In Vitro Behavior of Human Intestinal Mucosa

THE INFLUENCE OF ACETYL CHOLINE ON ION TRANSPORT

P. E. T. Isaacs, C. L. Corbett, A. K. Riley, P. C. Hawker, and L. A. Turnberg

From the Department of Medicine, Hope Hospital, University of Manchester School of Medicine, Eccles Old Road, Salford, M6 8HD, United Kingdom

Abstract The possibility that the autonomic nervous system may influence the function of intestinal mucosa was investigated by assessing the effect of acetyl choline on ion transport in human intestine. Isolated pieces of stripped ileal mucosa were mounted in Perspex flux-chambers and bathed in isotonic glucose Ringer's solution. Acetyl choline caused a rise in mean potential difference (8.8–12.3 mV, $P < 0.002$) and short circuit current (287.7–417.2 $\mu A \cdot cm^{-2}$, $P < 0.01$) ($n = 12$), observable at a concentration of 0.01 mM and maximal at 0.1 mM. This effect was enhanced by neostigmine and blocked by atropine. Isotopic flux determinations revealed a change from a small mean net Cl absorption (+ 0.58) to a net Cl secretion (− 4.3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ $P < 0.001$) due predominantly to an increase in the serosal to mucosal unidirectional flux of Cl (10.63–14.35 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ $P < 0.05$) and a smaller reduction in the mucosal to serosal flux (11.22 to 10.02 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ $P < 0.05$). Unidirectional and net Na transport was unaffected. A similar electrical and ion transport response was observed in a single study of two pieces of jejunal mucosa. In the absence of glucose net chloride secretion was produced and again an insignificant effect on net sodium transport was noted. Acetyl choline did not provoke a sustained effect on mucosal cyclic adenine nucleotide levels although a short-lived cyclic adenine nucleotide response was seen in some tissues 20–30 s after drug addition.

These studies demonstrate that acetyl choline does influence human intestinal ion transport by stimulating chloride secretion and suggest a possible mechanism by which the parasympathetic nervous system could be concerned in the control of ion transport.

A preliminary report of this work was presented at the British Society of Gastroenterology, Southampton, April 1975 and at the European Society for Clinical Investigation, Rotterdam, April, 1975.

Received for publication 20 August 1975 and in revised form 13 May 1976.

INTRODUCTION

It has long been thought that the autonomic nervous system may influence intestinal absorption and secretion. Sympathetic denervation in the dog and the cat caused an intestinal secretion which was blocked by atropine (1) and the spontaneous secretion of Thirty-Vella loops of dog ileum was similarly prevented by atropine (2). More recently Caren and co-workers (3) showed that the secretion provoked by tactile stimulation in such isolated loops was inhibited by atropine and hexamethonium. Pilocarpine (4) and eserine (5) caused intestinal secretion and bethanechol, a cholinergic agent, was shown to induce a net secretion of chloride in dog jejunum (6). In view of these suggestive pieces of evidence the present investigations were undertaken to examine in more detail the influence of acetyl choline on human intestinal ion transport. The results indicate that acetyl choline does influence ion transport in vitro and suggest a possible mechanism by which the parasympathetic nervous system could be involved in the control of intestinal transport.

METHODS

Experiments were performed on isolated pieces of human ileal mucosa from fresh surgical specimens removed at operation for colonic carcinoma, ulcerative colitis, Crohn's disease, or trauma. All specimens used were separated from diseased bowel and were macroscopically normal. Histological sections, examined subsequently, were also normal. A segment of intestine rapidly immersed in chilled, oxygenated buffer solution was opened along its mesenteric border and the mucosa dissected off from the muscle layers. A preparation consisting of epithelium, muscularis mucosa, and tags of submucosal connective tissue was obtained. Within 15 min of excision sheets of mucosa were clamped between Ussing-type Perspex half-chambers exposing an area of 1.77 cm^2 to the bathing solutions. 10-ml vol of iso-

tonic buffer solutions were placed on both sides of the membrane and these were oxygenated and stirred with 95% O_2/5% CO_2 by a gas-lift circulating system and maintained at 37°C by water jackets. Bathing solutions contained Na 146, K 4.2, Mg 1.2, Ca 1.2, Cl 127, HCO_3 27.6, HPO_4 1.2, H_2PO_4 0.2 meq/l (pH 7.4). 15 mM glucose was present in both bathing solutions in most experiments but was omitted in some. Two to four chambers were set up simultaneously using tissue from one surgical specimen.

Electrical measurements. The transmucosal electrical potential difference (PD) [1] was measured through narrow-tipped bridges placed close to either side of the tissue. These contained saturated KCl in agar and were connected to a high impedance digital voltmeter (Solartron A240 Solartron Electronic Group Ltd., Farnborough, Hants, United Kingdom) via matched calomel electrodes. A current sufficient to nullify the transmucosal PD was passed from an external dry battery via silver-silver chloride electrodes and narrow-tipped bridges containing 1 M NaCl in agar placed at opposite ends of the chambers. This short-circuit current (SCC) was adjusted manually at 3–5 min intervals (7) and a correction was made for the PD drop between the potential-measuring bridges and the mucosa according to the technique described by Field et al (8). Mucosal resistance was calculated from the open circuit PD and the SCC as PD.A/SCC where A is the area of exposed mucosa. SCC measurements in μA were converted to net ion fluxes in μeq·cm^{-2}·h^{-1} by multiplying by a factor of 0.02108 (3.6×10^3/A.F, where F is the Faraday constant).

Radioisotope fluxes. Fluxes of Na and Cl were measured after the addition of 0.5 μCi ^{22}Na and 2.5 μCi ^{36}Cl (The Radiochemical Centre, Amersham, Bucks, England) to either the mucosal or serosal solutions of each chamber. After an equilibration period of 30 min, both the mucosal and serosal reservoirs were serially sampled at 10 min intervals for 50 min, and the 1-ml samples removed were replaced with unlabeled solution at 37°C. Samples were counted for ^{22}Na on a well-type gamma counter together with a 1-ml ^{22}Na standard. 0.8-ml vol of each sample and standard were then transferred to scintillation vials containing 5 ml Bray's solution (Nuclear Enterprises, Sighthill, Edinburgh) and counted in a liquid scintillation counter (Mk 11 Nuclear-Chicago Corp., Des Plaines, Ill.) for the combined ^{22}Na and ^{36}Ci activity. The activity of ^{22}Na, assayed in the gamma counter, multiplied by a factor for the relative efficiency of the two counters for ^{22}Na, was then subtracted from the total to yield the ^{36}Cl counts. All samples were counted to 10,000 counts and background counts subtracted.

The unidirectional fluxes of Na and Cl were calculated as:-

$$J = \left(\frac{C_2}{P_2^*} - \frac{C_1}{P_1^*}\right)\frac{V}{At}$$

where C_1 and C_2 are the activities (in cpm·ml^{-1}) in consecutive samples from the unlabeled side, p_1^* and p_2^* are the specific activities (in cpm·ml^{-1}·μeq^{-1}) in consecutive samples from the labeled side, V the volume of bathing solution (10 ml), t the intervals between samples (hours) and A the area of exposed mucosa (1.77 cm^2).

For the ion flux experiments only pairs of mucosae from the same specimen differing in resistance by less than 25% were accepted. Net fluxes were calculated as the difference between oppositely directed unidirectional fluxes in paired tissues. The electrical measurements from the two mucosae were summed and averaged to yield single values for each tissue pair. Fluxes were measured over 10-min periods for 20 min before and 30 min after the addition of acetyl choline and neostigmine, consecutively, in the same paired tissues. Fluxes were also measured over a similar time period in control tissues to which no drug had been added.

In initial experiments PD responses to various concentrations of acetyl choline chloride added to either mucosal or serosal solutions were assessed. PD rose in response to a concentration of 0.1 mM added to either serosal or mucosal side, but at higher concentrations serosal addition produced a response different from that produced by mucosal addition probably due to the asymmetrical chloride concentrations associated with unilateral addition of acetyl choline chloride. This agent was added to both sides of the mucosa in all subsequent experiments to obviate this problem.

The influence of neostigmine (0.3 mM) on the dose response curve to acetyl choline was determined in untreated mucosa and in mucosa pretreated with atropine (0.3 mM).

The relationship between the response to acetyl choline and the response to the cyclic nucleotide, cyclic adenine nucleotide, was investigated in further experiments in which theophylline (10 mM) was added to one tissue of a pair and, at the peak PD response, acetyl choline (0.1 mM) was added to both tissues and the responses compared.

Fresh solutions of acetyl choline chloride, neostigmine, atropine, and theophylline (Sigma Chemical Co., Kingston-upon-Thames, Surrey, KT2, 7BH, England) were used in these experiments.

All the above electrical and ion transport studies were performed in glucose-containing buffer. The response of ileal mucosa to acetyl choline in glucose-free buffer was also examined to assess the possibility that glucose might influence the response.

Cyclic adenine nucleotide (cAMP) concentrations in stripped mucosa were measured by a competitive protein binding assay (9). Pieces of mucosa, wet weight 150–200 mg, were each incubated in 10 ml of buffer at 37°C bubbled with O_2/CO_2 (95/5%). Theophylline (final concentration 10 mM) or acetyl choline plus neostigmine (final concentrations 0.1 mM and 0.3 mM respectively) were added to each tissue incubation, and at timed intervals after drug addition the tissues were frozen rapidly in a methanol-cardice mixture and stored at −4°C.

Each frozen specimen was homogenized in 2.5 ml of ice cold trichloracetic acid (6% wt/vol) with a rotating blade homogeniser (model X1020, Internationale Laboratoriums, Dottingen, West Germany) at 25,000 rpm for 10 s. After centrifugation the supernate was washed with ether and 1 ml of the extract dried at 50°C with a rotary evaporator. The dried residue was taken up in 0.5 ml of 50 mM Tris buffer pH 7.5, containing 4 mM EDTA. The cAMP content of 50-μl samples of this was measured by the method of Brown et al. (9), all assays being performed in duplicate. All materials for the assay were supplied by the Radiochemical Centre, Amersham, Bucks, England.

In all experiments statistical comparisons were made by Student's *t* test, and results are expressed as the mean with 1 SE.

[1] *Abbreviations used in this paper:* Jms, mucosal to serosal flux; Jsm, serosal to mucosal flux; Jnet, net flux; JRnet, residual ion flux; PD, potential difference; SCC, short-circuit current.

RESULTS

Ileal response. The PD responses of mucosa, bathed in glucose containing buffer, to acetyl choline alone, and after treatment with neostigmine or atropine are shown in Fig. 1. The maximal PD response was achieved with a concentration of 0.1 mM acetyl choline alone (+ 1.42 ±0.27 mV, $n = 19$) or with neostigmine (+ 2.58±0.29 mV, $n = 51$) and this response persisted for at least 30 min. A significant PD response was still observed at a concentration of 0.001 mM in the presence of neostigmine (+ 0.65±0.28, $n = 10$) and at 0.01 mM in its absence (+ 0.39±0.17 mV, $n = 9$). A marked fall in PD was observed at a concentration of 100 mM acetyl choline and this was unaffected by neostigmine and atropine. Fig. 2 shows the SCC response to acetyl choline (0.1 mM) and neostigmine (0.3 mM) in 12 ileal tissues. SCC rose from 287.7±36.6 to 417.2±43.5 $\mu A \cdot cm^{-2}$, and PD from 8.8±0.9 to 12.3±0.9 mV. Both PD and SCC then fell somewhat but remained elevated above base-line levels for at least 30 min. There was no significant change in tissue resistance.

Ion fluxes in the two 10-min periods before drug addition did not differ significantly, and Table I shows the effect of acetyl choline (0.1 mM) and neostigmine (0.3 mM) on ion fluxes in the 10-min period before, and the first and third 10-min periods after drug addition. Sodium fluxes were unaltered during the 30–40 min after acetyl choline, but an insignificant net chloride absorption (+ 0.58±0.44 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) was changed to a net chloride secretion (− 4.33±0.49 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ $P <$ 0.001). This was due to a small decrease in mucosal to serosal flux (Jms) and a larger increase in Jsm and this response persisted for at least 30 min (Table I). Residual ion flux was not significantly affected. Control studies in ileal tissues not treated with acetyl choline are shown in Fig. 3 and indicate that no spontaneous change in ion fluxes occurred during this time.

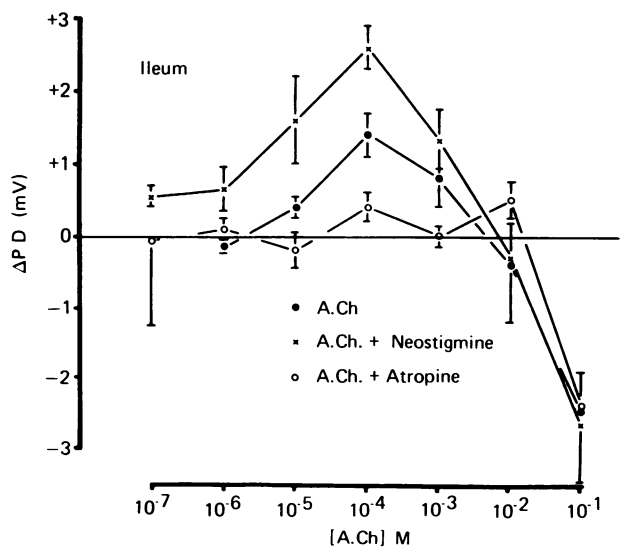

FIGURE 1 Relationship between PD response and dose of acetyl choline in ileal mucosa. Neostigmine (0.3 mM) enhanced and atropine (0.3 mM) inhibited the response to concentrations of 1 mM or less but did not influence the response to higher concentrations (n = between 7 and 51 for each point) (SE shown).

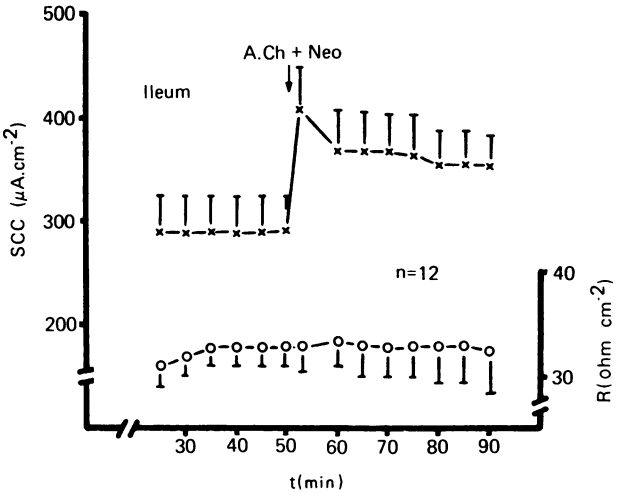

FIGURE 2 Response of SCC and resistance in ileal tissues treated with acetyl choline (0.1 mM) and neostigmine (0.3 mM) ($n = 12$).

The electrical responses to acetyl choline plus neostigmine in glucose-free solutions are shown in Fig. 4. The response differed from that in glucose-containing solutions in that by 30 min both PD and SCC had returned to base-line levels. There was also a slight rise in tissue resistance, maximal 20 min after drug additon. Ion flux measurements (Table II) showed that as in the presence of glucose, net sodium transport was not significantly affected, although in these studies control net sodium movement was zero and a small insignificant net secretion was noted with acetyl choline. A significant chloride secretion occurred due to a fall in Jms and a rise in

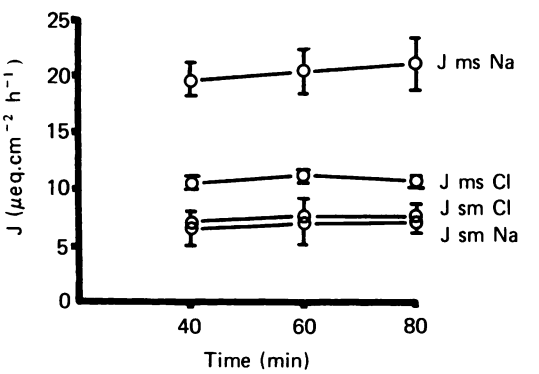

FIGURE 3 Unidirectional fluxes in ileal tissues exposed to glucose buffer solution alone (n = six tissue pairs).

TABLE I

Effect of Acetyl Choline (0.1 mM) and Neostigmine (0.3 mM) on Unidirectional and Net Flux and Isc (in $\mu eq \cdot cm^{-2} \cdot h^{-1}$) in Ileal Mucosal Samples in Glucose-containing Ringer (n = 6 Tissue Pairs, from Five Different Patients). Flux in the 10-min Period before Drug Addition, in the 10 min Immediately after, and 20–30 min after Drug Addition

	Na			Cl			Residual ion	
	Jms	Jsm	JNet	Jms	Jsm	JNet	JRnet	I_{sc}
A Control, *10 min*	19.37 ±1.88	9.63 ±1.26	+9.73 ±1.98	11.2 ±0.68	10.63 ±0.92	+0.58* ±0.44	+1.6* ±0.96	10.72 ±2.04
B Ac.Ch., *1st 10-min period*	17.95 ±1.61	9.32 ±1.32	+8.63 ±1.75	10.02 ±0.72	14.35 ±0.94	−4.33 ±0.49	+2.77* ±0.88	15.73 ±2.42
C Ac.Ch., *3rd 10-min period*	19.35 ±2.65	8.8 ±1.06	+10.55 ±2.52	11.1 ±0.87	16.13 ±2.52	−5.03 ±1.78	−1.78* ±1.72	13.80 ±2.19
P (A, B)	NS	NS	NS	<0.05	<0.01	<0.001	NS	<0.01
P (A, C)	NS	NS	NS	NS	<0.025	<0.02	NS	<0.02

Abbreviation: I_{sc}, short-circuit current.
+, absorption; −, secretion.
* NS different from zero.

Jsm. JRnet, compatible with bicarbonate secretion in the control period, changed to a small net absorption after acetyl choline. As with the electrical responses, these flux changes were short-lived, JRnet and Cl fluxes not being significantly different from control values when measured from 20 to 30 min after drug addition. The transient nature of these flux changes raises the possibility that they are artefactual and due to a failure to achieve steady-state conditions after adding acetyl choline. However, this transient response was very similar to the persistent steady-state response produced in the presence of glucose suggesting that it was probably not artefactual.

Jejunal response. In a single study of two pieces of jejunal mucosa, acetyl choline (0.1 mM) with neostigmine (0.3 mM) caused a rise in PD and SCC similar to that demonstrated in ileal tissue (Fig. 5). Flux measurements in this tissue revealed that unidirectional and net Na fluxes were unchanged by acetyl choline while Cl flux changed in a similar manner to ileal Cl flux (Jms Cl from

TABLE II

Effect of Acetyl Choline (0.1 mM) and Neostigmine (0.3 mM) on Unidirectional and Net Flux and I_{sc} (in $\mu eq \cdot cm^{-2} \cdot h^{-1}$) in Ileal Mucosal Samples in Glucose-free Buffer (n = Four Tissue Pairs, from Two Patients). A, Flux in the 10-min Period before Drug Addition; B, Flux in the 10-min Period Immediately after Drug Addition

	Na			Cl			Residual ion	
	Jms	Jsm	JNet	Jms	Jsm	JNet	JRnet	I_{sc}
A	9.49 +0.32	9.49 ±0.30	0 ±0.28	8.30 ±0.65	8.84 ±0.40	−0.55* ±0.58	+1.48 ±0.52	2.03 ±0.14
B	8.75 ±0.90	9.34 ±0.49	−0.59* ±1.21	5.46 ±0.45	11.89 ±0.95	−6.44 ±1.25	−1.42* ±0.91	4.43 ±0.59
P	NS	NS	NS	<0.005	<0.05	<0.005	<0.01	<0.025

Abbreviation: I_{sc}, short-circuit current.
+, absorption; −, secretion.
* NS different from zero.

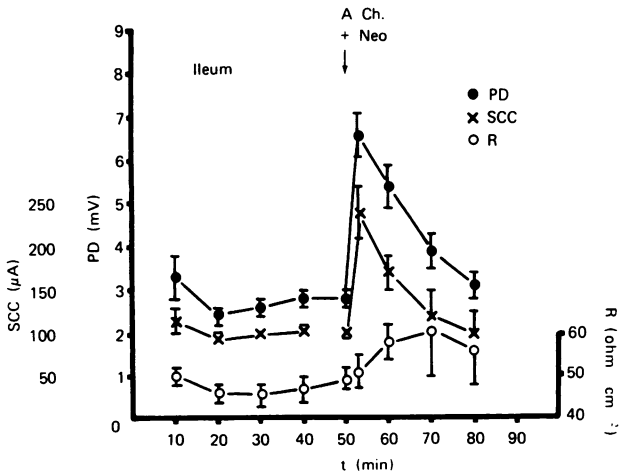

FIGURE 4 Effect of acetyl choline (0.1 mM) and neostigmine (0.3 mM) on PD, SCC and R in ileal mucosa in glucose-free Ringer.

7.8 to 6.8, Jsm Cl from 8.3 to 10.4, and J net Cl from -0.5 to -3.6 $\mu eq \cdot cm^{-2} \cdot h^{-1}$). The residual ion flux was unaffected.

Theophylline and acetyl choline interrelationships in ileal mucosa. Theophylline (10 mM) induced a rise in PD similar to that produced by acetyl choline (0.1 mM). Adding acetyl choline and neostigmine to the bathing solutions immediately after the maximal PD response to theophylline did not produce any further, additive rise although PD and SCC, which had begun to fall, returned to the maximal theophylline-stimulated level. A similar response was seen when theophylline was added after acetyl choline. These findings suggested the possibility either that acetyl choline was acting through cAMP (which presumably mediates the theophylline effects) or that both acetyl choline and cAMP stimulated a common ion transport mechanism.

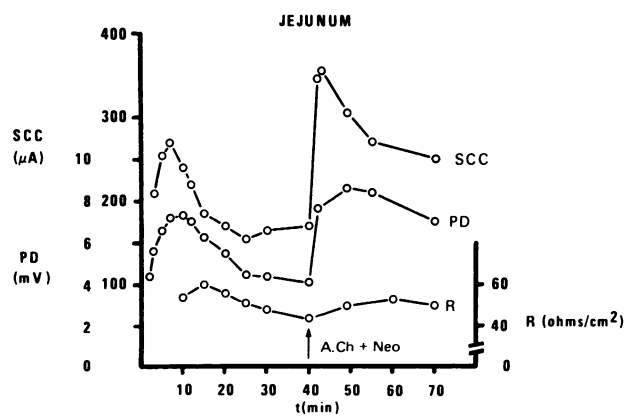

FIGURE 5 PD, SCC and R response of jejunal mucosa to acetyl choline (0.1 mM) with neostigmine (0.3 mM) ($n = 2$).

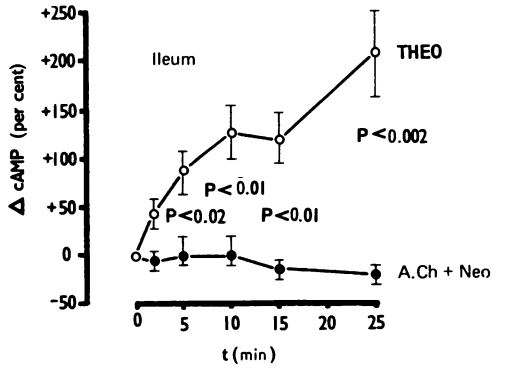

FIGURE 6 Levels of cAMP as a percentage of initial values in ileal mucosal tissue after incubation with either acetyl choline (0.1 mM) plus neostigmine (0.3 mM) or theophylline (10 mM). Measurements were made at varying time intervals between 2 and 30 min ($n = 4$).

cAMP measurements. To examine the possibility that the acetyl choline effects were mediated by cAMP, cAMP levels were measured in ileal mucosal specimens incubated with acetyl choline (0.1 mM) and neostigmine (0.3 mM). Basal levels of cAMP in ileal mucosa were 4.64 ± 0.38 pmol/mg protein ($n = 10$). No change occurred between 2 and 30 min after addition of acetyl choline (Fig. 6) although a short-lived peak of increased cAMP levels was seen in some tissues at 20–30 s and these returned to base-line values by 2 min (Fig. 7). Theophylline increased cAMP levels, as expected, in simultaneously incubated specimens (Fig. 6). Mucosal cAMP levels after stimulation with theophylline reached 13.98 ± 2.51 pmol/mg protein ($n = 4$) 15 min after drug addition. Acetyl choline failed to influence cAMP levels

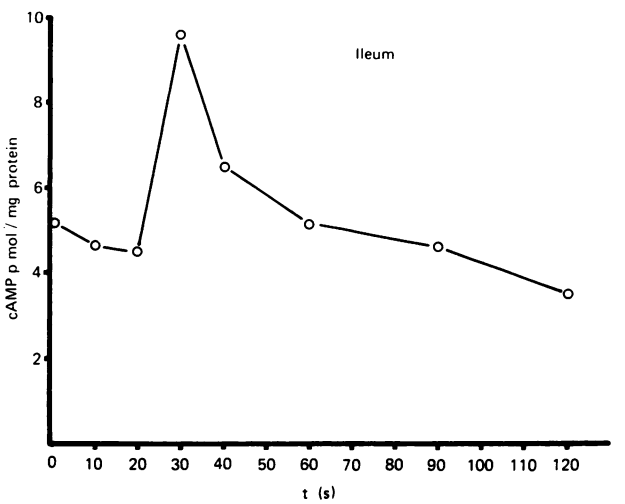

FIGURE 7 Levels of cAMP in ileal mucosa in response to acetyl choline plus neostigmine. Measurements were made at intervals between 0 and 120 s after drug addition ($n = 5$).

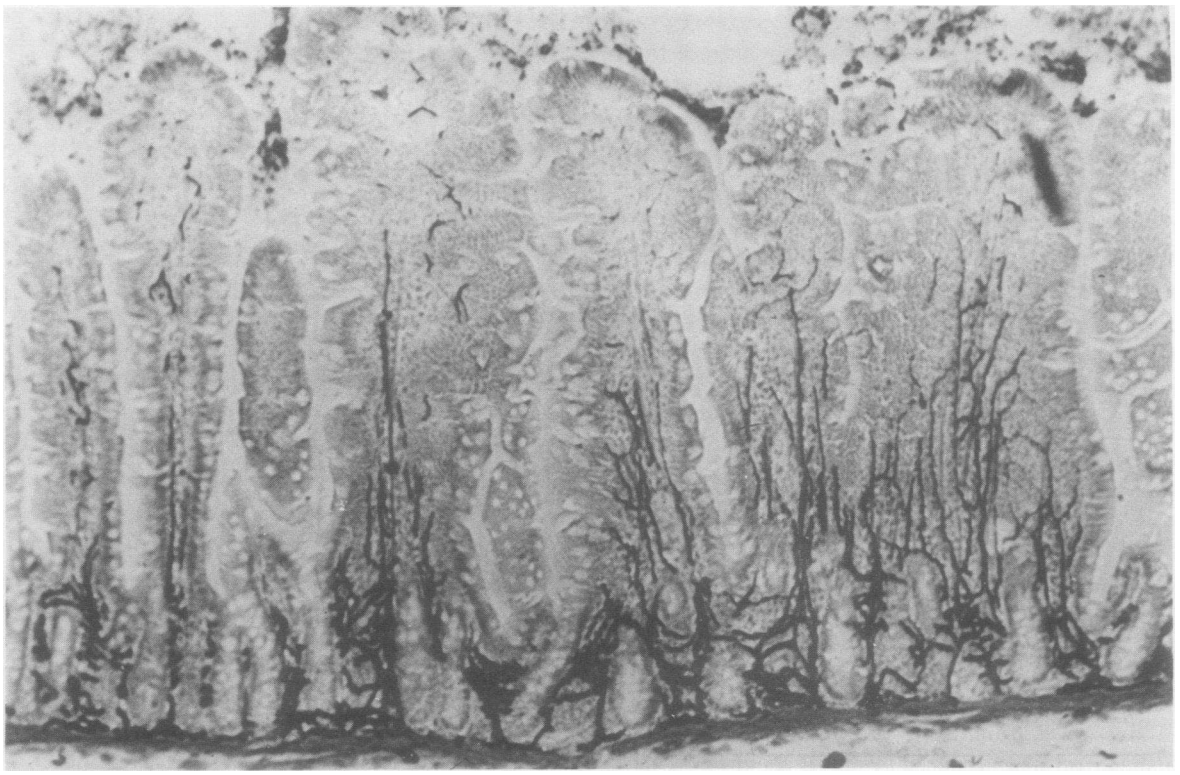

FIGURE 8 Histological section of human jejunal mucosa stained by thiocholine technique for cholinesterase and demonstrating cholinergic fibres around the crypts and extending up the villi.

in four jejunal experiments while theophylline caused a rise similar to that observed in ileum. Basal cAMP levels in jejunal mucosa were 4.73±0.49 pmol/mg protein ($n = 8$). Theophylline stimulated levels were 13.56 ±1.4 pmol/mg protein ($n = 4$) 15 min after drug addition. Control specimens of ileal and jejunal mucosa showed either no change or a slight fall in cAMP content over this time.

DISCUSSION

Studies of the autonomic nervous system in the alimentary tract have focussed on the rich innervation of the muscle coats and little attention has been paid to the possibility that the surface epithelium may also receive a nerve supply. Yet occasional reference has been made in the anatomical literature (10, 11) to the presence of nerve fibers surrounding the crypts and extending up into the villi. Fig. 8 illustrates the distribution of cholinergic fibers as demonstrated by a cholinesterase stain, in a section of human jejunal mucosa. Dense staining is visible, especially around the crypts and also in the villi. We have commonly seen fibers close to villous epithelial cells. The possibility that these fibers may have a role to play in function, particularly in the control of salt and water transport, is suggested by several pieces of evidence. Sympathetic denervation in animals induced intestinal secretion which was blocked by atropine (1, 12) and the spontaneous secretion of dog Thirty-Vella loops, and that induced by tactile stimulation, was also inhibited by atropine (2, 3). These observations suggest that cholinergic nervous stimulation may induce intestinal secretion and a number of cholinergic agents have also been shown to have a similar effect. Thus, pilocarpine (4), eserine (5), and bethanechol (6) all induce a net intestinal secretion.

The present investigations demonstrate that acetyl choline is capable of inducing a net secretion of chloride in isolated human ileal mucosa, an effect which is in broad agreement with Tidball's (6) observation that bethanechol induced chloride secretion against an electrochemical gradient in dog jejunum.

Hardcastle and Eggenton (13) also demonstrated an increase in PD in response to acetyl choline in loops of rat intestine but described a sigmoid dose-response curve, the PD response increasing with increasing concentrations of acetyl choline. Since the preparation of acetyl choline used was the chloride salt, and this was added only to the serosal side of the tissue, it seems likely that the rise in PD seen with high concentrations of acetyl choline was due to a diffusion potential produced by the asymmetrical chloride concentrations as noted in the present studies. We also noted that PD

changes in response to high concentrations of acetyl choline were not affected by neostigmine or atropine, confirming that they were not mediated by muscarinic cholinergic receptors.

The observation that neostigmine enhanced and atropine blocked the effects of acetyl choline at doses lower than 1 mM suggests that these were muscarinic effects and that muscarinic receptors were involved.

While cAMP, and agents which increase its concentration in epithelial cells, also induce a secretion of chloride (14, 15), the major difference between the cAMP and acetyl choline responses appears to be the failure of acetyl choline to influence sodium transport. It has been suggested that theophylline, probably via cAMP, inhibits a neutral sodium chloride absorptive pump on the luminal membrane of epithelial cells (16). Net chloride secretion is induced together with either inhibition or reversal of sodium absorption. Acetyl choline on the other hand appears to have little or no effect on sodium transport. This certainly seems to be the case in those experiments in which glucose was present but in the absence of glucose the effect on chloride transport was observed only during the first 10 min after acetyl choline was added, that is during a period in which steady-state conditions may not have been achieved. Thus these data cannot be taken as definitive but they do hint that Cl transport may be affected without a coincidental change in Na transport, as in the experiments with glucose present.

cAMP reduces tissue conductance in the absence of glucose (15) and increases it in its presence.[2] Acetyl choline however, did not apparently influence tissue conductance in glucose-containing solutions although a small rise in tissue resistance was seen in glucose-free solutions. In view of these differences in Na transport and tissue conductance responses it is not surprising to find that steady-state levels of cAMP were uninfluenced by acetyl choline and a similar lack of effect has been noted with carbachol (17). Although the electrical response to acetyl choline after a maximal response to theophylline was trivial, PD and SCC which had fallen slightly, did return to the maximum level induced by theophylline. This type of response is not usually seen with consecutive additions of stimulants which act through the same mechanism. For example, a second addition of theophylline induces no secondary response whatsoever. This observation suggests the possibility that acetyl choline was not acting through exactly the same mechanism as theophylline. However, the failure of acetyl choline to raise PD above the peak level after theophylline does suggest that a common, if not identical, mechanism is involved. The possibility cannot be entirely excluded that acetyl choline does act through cAMP by stimulating a small isolated part of the total cAMP pool which mediates its effect on ion transport while total cAMP levels remain apparently unchanged (18, 19). The short-lived peak in cAMP levels observed 20–30 s after acetyl choline addition in some tissues may be relevant in this connection, although its relationship to electrical and ion transport responses which persist for many minutes is unclear.

The relative roles of villi and crypts in the production of intestinal secretion remains unresolved and it is interesting to note in this context the distribution of cholinergic nerve fibres in the intestinal mucosa (Fig. 8). These fibres seem to be particularly dense around the crypts and Trier (20) has noted changes in the morphology of undifferentiated crypt cells and Paneth cells in response to parenteral pilocarpine in human subjects. It is possible therefore that acetyl choline induced secretion may derive from the crypts but we have no evidence for or against this localization.

Levels of the cyclic nucleotide guanosine 3′5′-cyclic monophosphate have been shown to rise in a number of tissues in response to cholinergic stimulation (21–23) and this nucleotide has been held to be a mediator of the cholinergic response in these tissues. The possibility that cyclic nucleotide guanosine 3′5′-cyclic monophosphate is involved in mediating the intestinal mucosal ion transport response to acetyl choline demonstrated here has to be considered and it has recently been reported that carbachol produces a transient increase in cyclic nucleotide guanosine 3′5′-cyclic monophosphate levels in rabbit ileal mucosa (24). This question is currently being investigated in our laboratory.

It is conceivable that acetyl choline liberates some other humoral agent from the tissue which then induces the effects noted on ion transport. It is unlikely however that two possible candidates, vasoactive intestinal peptide and prostaglandins, are involved since these are known to induce persistent increase in cAMP concentrations (17, 25) and these were unaffected in this study.

It is uncertain whether these in vitro effects of acetyl choline indicate a physiological activity. Certainly the concentrations used here appear high. However, it is clear that abundant choline esterases exist in the submucosal tissues and these may allow only much smaller concentrations of acetyl choline to reach the epithelial layer. The early in vivo experiments demonstrating effects of atropine and nerve section on intestinal secretion in a number of animals support a physiological role for this parasympathetic neurotransmitter, but further in vivo studies in man are now required to confirm or refute this possibility.

ACKNOWLEDGMENTS

We are indebted to Mr. H. B. Torrance, Mr. N. Keddie, Mr. J. B. Elder, and Professor M. H. Irving for providing the surgical specimens.

[2] Unpublished observations.

This work was supported by grants from the Medical Research Council and the Welcome Trust.

REFERENCES

1. Florey, H. W., R. D. Wright, and M. A. Jennings. 1941. The secretions of the intestines. *Physiol. Rev.* **21**: 36–69.
2. Tidball, C. S., and M. E. Tidball. 1958. Changes in intestinal net absorption of a sodium chloride solution produced by atropine in normal and vagotomized dogs. *Am. J. Physiol.* **193**: 25–28.
3. Caren, J. F., J. H. Meyer, and M. I. Grossman. 1974. Canine intestinal secretion during and after rapid distention of the small bowel. *Am. J. Physiol.* **227**: 183–188.
4. Hamburger, C. 1895. Vergleichende untersuchung uber die einwirkung des speichels, des pankreas—und darmsaftes, sowie des blutes auf starkekleister. *Pflugers Archiv. Gesamte Physiol. Menschen Tiere.* **60**: 543–577.
5. Molnár, B. 1909. Zur Analyse des Erregungs- und Hemmungsmechanismus der Darmsaftsekretion. *Deutsche. Med. Wochnschr.* **35**: 1384–1385.
6. Tidballs, C. S. 1961. Active chloride transport during intestinal secretion. *Am. J. Physiol.* **200**: 309–312.
7. Clarkson, T. W., and S. R. Toole. 1964. Measurement of short-circuit current and ion transport across the ileum. *Am. J. Physiol.* **206**: 658–668.
8. Field, M., D. Fromm, and I. McColl. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. *Am. J. Physiol.* **220**: 1388–1396.
9. Brown, B. L., J. D. M. Albano, R. P. Ekins, and A. M. Sgherzi. 1971. A simple and sensitive saturation assay method for the measurement of adenosine 3′: 5′-cyclic monophosphate. *Biochem. J.* **121**: 561–562.
10. Schofield, G. C. 1960. Experimental studies on the innervation of mucous membrane of the gut. *Brain.* **83**: 490–514. And plates LXXVII and LXXVIII.
11. Stach, W. 1973. Uber die Nervengeflechte der Duodenalzotten. Licht- und elektronenmikroskopische Untersuchungen. *Acta Anat.* **85**: 216–231.
12. Wright, R. D., M. A. Jennings, H. W. Florey, and R. Lium. 1940. The influence of nerves and drugs on secretion by the small intestine and an investigation of the enzymes in intestinal juice. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **30**: 73–120.
13. Hardcastle, P. T., and J. Eggenton. 1973. The effect of acetylcholine on the electrical activity of intestinal epithelial cells. *Biochim. Biophys. Acta.* **298**: 95–100.
14. Field, M. 1971. Ion transport in rabbit ileal mucosa. II. Effects of cyclic 3′,5′-AMP. *Am. J. Physiol.* **221**: 992–997.
15. Powell, D. W. 1974. Intestinal conductance and permselectivity changes with theophylline and choleragen. *Am. J. Physiol.* **227**: 1436–1443.
16. Nellans, H. N., and R. A. Frizzell, and S. G. Schultz. 1974. Brush-border processes and transepithelial Na and Cl transport by rabbit ileum. *Am. J. Physiol.* **226**: 1131–1141.
17. Schwartz, C. J., D. V. Kimberg, H. E. Sheerin, M. Field, and S. I. Said. 1974. Vasoactive intestinal peptide stimulation of adenylate cyclase and active electrolyte secretion in intestinal mucosa. *J. Clin. Invest.* **54**: 536–544.
18. Field, M., H. E. Sheerin, A. Henderson, and P. L. Smith. 1975. Catecholamine effects on cyclic AMP levels and ion secretion in rabbit ileal mucosa. *Am. J. Physiol.* **229**: 86–92.
19. Flores, J., P. A. Witkum, B. Beckman, and G. W. G. Sharp. 1975. Stimulation of osmotic water flow in toad bladder by prostaglandin E_1. Evidence for different compartments of cyclic AMP. *J. Clin. Invest.* **56**: 256–262.
20. Trier, J. S. 1964. Studies on small intestinal crypt epithelium. II. Evidence for and mechanisms of secretory activity by unidifferentiated crypt cells of the human small intestine. *Gastroenterology.* **47**: 480–495.
21. George, W. J., J. B. Polson, A. G. O'Toole, and N. D. Goldberg. 1970. Elevation of guanosine 3′5′-cyclic phosphate in rat heart after perfusion with acetylcholine. *Proc. Natl. Acad. Sci. U. S. A.* **66**: 398–403.
22. Lee, T. P., J. F. Kuo, and P. Greengard. 1972. Role of muscarinic cholinergic receptors in regulation of guanosine 3′: 5′-cyclic monophosphate content in mammalian brain, heart muscle and intestinal smooth muscle. *Proc. Natl. Acad. Sci. U. S. A.* **69**: 3287–3291.
23. Eichhorn, J. H., E. W. Saltzman, and W. Silen. 1974. Cyclic GMP response in vivo to cholinergic stimulation of gastric mucosa. *Nature (Lond.).* **248**: 238–239.
24. Brasitus, T. A., D. V. Kimberg, and M. Field. 1975. Intestinal mucosal cyclic GMP: possible role in stimulating active ion absorption. *Clin. Res.* **23**: 391 A. (Abstr.)
25. Kimberg, D. V., M. Field, J. Johnson, A. Henderson, and E. Gershon. 1971. Stimulation of intestinal mucosa adenyl cyclase by cholera enterotoxin and prostaglandins. *J. Clin. Invest.* **50**: 1218–1230.