J. Physiol. (1986), **376**, *pp.* 531–542
With 4 plates and 3 text-figures
Printed in Great Britain

MUCOSAL PLEXUS AND ELECTROLYTE TRANSPORT ACROSS THE RAT COLONIC MUCOSA

BY R. J. BRIDGES, M. RACK*, W. RUMMEL AND J. SCHREINER

*From the Institut für Pharmakologie und Toxikologie and the *Institut für Physiologische Chemie, der Universität des Saarlandes, D6650 Homburg/Saar, F.R.G.*

(*Received 6 August 1985*)

SUMMARY

1. Histological and functional studies were performed on a preparation of rat colonic mucosa from which the myenteric and submucosal plexus were removed. This preparation, referred to as the mucosa preparation, was used to investigate the potential influence of the mucosal plexus on electrolyte transport. Two neuropharmacologically active agents were used: sea anemone toxin (ATX II) to stimulate the fibres of the mucosal plexus and tetrodotoxin (TTX) to block the fibres of the mucosal plexus.

2. The morphology of the neuronal network of the mucosal plexus was visualized after the epithelium was removed and whole mount preparations of the lamina propria and circular muscle layer of muscularis mucosae were stained histochemically for acetylcholinesterase activity. Several levels of organization within the mucosal plexus were seen. Each crypt is encircled by a thin bundle of fibres near the top. These thin fibres connect with thicker bundles of fibres that encircle groups of two to five crypts in a broad band. These bundles of fibres are in turn connected to larger bundles of fibres which lie in a flat plane just below the crypts along the circular muscle layer of muscularis mucosae. In addition perikarya and ganglia were revealed within the mucosal plexus.

3. The base-line net transport of Na^+ and Cl^- across the mucosa preparation was completely inhibited by ATX II (10^{-6} M). This effect of ATX II on net Na^+ and Cl^- transport was accompanied with an increase in the short-circuit current (I_{sc}), transmural conductance, and open-circuit potential difference across the mucosa preparation. The effect of ATX II on I_{sc} was dose dependent with a half-maximal effective concentration at 5×10^{-8} M-ATX II and a maximal effective concentration of 10^{-7} M. ATX II was effective only when added to the serosal solution.

4. Net Na^+ and Cl^- transport was restored by TTX (10^{-6} M) to base-line values in ATX II-treated tissue. In addition the value of all three electrical parameters rapidly returned to the values measured before the addition of ATX II. TTX was effective in antagonizing the effects of ATX II only when added to the serosal solution.

5. The results suggest that the regulation of electrolyte transport across the epithelium is at least one function of the mucosal plexus. Stimulation of the neurones

within the mucosal plexus leads to the inhibition of electrolyte absorption. The neurones of the mucosal plexus appear to be spontaneously inactive and must be continuously stimulated in order to inhibit electrolyte absorption. This stimulation may be achieved with neuro-pharmacologically active agents or physiologically by the spontaneously active neurones extending from the submucosal plexus. Once this inhibitory influence is blocked the epithelium quickly returns to a state at or near maximal absorption suggesting that this absorptive state is the functional intrinsic set point of the epithelium.

INTRODUCTION

The presence of nerve fibres within the mucosal layer of the gastrointestinal tract has been known for more than 120 years (Billroth, 1858). Numerous investigators have since demonstrated in all regions of the gastrointestinal tract including the oesophagus, stomach, small and large intestine and rectum the existence of a dense network of neuronal fibres within the mucosa (for references see Hillarp, 1946; Kuntz, 1953; Schofield, 1968; Keast, Furness & Costa, 1986). Collectively this network has been named the mucosal plexus. It has been divided by some authors into a subglandular plexus lying below the crypts near the circular smooth muscle layer of muscularis mucosae, a periglandular plexus surrounding the crypts and an intravillous plexus extending into the villi. The nerve fibres of the mucosal plexus are thought to originate from both extra- and intra-mural sites. It is not known whether any of the fibres are sensory fibres. Although it was suggested last century there were perikarya within the mucosal plexus (Drasch, 1881), contemporary histologists have not confirmed this point. Thus the mucosal plexus is presently considered to be an aganglionated plexus free of perikarya.

The functional role of the mucosal plexus in the digestion and absorption of food and water is unknown. Possible functions include the regulation of blood flow, intestinal motility, and epithelial solute and water transport. These three possibilities are not mutually exclusive, however, since all three must be regulated in a coordinated manner for the gastrointestinal tract to function properly. Here we report experimental evidence that the transport of electrolytes across the colonic mucosa can be influenced by the mucosal plexus.

To investigate the potential influence of the mucosal plexus on electrolyte transport we used a preparation of rat colonic mucosa from which the myenteric and submucosal plexus were completely removed (Andres, Bock, Bridges, Rummel & Schreiner, 1985). Thus any neuronal-mediated effects on electrolyte transport must be attributed to the mucosal plexus. Two neuro-pharmacologically active agents were used to stimulate or block the mucosal plexus. Sea anemone toxin (ATX II) is a purified polypeptide toxin isolated from *Anemonia sulcata* (Béress, Béress & Wunderer, 1975*a*, *b*). ATX II has been shown in isolated nerve preparations to cause an increase in the spontaneous firing rate, an increase in the duration of the action potential, and an increase in neurotransmitter release (Béress, 1985) by a mechanism similar to other neuro-pharmacologically active agents of this class (e.g. batrachotoxin, scorpion venom, veratridine). A second neuro-toxin, tetrodotoxin (TTX), a known inhibitor of neuronal transmission and an antagonist of ATX II (Catterall, 1980) was used to

block pharmacologically the fibres of the mucosal plexus. Finally, because relatively few histological studies on the mucosal plexus from rat colon descendens have been reported, the histology of the mucosal plexus was investigated. The results will show that the mucosa of rat colon descendens is densely innervated and that perikarya and ganglia are also present within the mucosal plexus. Net Na^+ and Cl^- transport across the mucosa was completely inhibited by the addition of ATX II to the serosal bathing solution and this effect of ATX II was blocked by TTX.

METHODS

Animals

Female Sprague–Dawley rats (200–220 g) were used. They were housed in wire-bottom cages and had free access to food (Altromin Diet No. 1320, Lage, F.R.G.) and water until the time of the experiments.

Histology

Cross-sections and whole mount preparations of the mucosa with and without the epithelium were stained for acetylcholinesterase. Frozen sections (15 μm) of the intact mucosa were cut and air-dried overnight on microscope slides. The epithelium was removed from the lamina propria according to the method of Bjerknes & Cheng (1981) with some modifications. The rat was killed by cervical dislocation, the colon exposed through a mid-line incision and flushed with 25 ml Ca^{2+}-free buffer solution, 37 °C. The descending aorta was cannulated below the renal arteries and the animal perfused (3 ml min^{-1}) with 20 mM-EDTA buffer solution at 37 °C for 15 min followed for 5 min with Ca^{2+}-free buffer solution. The colon was then removed and dissected to isolate the circular muscle layer of muscularis mucosae and the adherent lamina propria. The tissue was then mounted on a small plastic holder with a 2 cm^2 orifice, attached to an electrical vibrator, vibrated for 15 s and subsequently placed in a Ca^{2+}-containing buffer solution for 15 min. After this the tissue was cut from the holder and air-dried overnight on a microscope slide.

Sections or whole mounts were fixed for 1 min in 10% formalin buffered with 50 mM-sodium acetate (pH 7·4) at 4 °C and subsequently washed with distilled water. Acetylcholinesterase staining was conducted according to the method of Karnovsky & Roots (1964). Acetylthiocholine was used as the substrate and the reaction carried out at pH 6·0. The use of tetraisopropyl pyrophosphoramide (OMPA) 10^{-4} M to inhibit non-specific ('pseudo') cholinesterase and the duration of the staining reaction are indicated in the Plate legends. Staining was intensified with silver as described by Bock & Mühlen (1968).

Electrical and ion-flux measurements

A preparation of the mucosa consisting of the mucosa and circular muscle layer of muscularis mucosae was used for all transport studies. This preparation, referred to as the mucosa preparation, was obtained exactly as described in detail by Andres *et al.* (1985). Furthermore electrical and ion flux measurements were also conducted as described by Andres *et al.* (1985).

Solutions

The bathing solution contained (in mM): NaCl, 107; KCl, 4·5; $NaHCO_3$, 25; Na_2HPO_4, 1·8; NaH_2PO_4, 0·2; $CaCl_2$, 1·25; $MgSO_4$, 1·0 and glucose, 12. The solution was gassed with 5% CO_2 in O_2 and had a pH of 7·4. When $NaHCO_3$ was omitted from the solution it was replaced with NaCl and the solution gassed with O_2. ATX II (1 mg) was dissolved in 150 mM-NaCl and kept frozen (−20 °C) in small aliquots until used. ATX II was a generous gift from Dr Béress. A stock solution of TTX, 1 mM in 5 mM-disodium citrate, pH 5·3, was prepared and diluted with bathing solution as necessary. TTX was obtained from Sankyo Co, Ltd., Tokyo, Japan. Acetylthiocholine, OMPA, and physostigmine were obtained from Sigma.

Statistics

Results are given as the mean ± one S.E. of mean. Significances of differences were tested using a two-tailed Student's *t* test. Paired or unpaired tests were used.

RESULTS

Histology

A photomicrograph of a cross-section of the mucosa preparation stained for acetylcholinesterase is shown in Pl. 1. The epithelium, circular muscle layer of muscularis mucosae and short segments of nerve fibres are stained. If OMPA (10^{-4} M), an inhibitor of non-specific cholinesterases was included in the incubation solution only the nerve fibres stained. If acetylthiocholine, the substrate, was omitted from the incubation solution or if physostigmine (10^{-4} M), an inhibitor of acetylcholinesterase, and OMPA (10^{-4} M) were included in the incubation solution then none of the structures stained. The latter two observations were also true for the whole mount preparations. Pl. 1 shows a photomicrograph of a typical cross-section of the colonic mucosa. In areas where the lamina propria is particularly wide short segments of nerve fibres can easily be seen. Where the crypts lie in close apposition, however, it is difficult to see any fibres. Occasionally, when the lamina propria between two crypts has accidentally been spread apart, arborizing fibrous structures can be seen.

A quite different view of the mucosal plexus is shown in Pl. 2. Here the epithelium has been removed and the mucosal plexus photographed as one looks down upon it from the mucosal side. The vast network of nerve fibres seen here is in strong contrast to the fragmentary view afforded with cross sections. Pl. 2 corresponds to 0·013 cm^2. This area would be occupied by approximately 300 crypts. Among the notable features that are revealed by this view of the mucosal plexus is the presence of perikarya some of which are aggregated in small ganglia (Pl. 3*A–C*). Many of the ganglionic cells have the same size, shape, and staining characteristics as cells seen in the ganglia of the submucosal plexus (Andres *et al.* 1985). The ganglia of the mucosal plexus have a fewer number of cells per ganglia (two to five cells) and there are a fewer number of ganglia per unit surface area (approx. 200 cm^{-2}) than in the submucosal plexus (five to forty cells per ganglia, approx. 400 ganglia cm^{-2}).

One feature which is difficult to capture in a single photomicrograph is the different levels of apparent organization within the mucosal plexus. The ganglia and the largest fibres lie in a relatively flat plane near the circular muscle layer of muscularis mucosae. From this plane smaller fibres project upwards to surround groups of crypts, seen as vacant pits in the lamina propria where the crypts used to be. Approximately two to five crypts per group are encircled in a broad band of fibres extending from the lower plane to just below the top of the crypts. At this point smaller fibres extend inward just below the border cells to encircle each crypt within the group. An attempt to demonstrate these different levels is shown in Pl. 4*A–C*.

Electrical and ion-flux measurements

The effects of ATX II (10^{-6} M) on the electrical parameters across the short-circuited mucosa preparation are shown in Fig. 1. ATX II caused a rapid increase in the values of all three electrical parameters (half-time 1 min). The rapid increase in short-circuit current (I_{sc}) and open-circuit potential difference (p.d.) was followed by a small transient decrease and then a slow sustained increase until the values reached a plateau 40–45 min after the addition of ATX II. The dose-dependent effect of ATX II on the steady-state change in I_{sc} is shown in Fig. 2. ATX II caused a detectable

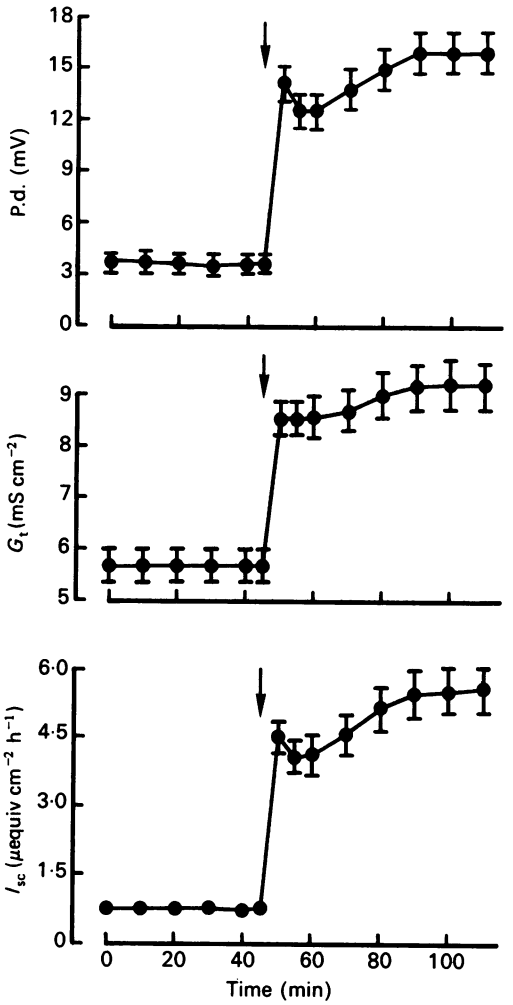


Fig. 1. Effect of ATX II added to serosal solution on the electrical parameters across the mucosa preparation from rat colon descendens. ATX II (10^{-6} M) was added at time = 45 min as indicated by the arrows. Values are the mean for thirteen preparations. Unless otherwise shown the S.E. of mean falls within the area of the symbol. P.d., open circuit potential difference; G_t, transmural conductance; I_{sc}, short-circuit current.

increase in I_{sc} at 10^{-8} M, a half-maximal effect at approximately 5×10^{-8} M, and a maximal effect at 2×10^{-7} M. The effect of ATX II on the electrical parameters was seen only when ATX II was added to the serosal solution. Addition of ATX II (10^{-6} M) to the mucosal solution had no effect on I_{sc}, transmural conductance, G_t, or p.d.

Unidirectional and net Na^+ and Cl^- fluxes across the mucosa preparation before and after the addition of ATX II (10^{-6} M) are given in Table 1. The substantial net transport of Na^+ (J^{Na}_{net}) and Cl (J^{Cl}_{net}) measured under control conditions was reduced by ATX II to values not statistically different from zero. The decrease in J^{Na}_{net} and J^{Cl}_{net} was due to a decrease in the mucosal to serosal flux and an increase in the serosal to mucosal flux of Na^+ and Cl^-. The net residual ion flux (J^{R}_{net}), calculated from

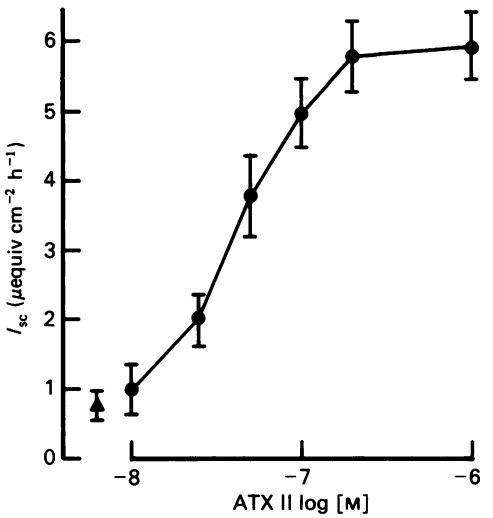


Fig. 2. Dose–response curve of the effect of ATX II added to the serosal solution on short-circuit current (I_{sc}) across the mucosa preparation. I_{sc} was measured after a plateau had been reached. The values are the mean ± the S.E. of mean for nine preparations before (▲) and after the addition of ATX II at each concentration (●).

the difference in I_{sc} and J^{Na}_{net} and J^{Cl}_{net}, was 2·8 ± 0·65 μequiv cm^{-2} h^{-1} under control conditions and 4·5 ± 0·78 μequiv cm^{-2} h^{-1} after the addition of ATX II. This increase in J^{R}_{net} was statistically significant ($P < 0·05$).

J^{R}_{net} is generally assumed to be due to the net secretion of HCO_3^-. If HCO_3^- was omitted from the bathing solution net ion fluxes under control conditions were: J^{Na}_{net} 6·7 ± 0·86, J^{Cl}_{net} 7·5 ± 1·25 and I_{sc} 0·8 ± 0·12 μequiv cm^{-2} h^{-1} and after the addition of ATX II they were: J^{Na}_{net} 2·5 ± 0·70, J^{Cl}_{net} −1·0 ± 1·26 and I_{sc} 4·8 ± 0·46 μequiv cm^{-2} h^{-1}. Thus the omission of HCO_3^- from the bathing solution reduced J^{R}_{net} under control conditions to 1·6 ± 0·74 μequiv cm^{-2} h^{-1} and to 1·2 ± 0·68 μequiv cm^{-2} h^{-1} after the addition of ATX II. These results support the conclusion that in normal HCO_3^--containing bathing solution J^{R}_{net} is, in large part, due to the secretion of HCO_3^- and that ATX II causes an increase in HCO_3^- secretion.

Addition of TTX to the serosal solution completely abolished the measured effects of ATX II on the mucosa preparation. A typical tracing of the effects of ATX II (10^{-6} M) and TTX (10^{-6} M) on I_{sc} is shown in Fig. 3. As can be seen TTX caused a rapid decrease in I_{sc} (half-time 0·5 min) to a value not different from the previous control value before the addition of ATX II. G_t and p.d. were also reduced by TTX to the control values (Table 2). Unidirectional and net Na^+ and Cl^- fluxes before and after the addition of TTX to ATX II-treated tissue are given in Table 2. TTX restored the net transport of Na^+ and Cl^- to values not significantly different from those measured under control conditions (Table 1). This restoration in J^{Na}_{net} and J^{Cl}_{net} by TTX was due to an increase in the mucosal to serosal flux and a decrease in the serosal to mucosal flux of Na^+ and Cl^-. J^{R}_{net} was reduced from 5·7 ± 0·79 μequiv cm^{-2} h^{-1} to 3·5 ± 0·73 μequiv cm^{-2} h^{-1} after the addition of TTX to the ATX II-treated tissue. This decrease in J^{R}_{net} was statistically significant ($P < 0·05$).

TABLE 1. Effect of ATX II on the unidirectional and net fluxes of Na^+ and Cl^- across the mucosa preparation from rat colon descendens

	$J_{ms}^{Na^+}$	$J_{sm}^{Na^+}$	$J_{net}^{Na^+}$	$J_{ms}^{Cl^-}$	$J_{sm}^{Cl^-}$	$J_{net}^{Cl^-}$	I_{sc}	G_t
Control	10·8±1·27	3·7±0·19	7·1±0·82	19·0±0·65	9·9±0·83	9·1±0·76	0·8±0·14	5·7±0·37
ATX II	5·8±0·82*	5·3±0·06*	0·5±0·52*	14·5±0·47*	14·7±0·78*	−0·2*±0·68	5·2**±0·51	9·0*±0·49

ATX II (10^{-6} M) was added to the serosal solution and fluxes before and after the addition of ATX II compared. Ion fluxes and short-circuit current (I_{sc}) are given in μequiv cm^{-2} h^{-1} and conductance, G_t, in mS cm^{-2}. Values are the mean±S.E. of mean for $n = 5$–7 preparations.

* Indicates significantly different when assessed with a paired Student's t test.

TABLE 2. Effect of TTX on unidirectional and net fluxes of Na^+ and Cl^- across the mucosa preparation treated with ATX II

	$J_{ms}^{Na^+}$	$J_{sm}^{Na^+}$	$J_{met}^{Na^+}$	$J_{ms}^{Cl^-}$	$J_{sm}^{Cl^-}$	$J_{net}^{Cl^-}$	I_{sc}	G_t
ATX II	5·1±0·29	4·7±0·40	0·4±0·36	15·5±0·72	14·7±1·17	0·8±1·01	5·3±0·57	9·5±0·51
TTX	9·6±0·44*	3·3±0·28*	6·3±0·36*	18·9±0·73*	9·9±0·56*	9·0±0·64*	0·8±0·11*	5·7±0·29*

TTX (10^{-6} M) was added to the serosal solution of tissue treated with ATX II (10^{-6} M) and fluxes before and after the addition of TTX compared. For other details see Table 1.

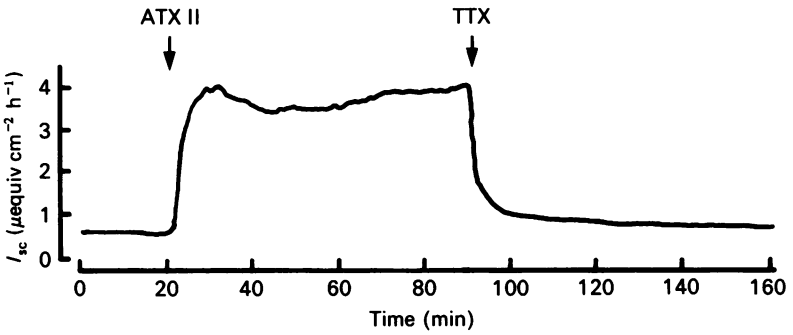


Fig. 3. Tracing of the effect of ATX II and TTX on short-circuit current I_{sc} across the mucosa preparation. ATX II (10^{-6} M) and TTX (10^{-6} M) were added to the serosal solution as indicated by the arrows.

DISCUSSION

Most histological studies of the mucosal plexus have made use of cross-sections of the mucosa with an intact epithelium. Results from these studies lead histologists to suggest that the mucosa is extensively innervated. However, to the untrained observer the fragmentary view afforded with cross-sections is rather unconvincing. Indeed the presence of the mucosal plexus has often been ignored by physiologists engaged in functional studies of the gastrointestinal mucosa. In contrast the view of the mucosal plexus shown in Pls. 2–4 should leave little doubt that the mucosa of the colon is extensively innervated. Furthermore when viewed in this manner it is also clear that within the mucosal plexus there are perikarya and ganglia. It is somewhat surprising that the latter observations have gone unnoticed for so long.

Drasch in 1881 used whole mount preparations of the mucosa from which the epithelium had been removed by prolonged incubation in citrate to study the mucosal plexus. The hand-drawn picture of the mucosal plexus presented by Drasch in his report is remarkably similar to the photomicrograph shown in Pl. 2. In his report Drasch indicated there were ganglion cells ('Ganglienknoten') within the mucosal plexus. Subsequent studies of the mucosal plexus by other investigators over the last century did not confirm this point. It is noteworthy that few studies of the mucosal plexus have made use of whole mount preparations of the mucosa. Furthermore until now, to our knowledge, there are no reports since the study by Drasch where the mucosal plexus was studied after first removing the epithelium. In our experience the presence of only a small portion of the epithelium completely obscures the lower level of the mucosal plexus and hence prevents one from seeing the perikarya and ganglia.

The acetylcholinesterase staining technique was used to demonstrate the presence of neurones within the mucosa. A positive reaction does not mean that the stained neurones are cholinergic since both cholinergic and non-cholinergic neurones can stain positively (Silver, 1974). In an ultrastructural study of the mouse colonic mucosa it has been reported that most of the neurones within the mucosa show a positive reaction when stained for acetylcholinesterase (Silva, Farrell & Smith, 1968). It is also important to point out that the fibres shown in Pls. 1–4 actually are bundles of fibres,

or fasciculi and not individual axons, or dendrites. In this same ultrastructural study of the mouse colon Silva and co-workers found that the diameter of individual fibres in the mucosa varied over a range of 0·15–1·6 μm. Most individual fibres would be below the resolving power of the light microscope and can therefore only be seen when aggregated in fasciculi. The average fasciculus in the mucosa of the mouse colon contained eight fibres (range two to forty-five) and there were approximately eighteen fasciculi around each crypt. Thus each crypt is surrounded by approximately 150 fibres.

Synaptic neurone endings such as those typically found in the central nervous system or at the motor end-plate have not been seen in ultrastructural studies of the enteric nervous system (for review see Gabella, 1981). Instead there are numerous varicosities (250–300 mm^{-1}) along the length of each fibre. These varicosities come close to, but not in contact with, other neurones, blood vessels, smooth muscle cells and the epithelium. Since the varicosities contain vesicles similar to those seen at synaptic terminals in other parts of the nervous system they are thought to be the sites at which neurotransmitters are released. In this way it is conceivable that one neurone can affect more than one cell or perhaps even more than one cell type along its length. The results of this study suggest that at least one cell type, namely the epithelial cell, is affected by the neurones of the mucosal plexus.

ATX II when added to the serosal solution caused a rapid and dose-dependent increase in I_{sc}, G_t, and p.d. across the mucosa preparation of the rat colon. The half-maximally effective concentration of 5×10^{-8} M-ATX II on the mucosa preparation agrees well with the K_a of 2×10^{-8} M reported for the effect of ATX II on isolated nerve preparations (Catterall, 1980). The increase in the values of the electrical parameters caused by ATX II was accompanied with a complete inhibition of net Na^+ and Cl^- transport. These effects of ATX II were rapidly and completely abolished by TTX. The most plausible explanation for these results is that ATX II causes the release of one or more neurotransmitters from the neurones within the mucosal plexus. The released neurotransmitters then directly or indirectly affect the epithelium to alter electrolyte transport. TTX, by blocking transmission of the neurones within the mucosal plexus, antagonizes the effects of ATX II on the neurones and restores electrolyte transport to base-line values.

The above interpretation is supported by several observations. (1) The mucosa is extensively innervated as shown in this report and by several other investigators. (2) Several neurotransmitters including vasoactive intestinal polypeptide (VIP), acetylcholine, noradrenaline, substance P, somatostatin, enkephalin, serotonin and others have been identified, by various techniques, in the mucosal plexus of the colon and some of these neurotransmitters have been shown to affect electrolyte transport by receptors on the epithelium (for review see the papers of Tapper, 1983; Gaginella, 1984; and Hubel, 1985). (3) ATX II and TTX were effective only when added to the serosal bathing solution, neither had any effect when added to the mucosal solution. (4) The only reported effects of ATX II or TTX are those resulting from changes in the excitable membranes of neurones, cardiac muscle and skeletal muscle (Narahashi, 1974; Catterall, 1980; Béress, 1986). There are no indications for a direct action of ATX II or TTX on non-excitable membranes. Given the well documented mechanisms of action of ATX II and TTX on excitable membranes and what is presently

understood about the mechanisms of ion transport across epithelia it is difficult to reason how ATX II or TTX could affect the epithelium directly to alter electrolyte transport in the observed manner. Thus it seems plausible to conclude that the effects of ATX II and TTX on electrolyte transport across the mucosa preparation are mediated by changes in the release of neurotransmitters from the neurones of the mucosal plexus.

In a previous report from this laboratory electrolyte transport across two preparations of the rat colon, one with (mucosa–submucosa preparation) and one without the submucosal plexus (mucosa preparation) was investigated (Andres *et al.* 1985). The results demonstrated that physical removal of the submucosal plexus or pharmacological blockade of the neurones within the mucosa–submucosa preparation by TTX enhanced the net transport of Na^+ and Cl^-. Based on these results it was suggested that spontaneously active neurones from the submucosal plexus have an inhibitory influence on the mucosa. Neurones or enterochromaffin cells within the mucosa must be continuously stimulated by neurones extending from the submucosal plexus to cause an inhibition of electrolyte absorption. Furthermore it was suggested that the intrinsic set point of the epithelium for electrolyte transport is set at or near maximal absorption and that regulatory mechanisms lower this set point causing an inhibition of electrolyte absorption.

The results reported here support and further extend these conclusions. ATX II, by stimulating the neurones of the mucosal plexus, caused an inhibition in Na^+ and Cl^- absorption, pharmacologically mimicking the action of the submucosal plexus. Indeed if a submaximal concentration of ATX II (5×10^{-8} M) is used then the electrical parameters, and Na^+ and Cl^- transport across the mucosa preparation are nearly the same as those measured in the mucosa–submucosa preparation (ATX II-treated mucosa preparation I_{sc} 3·2 μequiv cm^{-2} h^{-1}, G_t 8·3 mS cm^{-2}, p.d. 10·3 mV, J^{Na}_{net} 2·9, J^{Cl}_{net} 3·2 and J^{R}_{net} 3·5 μequiv cm^{-2} h^{-1} *versus* untreated mucosa–submucosa preparation I_{sc} 2·9 μequiv cm^{-2} h^{-1}, G_t 10·3 mS cm^{-2}, p.d. 10 mV, J^{Na}_{net} 2·7, J^{Cl}_{net} 4·3 and J^{R}_{net} 4·5 μequiv cm^{-2} h^{-1}). TTX caused a rapid decrease in I_{sc} and restored net Na^+ and Cl^- transport to base-line values in the ATX II-treated mucosa preparation. The latter observations indicate that the neurones stimulated by ATX II are TTX-sensitive neurones and therefore they are of the S/type 1 neurones (Wood, 1981). Furthermore, the rapid response to TTX indicates the neurotransmitter or transmitters released by the ATX II-stimulated neurones are rapidly inactivated. Once the transmitters are inactivated the epithelium returns to its intrinsic functional set point near maximal absorption. The transmitters affecting this regulation of ion transport remain to be identified.

We are indebted to Professor R. Bock for providing the laboratory facilities in which the histological studies were conducted and for his many helpful suggestions. The diligent technical assistance of Miss H. Andres and secretarial assistance of Miss B. Kilthau are a pleasure to acknowledge. This work was supported by Sonderforschungsbereich 38, Membrane Research.

REFERENCES

ANDRES, H., BOCK, R., BRIDGES, R. J., RUMMEL, W. & SCHREINER, J. (1985). Submucosal plexus and electrolyte transport across rat colonic mucosa. *Journal of Physiology* **364**, 301–312.

BÉRESS, L. (1986). Biologically active compounds from coelenterates. In *Poisonous and Venomous Marine Animals of the World*, ed. MAISTEAD, B. W. (in the Press).

BÉRESS, L., BÉRESS, R. & WUNDERER, G. (1975*a*). Isolation and characterization of three polypeptides with neurotoxic activity from *Anemonia sulcata*. *FEBS Letters* **50**, 311–314.

BÉRESS, L., BÉRESS, R. & WUNDERER, G. (1975*b*). Purification of three polypeptides with neuro- and cardiotoxic activity from the sea anemone *Anemonia sulcata*. *Toxicon* **13**, 359–369.

BILLROTH, T. (1858). Einige Beobachtungen ueber das ausgedehnte Vorkommen von Nervenanastomosen im Tractus intestinalis. *Archiv für Anatomie und Physiologie*, 148–158.

BJERKNES, M. & CHENG, H. (1981). Methods for the isolation of intact epithelium from the mouse intestine. *The Anatomical Record* **199**, 565–574.

BOCK, R. & MÜHLEN, K. AUS DER (1968). Beitraege zur funktionellen Morphologie der Neurohypophyse. *Zeitschrift für Zellforschung* **92**, 130–148.

CATTERALL, W. A. (1980). Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annual Review of Pharmacology and Toxicology* **20**, 15–43.

DRASCH, O. (1881). Beitraege zur Kenntniss des feineren Baues des Duenndarms, insbesondere ueber die Nerven desselben. *Sitzungsberichte der Akademie der Wissenschaften Wien* **82**, 168–198.

GABELLA, G. (1981). Structure of muscles and nerves in the gastrointestinal tract, Chapter 6. In *Physiology of the Gastrointestinal Tract*, ed. JOHNSON, L. R., pp. 197–241. New York: Raven Press.

GAGINELLA, T. S. (1984). Neuroregulation of intestinal ion transport. *Trends in Pharmacological Sciences* **5**, 397–399.

HILLARP, N. (1946). Structure of the synapse and the peripheral innervation apparatus of the autonomic nervous system. *Acta anatomica supplementum* **IV**, 1–50.

HUBEL, K. A. (1985). Intestinal nerves and ion transport: stimuli, reflexes, and responses. *American Journal of Physiology* **248**, G261–271.

KARNOVSKY, M. J. & ROOTS, L. (1964). A 'Direct-coloring' thiocholine method for cholinesterases. *Journal of Histochemistry and Cytochemistry* **12**, 219–221.

KEAST, J. R., FURNESS, J. B. & COSTA, M. (1986). Distribution of certain peptide-containing nerve fibres and endocrine cells in the gastrointestinal mucosa in five mammalian species. *Journal of Comparative Neurology* **234** (in the Press).

KUNTZ, A. (1953). *The Autonomic Nervous System*. Philadelphia: Lea and Febiger.

NARAHASHI, T. (1974). Chemicals as tools in the study of excitable membranes. *Physiological Reviews* **54**, 813–889.

SCHOFIELD, G. C. (1968). Anatomy of muscular and neural tissues in the alimentary canal. In *Handbook of Physiology*, vol. IV, ed. CODE C. F., pp. 1579–1629. Washington, D. C.: American Physiological Society.

SILVA, D. G., FARRELL, K. E. & SMITH, G. C. (1968). Ultrastructural and histochemical studies on the innervation of the mucous membrane of the mouse colon. *The Anatomical Record* **162**, 157–176.

SILVER, A. (1974). *The Biology of Cholinesterase*. Amsterdam: North-Holland.

TAPPER, E. J. (1983). Local modulation of intestinal ion transport by enteric neurons. *American Journal of Physiology* **244**, G457–468.

WOOD, J. D. (1981). Physiology of the enteric nervous system, Chapter 1. In *Physiology of the Gastrointestinal Tract*, ed. JOHNSON, L. R., pp. 1–37. New York: Raven Press.

EXPLANATION OF PLATES

PLATE 1

Photomicrograph of a cross-section of the mucosa preparation. Frozen cross-sections (15 μm) were stained for acetylcholinesterase activity. Sections were stained for 2 h without OMPA in the incubation solution. Both 'true' and 'pseudo' cholinesterase activity are stained. The bar represents 50 μm. Muscularis mucosae, m.m.; crypt, c.; nerve fibres, n.f.

PLATE 2

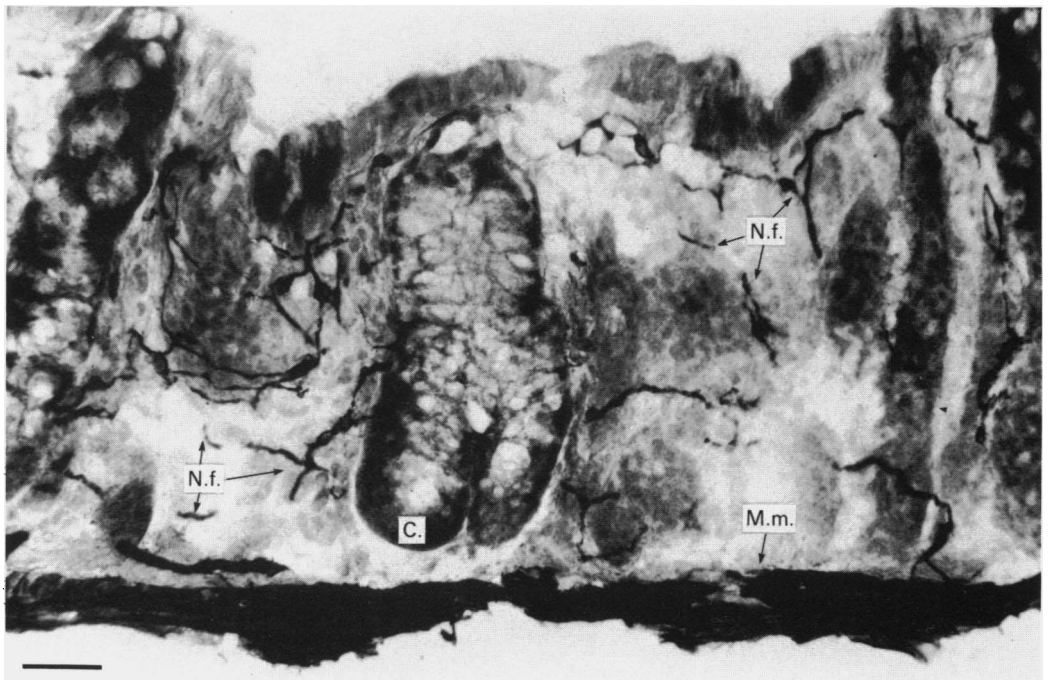
Photomicrograph of the mucosal plexus as seen from the luminal side. Air-dried whole mount of the mucosa preparation from which the epithelium was first removed was stained for acetylcholinesterase activity. Incubation was for 6 h with OMPA (10^{-4} M) in the incubation solution. The area corresponds to 0·013 cm^2. The bar represents 100 μm. Ganglia, g.; nerve fibres, n.f.

PLATE 3

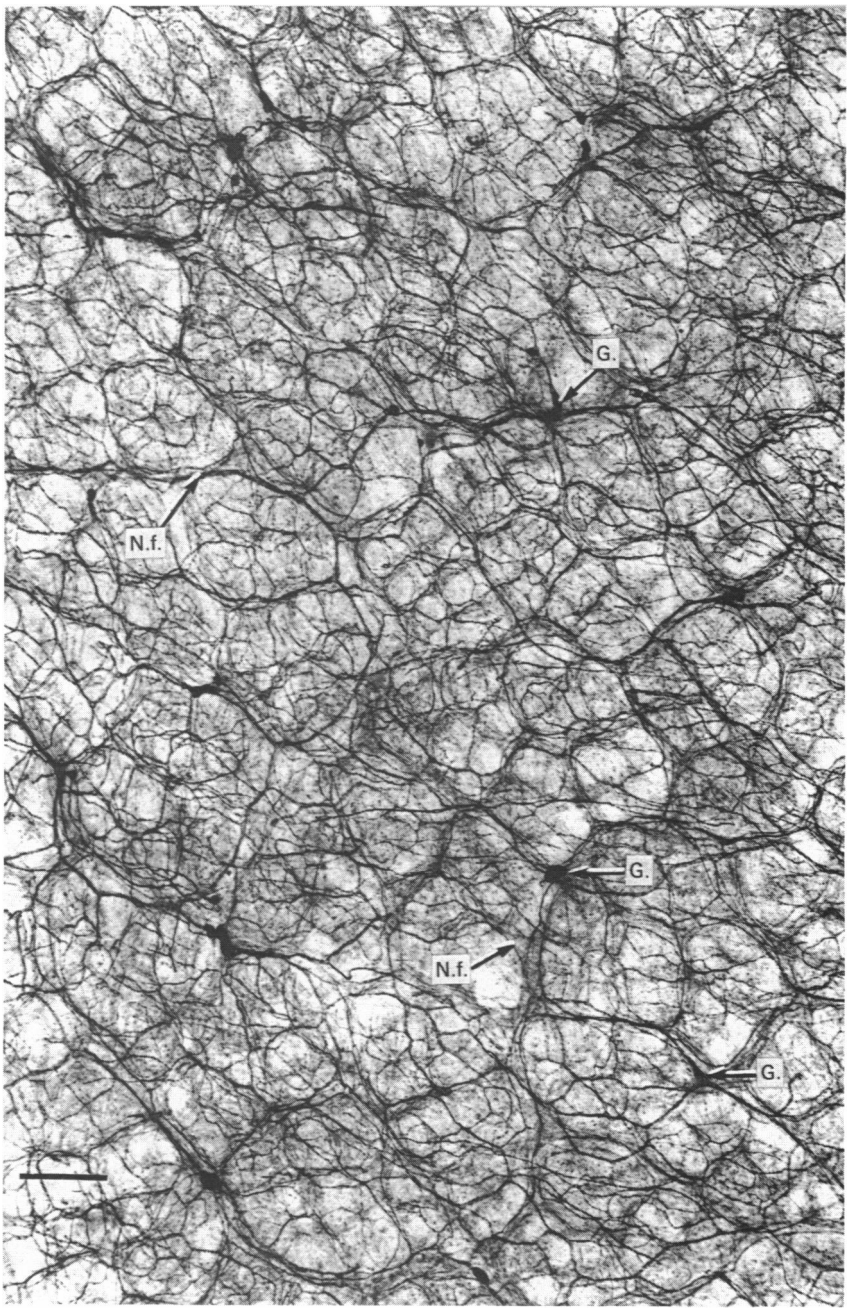
Ganglia of the mucosal plexus. Incubation was for 3 h with OMPA (10^{-4}) in the incubation solution. The bar represents 50 μm.

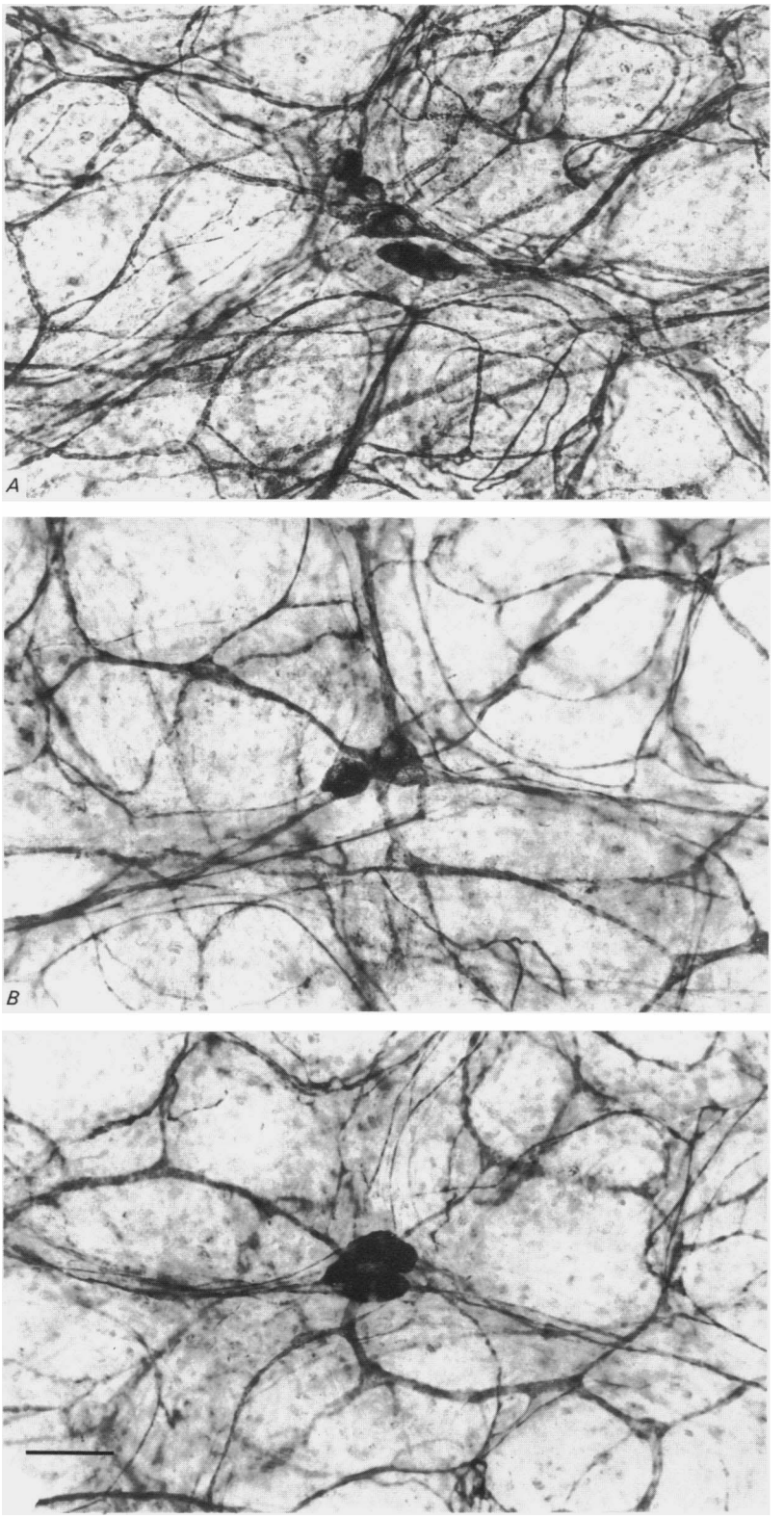
PLATE 4

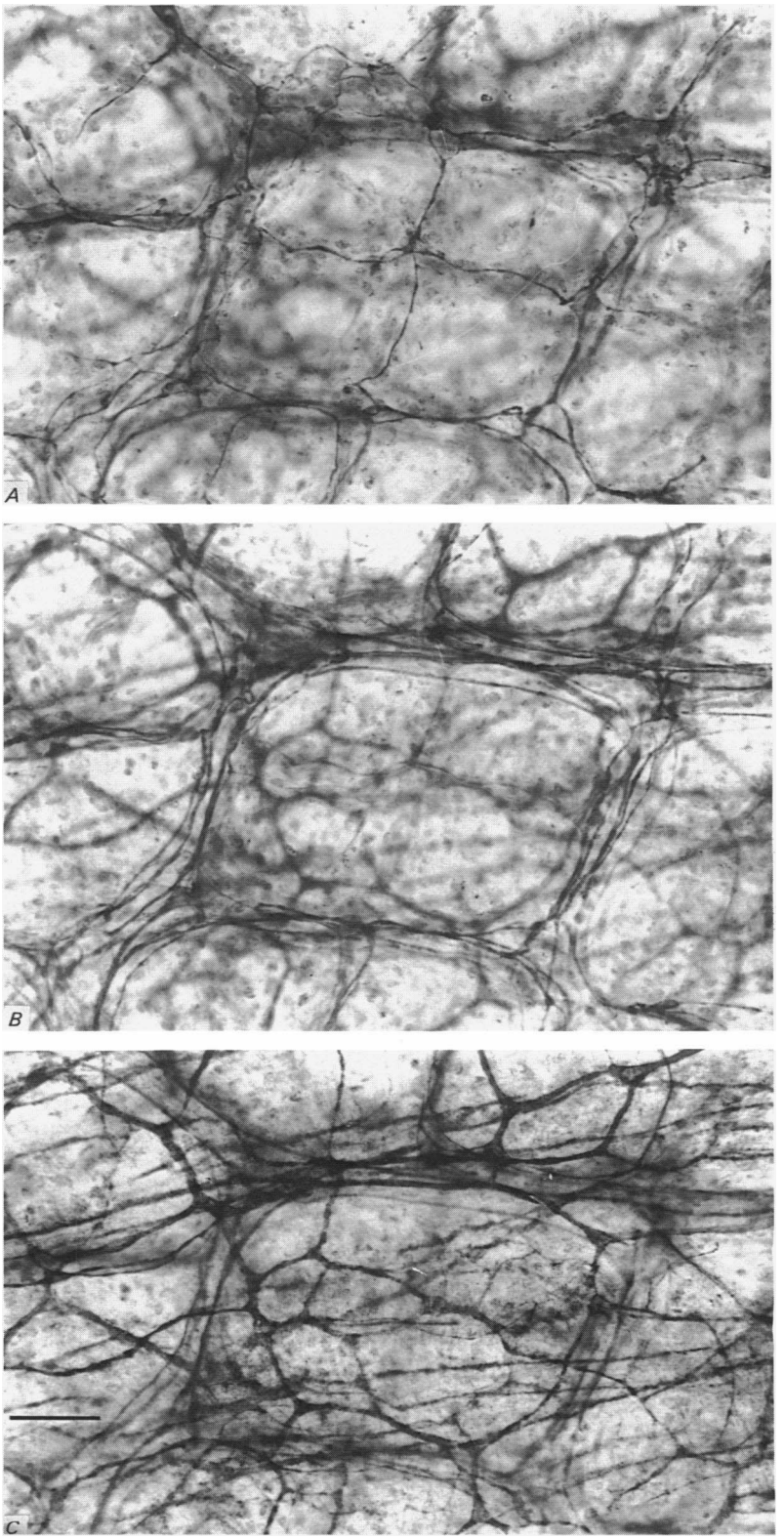
Photomicrographs of the mucosal plexus taken at different levels of focus. Details as for Pl. 2. *A*, upper level of the mucosal plexus. Thin fibres that surround each crypt are shown. *B*, middle level of the plexus. Thicker fibres that surround groups of crypts in a broad band are shown. *C*, lower level of the plexus. Larger fibres that extend below the bottom of the crypts along the circular muscle layer of muscularis mucosae are shown. Bar represents 50 μm.



(*Facing p.* 542)


G.
N.f.
G.
N.f.
G.


A
B


A
B
C