since infusion of the selective α_2-adrenoceptor agonist UK-14,304 was without effect upon basal fluid and electrolyte transport. The possibility therefore remains that the absorptive effect to noradrenaline might be associated with some other adrenoceptor activation such as β_2 or α_1. An electrogenic effect of noradrenaline via activation of α_1-adrenoceptors is unlikey, since *in-vitro* evidence presented in prevoius Chapters has suggested that such stimulation is prosecretory. However α_1-adrenoceptor activation in this same tissue has been suggested to stimulate electrically silent NaCl absorption (Williams, 1986), therefore such a response could be involved with the effect of noradrenaline in the colon. Care must be taken however when comparing effects in the large and small intestine, because of the heterogeneous nature of the intestinal tract, as described in Chapter 1. *In-vitro* evidence for an antisecretory β_2-adrenoceptor control of basal electrogenic electrolyte transport in the distal colon was presented in Chapter 6, thus raising the possibility that the *in-vivo* effects of noradrenaline might be associated with such β-adrenoceptor activation. However this needs further investigation, especially since β-adrenoceptor stimulation in the rabbit distal colon (Halm *et al*, 1983; Halm and Frizzell, 1986; Smith and McCabe, 1986) and raised cAMP in the rat proximal colon (Foster *et al*, 1983) are reported to stimulate active potassium secretion, which contrasts the present finding that noradrenaline elicited an inhibition of such secretion in the distal region, although the proximal and distal colonic regions are distinct in their transport function and there is documented species variation (Binder and Sandle, 1987).

Both theophylline and PGE_2 significantly stimulated fluid secretion in the colonic loop. Noticeable colonic lumenal mucus was observed in preparations treated with the above secretagogues, therefore in subsequent experiments using the secretagogue PGE_2, the lumenal fluid samples collected for analysis were treated with the mucolytic N-acetyl-L-cysteine (Norris *et al*, 1983), which degraded the mucus matrix and accounted for any experimental

errors in the gravimetric calculations and possible unequal electrolyte distribution between 'free fluid' and fluid trapped in the mucus matrix.

Although theophylline reduced the ongoing Na^+ absorption in samples not treated with N-acetyl-L-cysteine statistical significance was not reached. In contrast PGE_2 significantly ($P<0.01$) reduced Na^+ absorption in samples not treated with the mucolytic, however in samples treated with N-acetyl-L-cysteine, the reduction in Na^+ absorption was not statistically significant. No clear conclusion can be made as to the effect of PGE_2 upon Na^+ absorption, although an inhibitory effect cannot be discounted.

The level of Cl^- absorption in the presence of lumenal theophylline was not significantly different from the level in the absence of this secretagogue, which was surprising since theophylline is reported to inhibit Cl^- absorption and/or stimulate electrogenic Cl^- secretion (Frizzell and Schultz, 1979; Rao and Field, 1983). The assumption that there is an equal distribution of Cl^- ions through out the lumenal fluid sample might be incorrect and thus may account for this unusual observation, however Cl^- absorption was significantly reduced by the secretagogue PGE_2.

Both theophylline and PGE_2 significantly stimulated K^+ secretion in samples not treated with N-acetyl-L-cysteine, whilst the latter also evoked a similar response in N-acetyl-L-cysteine treated samples.

The present findings that PGE_2 acts as a potent secretagogue, evoking an intestinal fluid secretion whilst also inducing secretory and antiabsorptive influences over isotonic electrolyte transport is consistent with similar observations made by Diener *et al* (1988b). In addition PGE_1 and PGE_2 are reported to induce a fluid secretion in the rat (Nakaki *et al* (1982) and human jejunum (Bukhave and Rask-Madsen, 1980) respectively. Interestingly PGD_2

has been reported to have the opposite effect to that of PGE_2 in the rat distal colon, i.e., PGD_2 evokes an antisecretory response (Hill *et al*, 1988).

The infusion of UK-14,304 (i.v.) significantly reduced the stimulation of fluid and K^+ secretion evoked by PGE_2. No conclusion can be made about the effects of UK-14,304 upon Na^+ transport in preparations treated with PGE_2 since none of the effects were significant. Cl^- transport was not investigated, due to the lack of specificity of the Cl^- analyser, distinguishing between Cl^- ions and N-acetyl-L-cysteine.

It would therefore appear that although α_2-adrenoceptor activation is of little importance in the regulation of fluid transport under basal conditions in the distal colon, an antisecretory regulation is observed after secretagogue challenge by PGE_2. However, the lack of any basal α_2-adrenoceptor regulation might simply reflect the fact that there is only a minimal level of basal secretion, and so it is difficult to observe any antisecretory effect.

The present findings are consistent with the observation that clonidine is without effect upon basal fluid transport in the rat colon *in-vivo* (Bunce and Spraggs, 1983). Furthermore a greater importance of an α_2-adrenoceptor regulation after secretagogue challenge in the distal colon *in-vivo,* is also consistent with the similar suggestion made for jejunal transport *in-vitro* (Chapter 3).

CHAPTER 8: GENERAL DISCUSSION

A role for the sympathetic nervous regulation of intestinal absorption and secretion has been established since the 1940s (Florey *et al*, 1941; Wright *et al*, 1940). The sympathetic nervous system is known to promote absorption and inhibit secretion through the release of the catecholamine noradrenaline (Field and McColl, 1973), responses which are mediated through α-adrenoceptor activation (Dietz and Field, 1973; Racusen and Binder, 1979). The development of selective agonists and antagonists for α-adrenoceptor sub-types, has lead to investigations to try and identify the α-adrenoceptor sub-types influencing intestinal fluid and electrolyte transport. The characterization of such receptor regulation and the development of selective regulatory agents, has immense therapeutic potential for the treatment of diseased states with associated conditions of either severe diarrhoea or constipation.

Prior to the present investigation, the evidence was unclear as to which α-adrenoceptor sub-type has the preponderant regulatory role over intestinal fluid and electrolyte transport. It has been suggested that there is an α_2-adrenoceptor antisecretory and pro-absorptive regulation of intestinal fluid and electrolyte transport in the rabbit ileum *in-vitro* (Chang *et al*, 1982; Dharmsathaphorn *et al*, 1984; Durbin *et al*, 1982; Fondacaro *et al*, 1988). In contrast Cotterell *et al* (1983), and Parsons *et al* (1983), have suggested that an α_1-adrenoceptor regulates fluid absorption in the rat small intestine *in-vitro*. In addition an α_1-adrenoceptor mechanism has been suggested to mediate increases in rat jejunal basal fluid and electrolyte transport (Levens, 1983; Levens *et al*, 1981b), and reflex increases in fluid absorption in response to haemorrhage (Levens, 1984a) or dehydration (Levens, 1984b). The latter two responses are thought to be mediated through the release of angiotensin and subsequent enhanced release of the sympathetic neurotransmitter noradrenaline.

As well as an α-adrenoceptor regulation of small intestinal fluid absorption, there is evidence of a similar regulation in the large intestine (Sellin and DeSoignie, 1984; Racusen and Binder, 1979). An α_2-adrenoceptor antisecretory and pro-absorptive regulation of large intestinal fluid and electrolyte transport has been suggested by Dharmsathaphorn *et al* (1984), whereas Bunce and Spraggs (1983a), and Durbin *et al* (1982), have failed to demonstrate such regulation. Racusen and Binder (1979) have also proposed that colonic β-adrenoceptor activation exhibits antisecretory and pro-absorptive responses.

It is unclear whether there is any nervous adrenergic tone to the intestinal tract which regulates fluid and electrolyte transport. Chang *et al* (1983a) have suggested a level of adrenergic tone based on circumstantial evidence, ie., diabetic rats exhibit malabsorption of ions, secretory diarrhoea and often have damage of sympathetic nerve terminals innervating the intestine.

The overall objectives of the present study were to characterise the adrenoceptor subtypes which may influence intestinal fluid and electrolyte transport in the rat. An *in-vitro* study made use of the Ussing chamber technique to investigate rat jejunal electrolyte transport, whereas additional investigations were also undertaken in the rat distal colon. The possibility of adrenergic nervous tonic regulation of intestinal fluid and electrolyte transport was investigated using a distal colonic loop *in-vivo* model. The rat was used as an experimental model for intestinal fluid and electrolyte transport because of its ease of availability and relative inexpensiveness.

Dettmar *et al* (1986a) and Williams (1986) have suggested that α_2-adrenoceptor stimulation might mediate antisecretory responses upon basal rat jejunal electrogenic Cl^- secretion associated with a decreases in SCC. In the present study possible adrenoceptor influences upon piretanide sensitive theophylline elevated SCC was investigated. The results presented in

Chapter 3 suggest that it is α_2-adrenoceptors which exhibit antisecretory regulation of secretagogue stimulated electrogenic Cl^- secretion. Williams (1986) showed that these responses to noradrenaline were associated with a direct effect upon the transporting epithelium, as reflected in the lack of effect of the neurotoxin tetrodotoxin.

The present *in-vitro* evidence of an α_2-adrenoceptor antisecretory effect upon electrolyte transport in the rat jejunum, is also consistent with similar regulatory processes based on *in-vivo* observations (Bunce and Spraggs, 1983a and 1983b; Nakaki *et al*, 1982a and 1982b). Fondacaro *et al* (1988) have suggested that α_2-adrenoceptor agonists may be useful in converting the hypersecreting mammalian small bowel to its normal state. α_2-Adrenoceptor agonists could possibly have a wide spectrum of action in that they may be effective against many secretagogues having different mechanisms of action (e.g., mediation through intracellular cAMP, Ca^{2+} etc.).

Clinical trials have demonstrated beneficial antisecretory effects of α_2-adrenoceptor agonists in patients suffering from diabetic diarrhoea (Fedorak *et al*, 1985; Goff, 1984), and diarrhoea associated with either bronchogenic carcinoma (McArthur *et al*, 1982) or opiate withdrawal (Gold *et al*, 1978 and 1990; Henry, 1974; Jaffe, 1980). Unfortunately unpleasant and intolerable side effects such as lethargy, orthostatic hypotension (not in diabetic diarrhoea therapy), and α_2-adrenoceptor withdrawal syndromes, have been associated with the treatment of diarrhoea using α_2-adrenoceptor agonists. Obviously the development of greater gastrointestinal selectivity would be advantageous in the reduction of these unwanted side effects. In this respect Dharmsathaphorn *et al* (1984) reported that methoxy substitutions on the phenyl ring of imidazoline derivatives increased gut verses brain selectivity

Both α_2- (Cotterell *et al*, 1982 and 1984; Nakaki *et al*, 1983) and α_1-adrenoceptors (Cotterell *et al*, 1982, 1983 and 1984) have been identified on rat jejunal epithelial cell membranes using radio ligand binding techniques,

however there is less information about a functional regulatory role for α_1-adrenoceptors (Cotterell *et al*, 1984; Parsons *et al*, 1983).

Bunce and Spraggs (1983b) have shown that high doses of the selective α_1-adrenoceptor agonist phenylephrine induces antisecretory responses upon fluid absorption in the rat jejunum *in-vivo* through non-specific activation of α_2-adrenoceptors. Furthermore an antisecretory response induced by a high concentration of phenylephrine upon rat jejunal basal SCC *in-vitro*, is sensitive to α_2-adrenoceptor antagonism, revealing a transient increase in SCC (Dettmar *et al*, 1986a). The results presented in Chapter 4 suggest that this transient increase in SCC observed after α_2-adrenoceptor antagonism, is mediated through direct activation of α_1-adrenoceptors in the rat jejunum, and that this response is associated with a stimulation of electrogenic Cl^- secretion, although a contributory component from electrogenic Na^+ transport cannot be totally excluded. There appears to be little involvement of electrogenic HCO_3^- transport in the response. α_2-Adrenoceptor antisecretory influences appear to predominate and have to be antagonised before α_1-adrenoceptor mediated secretory activity can be observed. Since the selective α_1-adrenoceptor agonist phenylephrine exhibited a relatively low potency at these jejunal α_1-adrenoceptors, and showed a higher affinity for the α_2-adrenoceptors, this might suggest that these jejunal α_1-adrenoceptors may be subtly different to the classical α_1-adrenoceptors classified in other tissues. Furthermore the selective α_1-adrenoceptor agonist cirazoline appeared to act as an antagonist rather than an agonist at these α_1-adrenoceptors. A small population of α_1-adrenoceptors compared to α_2-adrenoceptors in this tissue could also account for the lack of potency of phenylephrine, but this is improbable since radio-ligand binding evidence suggests that α_1-adrenoceptors predominate on rat jejunal epithelial cell membranes (Cotterell *et al*, 1984). Further characterization of these unusual α_1-adrenoceptors might lead to the development of intestinal selective agents

which could manipulate secretory processes with obvious therapeutic potential. Hemlin (1987) has observed that the selective α_1-adrenoceptor antagonist, prazosin is pro-absorptive in the rat jejunum *in-vivo*.

In contrast to the present *in-vitro* evidence for an electrogenic pro-secretory regulation of electrolyte transport by α_1-adrenoceptor activation in the rat jejunum, *in-vivo* evidence from investigations into the effects of haemorrhage and dehydration, suggest that angiotensin elicits enhanced noradrenaline release from sympathetic nerve terminals, resulting in stimulation of α_1-adrenoceptors and pro-absorptive responses (Levens *et al*, 1981a; Levens, 1983, 1984a and 1984b). Levins *et al* (1979) have suggested that effects of angiotensin upon intestinal transport are electrically silent, since they occur in the absence of any changes in transmural potential difference. This is consistent with the suggestion that the antisecretory effects of α_1-adrenoceptor stimulation in the jejunal mucosa probably reflect a stimulation of electrically silent sodium chloride absorption Williams (1986).

The local autocoid and neurotransmitter, 5-hydroxytryptamine (5-HT) is well established to be a potent intestinal secretagogue (Allbee and Gaginella, 1985; Ball *et al*, 1988a and 1988b; Baird and Cuthbert, 1987; Donowitz *et al*, 1977,1979 1980a and 1980b; Hardcastle *et al*, 1981; Keast *et al*, 1985), although less is known about its actual mechanism of action. Most of the 5-HT in the body is located in the gastrointestinal tract, particularly in the pyloris of the stomach and the upper regions of the small intestine (Bowman and Rand, 1980b). It is confined to the mucosal enterochromaffin cells where it functions as a local hormone (Cassuto *et al*, 1981a, 1981b, 1982a, 182b, 1982c and 1983; Cooke, 1986) and to enteric neurones where it operates as a putative neurotransmitter (Furness and Costa, 1982; Keast *et al*, 1984; Wade and Wood, 1988; Wood, 1984). Under certain diseased states such as inflammatory bowel disease, cholera toxin exposure and Carcinoid Syndrome (carcinoid

tumours of the enterochromaffin cells of the intestinal mucosa), a secretory diarrhoea is often observed, in which 5-HT release might be a contributory factor (Bowman and Rand, 1980b; Cassuto *et al*, 1981a, 1981b, 1982a, 182b, 1982c and 1983). Therefore a greater understanding of the enteric pharmacology of 5-HT, especially that associated with intestinal fluid and electrolyte secretion has important clinical implications.

It was considered pertinent to investigate any possible interactions between 5-hydroxytryptaminergic systems and the jejunal α_1-adrenoceptors regulating electrogenic ion secretion. 5-HT elicited a tetrodotoxin sensitive transient increase in rat jejunal SCC (Chapter 5), which is consistent with similar findings by Hardcastle *et al* (1981), also in the jejunum, and Donowitz *et al* (1979) in the ileum of the rat. A small residual 5-HT evoked response was observed in the presence of tetrodotoxin, and was resistant to antagonism by methysergide. This residual response might reflect an incomplete blockade by tetrodotoxin at the neuronal sodium channel, however a non-specific action by 5-HT or a direct action on the transporting epithelium cannot be totally excluded.

Circumstantial evidence implies an indirect action of 5-HT in evoking intestinal secretory responses. Radio-ligand binding studies have been unable to detect any 5-HT receptors on rat intestinal epithelial cell membranes (Gaginella *et al* (1983). The sparse distribution of 5-hydroxytryptaminergic fibers in the mucosa and their presence in the submucosal ganglia reduces the possibility that these neurones influence mucosal function directly by releasing 5-HT at the neuroenterocyte junction (Keast *et al*, 1984). In addition the morphology of 5-hydroxytryptaminergic neurones with their single long axons that project over relatively long distances down the intestine suggests that they are involved in transmitting information down the gut to the submucosal plexus. Wood (1984) has proposed that activation of enteric 5-HT neurones stimulates synaptically coupled neurones which regulate the secretory epithelium of the intestine.

Branchek *et al* (1984) have described high affinity, saturable, reversible binding sites for [^{3}H] 5-HT in enteric neural membranes. These sites are different from either the 5-HT_1 or the 5-HT_2 class of central nervous system 5-HT receptor, and the structure-activity requirements of indoles for the enteric binding sites parallel their requirements for the pharmacological activity at M receptors. Furthermore Mawe *et al* (1986) have suggested that there are two classes of enteric neural 5-HT receptors located on myenteric neurones, i.e., 5-HT_{1P} and 5-HT_{2P}, which are selectively antagonised by 5-hydroxytryptophenyl-5-hydroxytryptophan amide and ICS 205930 respectively. Buchheit (1989) has reported that the 5-HT_3 receptor antagonist, ICS 205930 reduces cholera toxin-induced intestinal secretion in mice. Ball *et al* (1988b), have suggested that in the rat ileum, high concentrations of 5-HT stimulate neuronal 5-HT_3 receptors, whereas low concentrations stimulate non-neuronal benzamide-sensitive 5-HT-receptors, which are not 5-HT_1, 5-HT_2 or 5-HT_3-like in nature. In contrast Beubler and Burg (1989), have reported that 5-HT released by cholera toxin in the rat jejunum, stimulates both 5-HT_2 and 5-HT_3 receptors.

The results of the present study have shown that 5-HT evokes a transient increase in SCC in the rat jejunum *in-vitro*, a response which appears to be partly mediated through sympathetic nervous stimulation and subsequent activation of α_1-adrenoceptors, as demonstrated by the sensitivity of the response to reserpinization and α_1-adrenoceptor antagonism. The neural activation by 5-HT might possibly be post-ganglionic, and there appears to be an association with electrogenic HCO_3^- secretion. Results presented in Chapter 4 have shown that direct activation of jejunal α_1-adrenoceptors stimulates electrogenic Cl^- and not HCO_3^- secretion, thus there appears to be a pharmacological paradox. One possibility is that 5-HT stimulates sympathetic nerves which specifically innervate enteric cells which are predominantly associated with HCO_3^- secretion, and that direct α_1-adrenoceptor activation by exogenous agonists predominately stimulate enteric transporting cells which

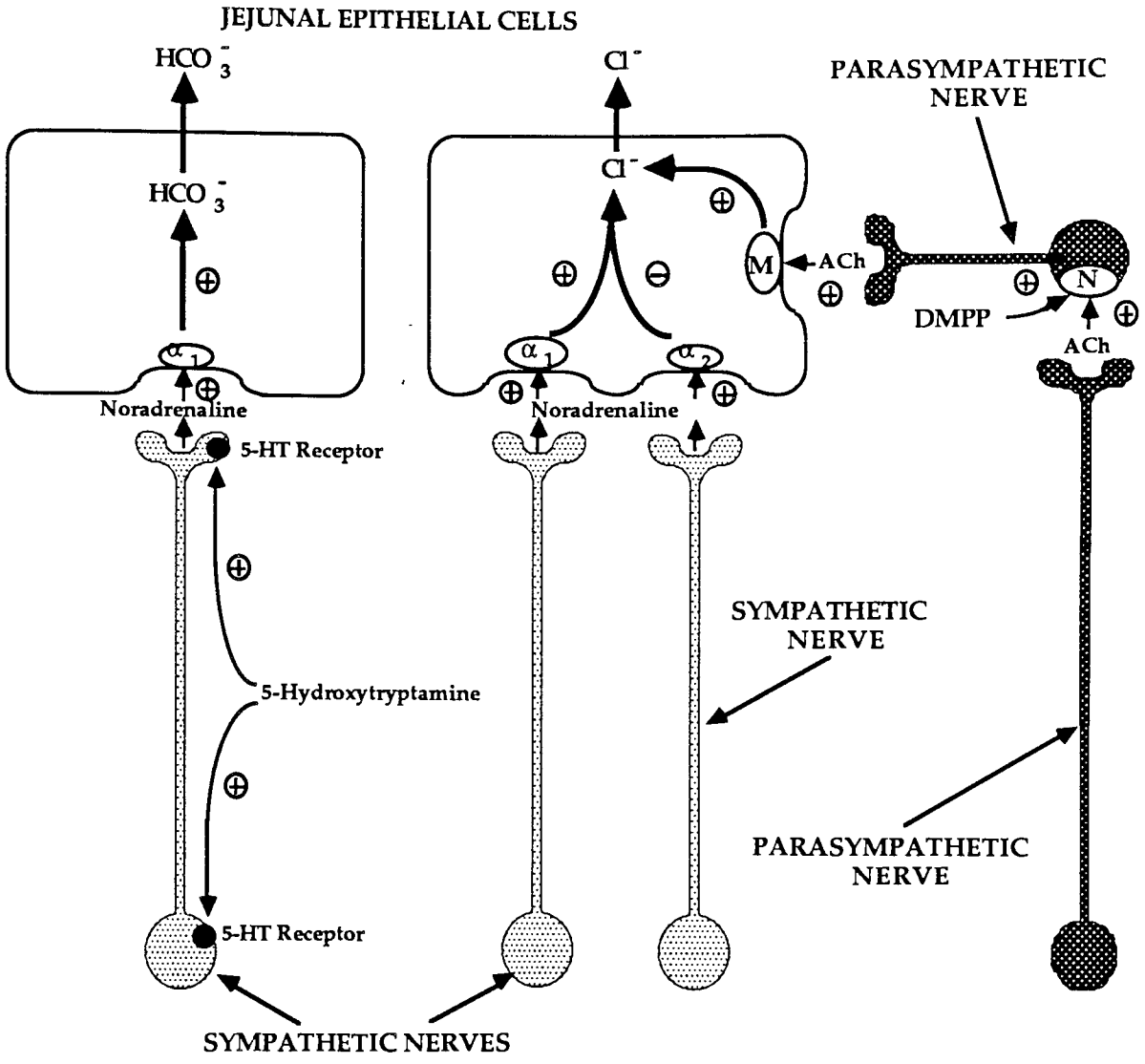

FIGURE 59 : A diagrammatic representation of the putative site of action and influence of 5-hydroxytryptamine upon rat jejunal HCO_3^- transport. 5-hydroxytryptamine is suggested to stimulate neurally located 5-hydroxytryptamine receptors which activate sympathetic transmission and release noradrenaline, specifically innervated enteric cells predominantly associated with HCO_3^- secretion are then activated by stimulation of α_1-adrenoceptors. The secretory effects of α_1-adrenoceptor and muscarinic cholinoceptor activation, and the antisecretory effect of α_2-adrenoceptor activation upon enteric cells predominantly associated with Cl^- secretion are also illustrated. ● = 5-Hydroxytryptamine receptor, M = muscarinic cholinoceptor, N= nicotinic cholinoceptor.

are preponderantly associated with Cl^- secretion (figure 59). Microelectrode studies of responses to secretagogues in both the rat ileum and jejunum *in-vitro,* have suggested that both crypt and villus cells have a secretory capability (Stewart and Turnberg, 1989). PGE_2 was found to increase transmural potential difference, depolarize the apical membrane and stimulate Cl^- secretion in both villus and crypt cells. Although the effects of 5-HT were not investigated in the jejunum, 5-HT did evoke increases in ileal transmural potential difference and induce depolarization of apical membranes, however an association with Cl^- secretion was not investigated. The possibility therefore exists that the two different ionic secretory responses by direct and indirect α_1-adrenoceptor activation in the rat jejunum, observed in the present study, could be due to different locations of these α_1-adrenoceptors, i.e., either on intestinal crypt or villus cells.

5-HT has been reported previously to have the ability to depolarize sympathetic neurones in the superior cervical ganglia of the rabbit, the effect was specifically and selectively antagonised by the neuronal 5-HT receptor antagonist ICS 205930 (Round and Wallis, 1986).

Since there is some evidence to suggest that these jejunal α_1-adrenoceptors activated indirectly by 5-HT are atypical (Chapter 5), the development of intestinal selective α_1-adrenoceptor antagonists could be of use in hyper-secretory states associated with 5-HT release, such as carcinoid syndrome. Suppression of the watery diarrhoea of the carcinoid syndrome by treatment with the $5\text{-}HT_3$-receptor antagonist, ICS 205-930 has been reported (Anderson *et al,* 1987).

There is considerable evidence supporting a physiological role for adrenergic regulation of large intestinal electrolyte transport, although there is some uncertainty about the particular regulatory adrenoceptor sub-types involved, which probably results from species variation and the

heterogeniety of this transporting epithelium.

Racusen and Binder (1979) have attributed antisecretory responses by adrenaline in the rat colon, to activation of both α- and β-adrenoceptors, the latter contrasting the observations of Dettmar *et al* (1986b) using the rat small intestine. Racusen and Binder (1979) have suggested that the effects of adrenaline are due to a direct action upon receptors located on the transporting epithelium. Work undertaken by Dharmsathaphorn *et al* (1984) using the rat distal colon *in-vitro*, has suggested that it is α_2-adrenoceptor activation which exhibits the antisecretory regulation over electrolyte transport. In addition Albin and Gutman (1980) have observed that α-adrenoceptor stimulation in the rabbit distal colon *in-vitro* enhances net sodium absorption. There is much less information concerning the role of β-adrenoceptors in the regulation of colonic electrolyte and fluid transport. Conley *et al* (1976), have reported that the non-selective β-adrenoceptor antagonist propranolol inhibits secretion evoked by deoxycholic acid in the rabbit colon *in-vivo*, which contrasts the findings of Hall *et al* (1981), who observed that propranolol was ineffective in the treatment of bile acid induced diarrhoea in humans. β-Adrenoceptor activation in the rabbit distal colon has been shown to elicit K^+ secretion (Halm *et al*, 1983; Halm and Frizzell, 1986; Smith and McCabe, 1986).

Prior to the present investigation (Chapter 6) there was little information about the particular β-adrenoceptor sub-type which has the antisecretory regulatory function over electrolyte transport in the rat distal colon. *In-vitro* evidence has been presented for a β_2-adrenoceptor rather than β_1-adrenoceptor antisecretory regulation of distal colonic electrogenic electrolyte transport, as reflected in a decrease in SCC. In addition some evidence has also been produced for a similar α_2-adrenoceptor mediated antisecretory regulation. A β_2-adrenoceptor colonic antisecretory regulation of electrogenic electrolyte transport contrasts the effects of such receptor stimulation reported

in the rat jejunum *in-vitro* (Dettmar *et al* (1986b). However β-adrenoceptor mediated antisecretory decreases in SCC in the rat colon, have been reported to be associated with both pro-absorptive and antisecretory responses upon NaCl transport (Racusen and Binder, 1979).

Further investigation is necessary to fully characterise these colonic regulatory β-adrenoceptors. Arunlakshana and Schild analysis of β-adrenoceptor antagonism, and the use of selective β-adrenoceptor agonists in the construction of log. concentration response relationships, to observe possible rank order of potency is required.

As described previously it is unclear whether there is any nervous adrenergic tone to the intestinal tract regulating fluid and electrolyte transport. Based on circumstantial evidence, Chang *et al* (1983a) have suggested the possible existence of adrenergic sympathetic tone regulating intestinal fluid and electrolyte transport. Destruction of sympathetic nerve terminals innervating the ileum and colon and a malabsorption of ions is often observed in diabetic rats. The α_2-adrenoceptor agonist clonidine can prevent naloxone-precipitated morphine withdrawal diarrhoea (Nakaki *et al*, 1981; Schreier and Burks, 1980), and can inhibit caster oil induced diarrhoea in rats (Lal and Shearman, 1981; Spraggs and Bunce, 1983), and acute opiate withdrawal diarrhoea in humans (Gold *et al*, 1978). In addition clonidine is reported to be therapeutically beneficial in the treatment of patients with diabetic diarrhoea and autonomic neuropathy. Furthermore clonidine has been shown to correct the dysfunction of fluid and electrolyte absorption, and the denervation supersensitivity seen in the chronically diabetic rats (Chang *et al*, 1986). Some of the adverse side effects of drugs might be associated with their effects upon intestinal fluid and electrolyte transport, e.g., reserpine and adrenergic blocking drugs such as guanethidine can induce diarrhoea (Bowman and Rand, 1980), whereas clonidine is known

to cause constipation (Schmitt, 1977).

Using an isolated colonic loop technique, an attempt was made to try and elucidate any adrenergic regulation of rat distal colonic electrolyte and fluid transport *in-vivo* (Chapter 2, Section 2). The possible role of adrenoceptors in both basal and secretagogue stimulated fluid and electrolyte transport in the rat distal colon was addressed (Chapter 7).

Large volumes of fluid are transported across the gut mucosa during normal digestion, however in diarrhoea very large quantities of fluid may be lost in the stools, e.g., in Asiatic cholera, patients may lose 1 litre/hour of fluid (Sjövall *et al*, 1987). Clearly, a physiological regulation of intestinal fluid and electrolyte transport would be advantageous in body fluid homeostasis. If there is continual anti-secretory/pro-absorptive tonic adrenergic regulation of colonic fluid and electrolyte transport, then adrenoceptor antagonism should either reduce any ongoing absorption and/or stimulate any ongoing secretion. Such antagonism would provide a simple way of determining if there is any adrenergic tone is present, and if this is the case there is the potential for the development of intestinal selective adrenoceptor antagonists for treating conditions such as constipation.

A significant ongoing distal colonic absorption of Na^+ and Cl^-, and secretion of K^+ was recorded under basal conditions, whereas a residual non-significant secretion of fluid was also observed (Chapter 7). These basal levels of transport were unaffected by α_1-adrenoceptor or α_2-adrenoceptor antagonism, suggesting little ongoing basal α-adrenergic tonic regulation. Possible β-adrenergic tone was not investigated and remains a possibility.

Noradrenaline infusion evoked a substantial effect upon basal fluid transport, converting a non-significant secretion into a highly significant

absorption. In addition a simultaneous highly significant stimulation of isotonic Na^+ and Cl^- absorption was also observed. These findings are consistent with observations made in the rat ileum and jejunum, where noradrenaline is also reported to enhance fluid and NaCl absorption (Hubel, 1976). In the present investigation the noradrenaline stimulated Na^+ and Cl^- absorption were closely matched, suggesting a possible stimulation of coupled NaCl transport, although radio labelled flux studies would be required for full confirmation. Sellin and DeSoignie (1984), using the rabbit colon and Racusen and Binder (1979), using the rat colon *in-vitro*, have both suggested that noradrenaline stimulates coupled NaCl absorption. In the present study as well as an effect of noradrenaline upon NaCl absorption, an antisecretory effect upon isotonic K^+ transport was also recorded. Colonic K^+ secretion is an electrogenic process (Chapter 1, Section 2D), and so the effect of noradrenaline upon K^+ transport is probably a reflection of an inhibition of this active transport process.

The effects of noradrenaline upon the transport of other ionic species not monitored in the present investigation cannot be excluded, e.g., an effect upon HCO_3^- transport. *In-vitro* evidence has shown that in the rabbit ileum, adrenaline can stimulate sodium-dependent HCO_3^- absorption (Smith *et al*, 1985), whereas α-adrenoceptor activation in the rat colon can evoke a decrease in HCO_3^- secretion (Racusen and Binder, 1979).

The effects of selective adrenoceptor antagonism were not investigated upon the influences of noradrenaline on distal colonic fluid and electrolyte transport *in-vivo*, however infusion of the selective α_2-adrenoceptor agonist UK-14,304 alone, was without effect upon the basal levels of transport. This is consistent with the findings of Bunce and Spraggs (1983a), who observed that the selective α_2-adrenoceptor agonist clonidine, was without effect upon the basal level of fluid absorption in the rat colon *in-vivo*. It is therefore unlikely that the effects of noradrenaline were mediated through α_2-adrenoceptor activation, since specific stimulation of these receptors was

without effect. Stimulation of other adrenoceptors such as α_1- or β could be involved. *In-vitro* evidence has been presented in Chapter 4 for an α_1-adrenoceptor secretory regulation of jejunal electrogenic electrolyte transport, in contrast, Williams (1986) has suggested that α_1-adrenoceptor activation in this tissue can evoke antisecretory/proabsorptive responses, probably mediated by a stimulation of electrically silent sodium chloride absorption. Noradrenaline therefore could be activating colonic α_1-adrenoceptors, thus stimulating coupled NaCl absorption. This requires further investigation, especially in light of the differences between that large and small intestine, as outlined in Chapter 1. Selective stimulation and antagonism of α_1-adrenoceptors with specific agents would therefore be advantageous in elucidating the mechanism of action of noradrenaline.

In-vitro evidence has been presented for a β_2-adrenoceptor mediated antisecretory response upon electrogenic electrolyte transport in the rat distal colon (Chapter 6). Therefore the observed effects of noradrenaline in the distal colon *in-vivo* might be mediated via β_2-adrenoceptor stimulation, however β_1-adrenoceptor activation in the rabbit distal colon (Smith and McCabe, 1986) and raised intra-cellular cAMP in the rat proximal colon (Foster *et al*, 1983) are reported to evoke a secretory response upon K^+ transport. Since the proximal and distal colonic regions have differences in their electrolyte transport function and there is also considerable species variation (Binder and Sandle, 1987), additional investigations are required to evaluate any possible role of β-adrenoceptors in the observed response by noradrenaline in the distal colon. The effects of selective β-adrenoceptor agonists and antagonists would be helpful in clarifying any possible β-adrenergic mechanism.

Theophylline and PGE_2, both significantly stimulated colonic fluid secretion, however noticeable colonic mucus was also observed within the intestinal lumenal samples after exposure with these secretagogues.

Therefore the mucolytic N-acetyl-L-cysteine which is documented to degrade the mucus matrix (Norris *et al*, 1983), was introduced to lumenal samples collected after PGE_2 treatment.

PGE_2 significantly reduced the level of isotonic Na^+ absorption in samples untreated by the mucolytic, however in those samples which were treated, no significant effect was observed, and therefore no real conclusion could be made about the effects of PGE_2 upon Na^+ transport.

Unfortunately the Cl^- analyzer used in the Cl^- assay of the colonic lumenal samples, also titrated N-acetyl-L-cysteine along with Cl^- ions, thus introducing an experimental error, therefore the effects of PGE_2 upon Cl^- transport could only be assessed in samples not treated with the mucolytic. PGE_2 significantly reduced the basal level of isotonic Cl^- absorption. In the rat proximal colon PGE_2 and theophylline are suggested to inhibit electrically neutral absorption of Na^+ and Cl^-, which constitutes an important pathogenic mechanism in secretory diarrhoea (Haag *et al*, 1985). In the present study in the distal colon, a stimulation of isotonic K^+ secretion (possibly electrogenic) was also observed by PGE_2 and theophylline, this might represent another important contribution to the pathogenic mechanism of secretory diarrhoea.

The selective α_2-adrenoceptor agonist UK-14,304 significantly decreased the secretory effects of PGE_2 upon distal colonic fluid and K^+ transport. It would have been interesting to see if the effects of UK-14,304 could be decreased by α_2-adrenoceptor antagonism. Bunce and Spraggs (1983a) have shown that clonidine reverses the reduced fluid absorption evoked by PGE_1/theophylline in the rat jejunum *in-vivo*, a response which is yohimbine sensitive, however they observed no such reversal by clonidine in the ileum or colon.

In conclusion, although a basal distal colonic absorption of Na^+ and Cl^-, and a secretion of K^+ was observed, these basal transport processes do not

appear to be under α-adrenergic tonic regulation, whilst other adrenoceptor mechanisms still remain possibilities. In contrast the secretory effects of PGE_2 are inhibited by α_2-adrenoceptor activation, whereas such stimulation does not evoke pro-absorptive responses upon basal transport, unlike noradrenaline.

APPENDIX I.

ELECTRONIC DEVICES ASSOCIATED WITH THE MONITORING OF TRANSEPITHELIAL PD AND SCC FROM INTESTINAL TISSUE MOUNTED IN USSING CHAMBERS.

The following equipment was designed and built in the Department of Pharmaceutical Sciences, Aston University by Mr. D. Briggs.

1) Electrode Offset Compensator (Potentiometer).

This device was needed to make adjustments to correct for slight differences in potential between the voltage measurement electrodes. It was comprised of a stable mercury battery (1.35V) which passed a small current through a large value resistor (3.3 MOhms) onto a bridge network of fixed resistors and a multi-turn potentiometer. The connections are such that, when the battery is switched on, the offset voltage obtainable between the wiper of the potentiometer and the mid-point of the resistor network is evenly adjustable between +3 and -3 mV. Across the entire adjustment range, the net impedence of the circuit, as seen by the voltage clamp, remains close to 10 KOhms.

2) High Resolution Digital Millivoltmeter.

The circuitry for this device is based on the use of digital multimeter integrated circuit (R.S. Components Ltd., Northants). This chip uses a sophisticated combination of analogue and digital techniques to obtain 4.5 digits of conversion. It also provides the control signals for the liquid crystal display and requires very little additional circuitry to make the complete meter. The display gives a reading between +199.99 and -199.99 mV with a resolution of 10 μV. The conversion technique used guarantees a zero reading for a zero signal input, which makes the meter useful for nulling the voltage clamp. To provide additional accuracy, a quartz crystal (100 KHz) is used to drive the converter clock. The display is updated at the end of each conversion, of which there are approximately 2 each second. The meter runs on a single 9V battery monitoring circuit which illuminates a 'Low Battery' annunciator on the display when the battery voltage drops below about 6.5V.

3) Automatic Voltage Clamp (Short-Circuit Current Amplifier).

A). Circuitry.

This voltage clamp design is very similar to that described by Rothe *et al* (1969). The circuitry for the device breaks down into three main sections. These are :-

(i) the voltage sensing differential amplifier;
(ii) the current measurement amplifier; and
(iii) the current generator.

Signals from the potential recording electrodes feed into the inputs of two high input impedence operational amplifiers which are arranged in a instrumentation amplifier configuration. This amplifier multiplies, by a factor of approximately 5, the difference between the potentials, before feeding two signals into a following differential amplifier. This amplifier also amplifies the signal, having a gain of about ten. This differential amplifier produces a single-sided output, one referenced to ground, which reflects the voltage difference across the membrane.

The current measurement amplifier is used to maintain the overall potential of the bath at a controlled level, and it also sucks any current injected into the bath out again. Current measurement is achieved by forcing the amplifier to use a 4.7 KOhm resistor in the current path. The amplifier always tries to maintain the bath end of this resistor at circuit ground potential. When current is passed into the bath, the voltage rises, and the amplifier has to draw more out again. This arrangement turns the current passing into a voltage signal, also referenced to the circuit ground, but inverted (i.e., a positive current produces a negative voltage).

The output from both signals of these circuits are added together by the current generator. The resultant signal is the clamp command signal. This is amplified by the current generator, which is also inverting, to give an integral control current which is passed out to the tissue bath.

B). Operation.

When no clamp current is flowing, the voltage amplifier senses the difference in voltage across the membrane. The resultant signal can be used as a command signal for the current generator. If the current path was completed and the short-circuit clamp switched on, this would have the effect of reducing the voltage across the membrane to zero. However when current flows through the saline in the bath, small voltages are developed as a direct consequence of the resistance of the saline itself. The current generator

actually clamps the voltage sensing electrodes, and not the tissue. The electrodes do not normally change position during an experiment, so if the resistance in the saline was known, it should be possible to compensate for it. This is determined in the following way:-

The bath is assembled without any tissue in it, but with all other conditions as for an experiment. A hand held adjusted potentiometer is then used to pass a varying current through the bath (with the clamp current disconnected). While monitoring the output voltage of the current generator, the proportion of the current signal (as measured by the current measurement amplifier) that is added to the voltage command signal can varied using a 'built in' multiturn potentiometer.

The object of this adjustment is to cancel out (just) the voltage changes which are being produced by the first potentiometer. When this has been done, then the proportion of the voltage signal which resulted from the resistance of the saline is exactly cancelled out. The multiturn dial is then locked in position and the first potentiometer removed from the circuit. Then, with a piece of tissue in place, a similar amount of saline will be present between the voltage sensing electrodes, and a similar proportion of the voltage seen will be produced current flowing in the clamp circuit. When the clamp is operating, any current that is passed through the tissue goes through an accurate 1 KOhm resistor first. The current will induce a voltage across the resistor and this is measured by another differential amplifier (which can be used to monitor the voltage difference as well). The current or voltage signal is fed out to the high resolution digital millivoltmeter. This output is a low impedence (approximately 1 KOhm) single ended signal, referenced to circuit ground. During operation of the clamp, the voltage signal obtained will not be zero, but can be used to check the clamp efficiency if the saline resistance is known. The automatic voltage clamp is mains powered.

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