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THE ROLE OF ACETYLCHOLINE IN THE REGULATION OF ION TRANSPORT BY RAT COLON MUCOSA

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SUMMARY

1. Acetylcholine increases the potential difference across rat proximal colon both *in vivo* and *in vitro*.

2. There is a sigmoid relationship between the change in potential difference and the logarithm of the dose of acetylcholine. The dose–response curve is shifted to the left by neostigmine and to the right by atropine, suggesting that the action of acetylcholine is mediated by a muscarinic type of receptor.

3. The dose-response curve for acetylcholine *in vivo* is not altered by the ganglion-blocking agents hexamethonium and pentolinium, suggesting a direct effect of this transmitter on the colon.

4. Acetylcholine causes an increase in potential difference, a small decrease in resistance and hence a rise in the current generated by both normal and stripped everted sacs of rat colon.

5. In the absence of sodium, the calculated current change produced by acetylcholine is reduced, and the removal of chloride has a similar inhibitory effect. The absence of bicarbonate does not significantly affect the response.

6. Acetylcholine virtually abolished net sodium movement and induced net chloride secretion and these changes accounted for the increased short-circuit current.

7. Acetylcholine had no effect on oxygen consumption by rings of colon.

8. Tracts staining for acetylcholinesterase were observed running from the submucous plexus towards the mucosal epithelium.

9. This study shows that acetylcholine can influence ion movement by rat colonic mucosa and suggests that the autonomic nervous system might be involved in the regulation of transport mechanisms in this tissue.

INTRODUCTION

The final stages of intestinal absorption take place in the colon. This region of the gastrointestinal tract is primarily involved in the conservation of salt and water, and to this end sodium is actively pumped into the blood with water following osmotically. The colon also has an important secretory role, potassium and bicarbonate ions being transported into its lumen (Edmonds, 1967). It is well established that ion transport processes in this tissue can be influenced by hormonal mechanisms (Cummings, 1975). However, little is known about the importance of nervous mechanisms in regulating colonic transport functions.

A number of observations suggest the possibility that intestinal ion transport mechanisms might be under cholinergic control, although evidence for this has been confined to the jejunum and ileum. Indirect evidence for cholinergic regulation has been provided by the observation that atropine alters fluid and electrolyte movement in the small intestine (Blickenstaff & Lewis, 1952; Tidball & Tidball, 1958), while more direct evidence has been obtained in experiments where the action of cholinergic drugs on intestinal transport has been measured. Cholinergic agents have been shown to increase the electrical activity of the small intestine (Hardcastle & Eggenton, 1973) and to inhibit sodium absorption while stimulating chloride secretion (Tidball, 1961; Hubel, 1976; Isaacs, Corbett, Riley, Hawker & Turnberg, 1976). These effects on ion transport explain the increased intestinal secretion observed by Wright, Jennings, Florey & Lium (1940) in response to the administration of acetylcholine.

The aim of this study is to investigate the possibility that ion transport mechanisms in the colon might also be under cholinergic control by determining the effect of acetylcholine on the transport activity of this tissue. A preliminary report of the results obtained has already been published (Browning, Hardcastle, Hardcastle and Sanford, 1976).

METHODS

Animals. Male albino rats bred in the Sheffield Field Laboratories and weighing between 230 and 250 g were used. Before experiments they were maintained on an unrestricted diet (diet 86, Oxoid, London) with free access to water. They were anaesthetized by an I.P. injection of sodium pentobarbitone.

Measurement of the potential difference in vivo. The preparation used to measure the potential difference across the colon *in vivo* is similar to that described for the small intestine by Hardcastle & Eggenton (1973). The proximal 2–3 cm of the colon were isolated by tying off at the distal end and inserting a cannula at the proximal end. This loop was then filled with 0·9 % saline. The mucosal salt bridge electrode was placed in the lumen of the colon loop, while a wick electrode connected the serosal salt bridge to the peritoneal cavity which was kept moist with 0·9 % saline. Drugs

were injected through a cannula into the jugular vein and each dose was washed in with 0·2 ml. 0·9% saline (w/v).

Measurement of the potential difference in vitro. The potential was measured across everted sacs of rat proximal colon using the method described by Barry, Dikstein, Matthews, Smyth & Wright (1964). Paired sacs of proximal colon, about 2 cm long, were taken from adjacent segments of the tissue. One sac then acted as the control, while the other sac was exposed to the test conditions. Initial experiments showed that there was no significant difference in the response of the two sacs to acetylcholine.

Measurement of the resistance. The resistance of everted sacs of rat proximal colon was determined by measuring the change in potential difference that occurred when a current of 1 mA was passed across the tissue. Since the colon behaves as an ohmic resistor (Edmonds & Marriott, 1968), the resistance can be calculated using Ohm's law. The current was applied using silver/silver chloride electrodes as described by Barry, Smyth & Wright (1965).

Stripped sacs. In some experiments the muscle layers were removed from the colon using the method of Parsons and Paterson (1960).

Measurement of short-circuit current and ion fluxes. Ion fluxes were measured across sheets of proximal colon which were short-circuited throughout the experimental period. The area of tissue was 1·925 cm^2 and it was bathed on each side with 5 ml. fluid. The salt bridge electrodes used to record the potential difference were placed within 1 mm of the mucosal and serosal surfaces of the tissue. The current was passed via silver/silver chloride electrodes inserted into salt bridges, external diameter 4 mm.

Influx and efflux measurements were made on adjacent pieces of tissue. These paired tissues were discarded if their resistances differed by more than 25%.

Sodium fluxes were determined using ^{22}Na. 1 μc was placed on one side of the tissue and its appearance in the other compartment was monitored. Collection periods of 10 min duration were started 25 min after the addition of the tracer by which time steady state fluxes had been achieved. In the first collection period the fluxes were measured under control conditions. In the second period acetylcholine was added to the serosal compartment to give a concentration of 10 mM and the fluxes again determined. At the end of each collection period the entire 5 ml. of the receiving compartment, together with three rinses, were removed and counted in a Gamma counter (Gamma Guard II, Tracerlab). The receiving compartment was then refilled with 5 ml. fresh medium containing no tracer.

A similar procedure was used in the measurement of chloride fluxes. 3 μc ^{36}Cl were initially placed on one side of the tissue and the appearance of the isotope in the other compartment was measured. At the end of each 10 min collection period a 1 ml. sample was taken from the receiving compartment. This was added to 10 ml. scintillation fluid (Bray, 1960) and counted on a liquid scintillation counter (Packard Tri-carb, model 3375). The remainder of the receiving compartment was emptied, rinsed and then filled with 5 ml. fresh, unlabelled medium.

Electrical equipment. The potential difference was measured on a Vibron electrometer. The electrodes consisted of salt bridges filled with 1M-potassium chloride solidified with agar. These were connected to the electrometer via calomel half-cells. The potential differences were displayed visually on a two-channel chart recorder (Telsec 700 series).

Measurement of oxygen consumption. The method for measuring the oxygen consumption of the colon was based on that used by Bronk & Parsons (1965). A 1 cm length of proximal colon was cut into rings 0·5 mm thick. These were then placed in 3 ml. Krebs bicarbonate saline and their oxygen consumption was determined

using an oxygen electrode (Yellow Springs Biological Oxygen Monitor, YS1 model 53) and the results expressed as μl. oxygen/mg dry weight.hr.

Incubation media. In vitro preparations of the colon were incubated in the bicarbonate saline of Krebs & Henseleit (1932), gassed with 95% oxygen/5% carbon dioxide and maintained at 37 °C. The composition of the medium was altered in experiments to determine the ionic basis of the acetylcholine response. In the sodium-free medium all the sodium was replaced with Tris (trihydroxymethylmethylamine). In the chloride-free medium all the chloride was replaced with sulphate and isotonicity maintained with mannitol. In chloride-free conditions the iodide salt of acetylcholine was used instead of the chloride salt. Preliminary experiments showed that there was no significant difference in the response of the colon to these two salts. When both sodium and chloride were replaced, Tris substituted for sodium and sulphate for chloride. Again the iodide salt of acetylcholine was used. To study the effect of the absence of bicarbonate, Krebs phosphate saline (Krebs, 1933) was used. This was gassed with 100% oxygen.

Demonstration of acetylcholinesterase. Fresh cryostat sections, approximately 50 μm thick, were obtained and fixed in a formal sucrose fixative at pH 6·7 (Pearson, 1963). Cholinesterase activity was determined using the method of Gomori (1952) and pseudocholinesterase activity eliminated using the inhibitor iso-OMPA (Bayliss & Todrick, 1956). A silver intensification method was used to enhance the acetylcholinesterase activity (Henderson, 1967).

Chemicals. Acetylcholine chloride, acetylcholine iodide, acetylthiocholine iodide, atropine sulphate and neostigmine bromide were obtained from Sigma Chemical Co., P.O. Box 14508, St Louis, Missouri 63168 U.S.A. Pentolinium as a 0·5% solution of pentolinium tartrate (Ansolysen) and hexamethonium bromide were obtained from May and Baker Ltd, Dagenham, England. The salts for the incubation media were all obtained from May and Baker Ltd, Dagenham, England. Tris and iso-OMPA (tetraisopropylpyrophosphoramide) were supplied by Koch-Light Laboratories, Colnbrook, Bucks., England. Radioactive tracers were supplied by the Radiochemical Centre, Amersham, Bucks., England.

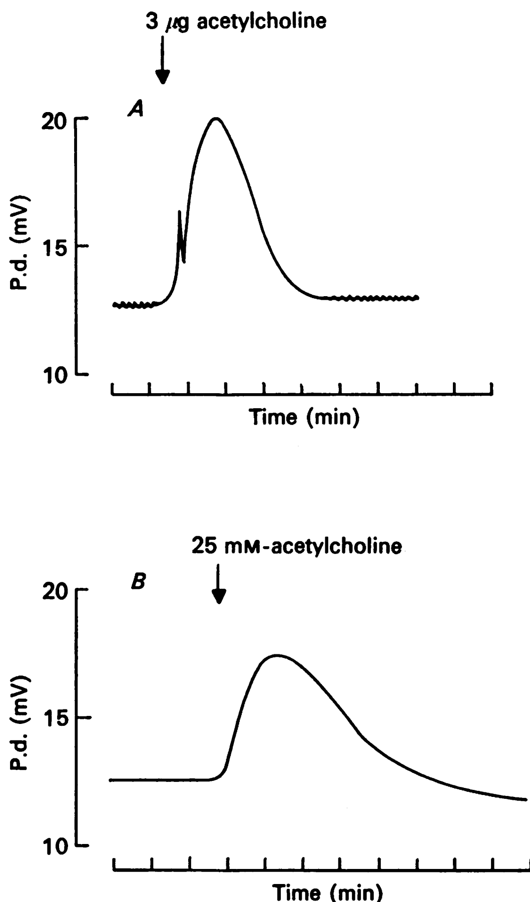
Statistical analysis. The experiments involving the effect of the ionic composition of the medium on the response to acetylcholine were performed on paired sacs of colon, one sac being incubated in normal Krebs bicarbonate saline and the adjacent sac in the modified medium. Acetylcholine was added to the serosal fluid of each sac and a paired t test used to determine whether there was a statistically significant difference in the response. Sodium and chloride fluxes were measured before and after the addition of acetylcholine and again a paired t test was used to evaluate the significance of the changes observed.

RESULTS

The colonic epithelium generates a potential difference of between 5 and 15 mV, the serosal side of the tissue being positive with respect to the mucosal side. Acetylcholine caused a transient increase in this potential difference both *in vivo* and *in vitro* and typical responses are shown in Text-fig. 1. In the *in vivo* preparation I.V. administered acetylcholine caused an initial peak in the potential difference which rapidly decayed and was followed by a more gradual rise, which reached a maximum in 1–1·5 min (Fig. 1*A*). The everted sac of rat colon was less sensitive to acetylcholine. There was no initial peak in response to acetylcholine,

although the more gradual rise in potential difference closely followed the pattern observed *in vivo* (Fig. 1*B*).

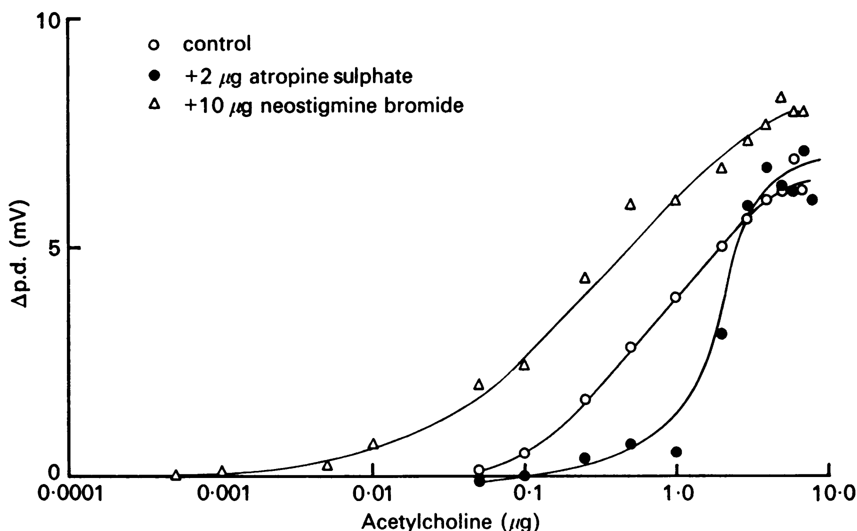
The response to acetylcholine in vivo. The relationship between the change in potential difference and the logarithm of the dose of acetylcholine was sigmoid (Text-fig. 2), a maximum response of $6{\cdot}5 \pm 0{\cdot}4$ mV (42) being obtained at a dose of $4{\cdot}7 \pm 0{\cdot}3$ μg (42).



Text-fig. 1. Typical responses showing the effect of acetylcholine on the potential difference (p.d.) across rat proximal colon *in vivo* (*A*) and *in vitro* (*B*). In the *in vivo* preparation acetylcholine was administered via the jugular vein. The *in vitro* preparation was the everted sac and acetylcholine was added to the serosal fluid to give the concentration indicated.

The sensitivity of the preparation was reduced by 2 μg atropine sulphate, as indicated by the shift of the dose–response curve to the right. However, the maximum response was unaltered.

On the other hand, 10 μg neostigmine bromide enhanced the sensitivity of the colon, shifting the dose-response curve to the left. The maximum response was also increased from $6{\cdot}7 \pm 0{\cdot}7$ (10) to $8{\cdot}8 \pm 0{\cdot}7$ mV (10), a change which was shown by a paired t test to be significant ($0{\cdot}05 > P > 0{\cdot}01$).



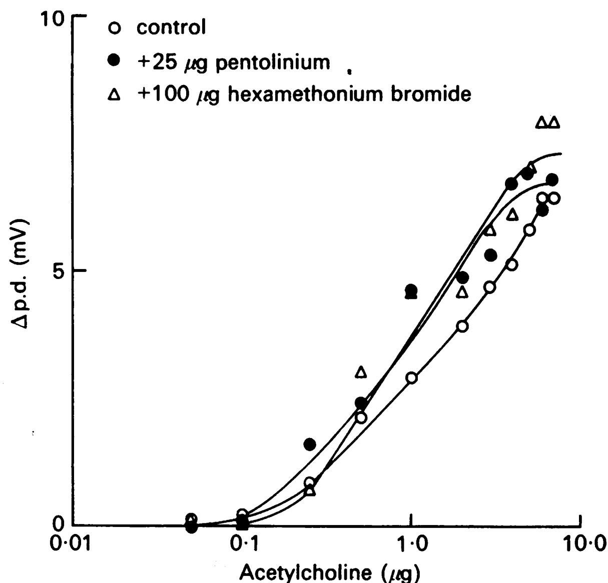
Text-fig. 2. Effect of acetylcholine on the potential difference across rat proximal colon *in vivo*. Drugs were injected via the jugular vein and each dose was washed in with 0·2 ml. 0·9 % saline. The change in potential difference (Δ p.d.) is plotted against the logarithm of the dose of acetylcholine and each point represents the mean of seventeen observations. The response to acetylcholine following the administration of 2 μg atropine sulphate (each point is the mean of seven observations) or 10 μg neostigmine bromide (each point is the mean of ten observations) is also shown.

To determine whether acetylcholine was acting directly on the colon or indirectly by modifying ganglionic transmission, the effects of ganglion-blocking agents on the response to this drug were studied. Although both 100 μg hexamethonium bromide and 25 μg pentolinium caused a profound drop in blood pressure, they had no significant effect on the colonic potential difference. Neither blocker altered the dose-response characteristics of acetylcholine (Text-fig. 3), which therefore seems to be acting directly on the colon.

Response to acetylcholine in vitro. Acetylcholine also increased the potential difference across rat proximal colon *in vitro*, showing a direct action of the drug on this tissue. Again the magnitude of the response was related to the dose of acetylcholine and a sigmoid relationship between the change

in potential difference and the logarithm of the acetylcholine concentration in the serosal fluid was obtained (Text-fig. 4).

The possibility that the addition of a high concentration of the chloride salt of acetylcholine to one side of the *in vitro* intestine might give rise to a chloride diffusion potential has been tested. Additional sodium chloride (50 mM) or sodium sulphate (50 mM) was added to the serosal fluid of an

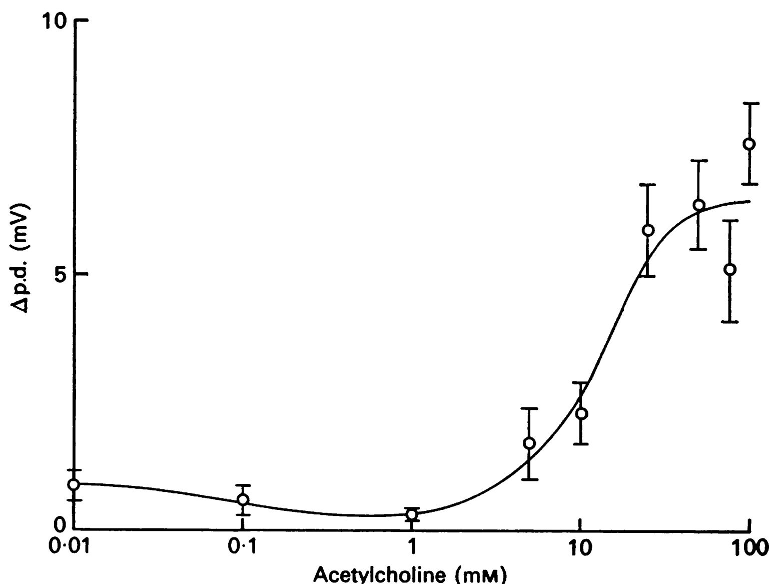


Text-fig. 3. Effect of 100 μg hexamethonium bromide and 25 μg pentolinium on the response of rat proximal colon to acetylcholine *in vivo*. Drugs were administered via the jugular vein and each dose was washed in with 0·2 ml. 0·9% saline. The change in potential difference (Δp.d.) is plotted against the logarithm of the dose of acetylcholine. In the control curve each point represents the mean of eleven observations, while in the curves with hexamethonium bromide and pentolinium each point is the mean of six and five observations respectively.

everted sac of colon. Sodium chloride decreased the potential difference by $0·9 \pm 0·4$ mV (6), while sodium sulphate produced a decrease of $1·2 \pm 0·2$ mV (6). There is no significant difference between these two changes ($P > 0·1$, paired t test).

The increased potential difference induced in the colon by acetylcholine may result from an alteration in net ion movement by this tissue. However, it could simply reflect a change in resistance. The effect of acetylcholine on the resistance of everted sacs of colon was therefore measured and the results are shown in Table 1. Acetylcholine increased the potential difference while causing a small decrease in tissue resistance. Since the colon

behaves as an ohmic resistor (Edmonds & Marriott, 1968), the current generated by the tissue, which is an index of net ion movement, can be calculated from potential difference and resistance measurements. Acetylcholine caused a marked rise in the calculated current and hence must have brought about an alteration in net ion movement by the colon.



Text-fig. 4. Effect of acetylcholine on the potential difference across everted sacs of rat proximal colon. Acetylcholine was added to the serosal fluid to give the concentrations shown. The change in potential difference (Δp.d.) is plotted against the logarithm of the acetylcholine concentration and each point represents the mean of at least six observations ± 1 S.E.

TABLE 1. Effect of acetylcholine (ACh) on the electrical activity of normal and stripped everted sacs of rat proximal colon. Acetylcholine was added to the serosal fluid to give a final concentration of 50 mM. Potential difference (p.d.) and resistance (R) measurements were made immediately before the addition of acetylcholine (initial) and at the peak of the response (+ACh). The current generated by the tissue ($I_{calculated}$) was determined from p.d. and R measurements using Ohm's Law. The response to acetylcholine is the difference between the initial and +ACh values and is given as the mean value ± 1 S.E. of the mean

	Number		P.d. (mV)	R (Ω/6 cm sac)	$I_{calculated}$ (mA/6 cm sac)
Normal sacs	8	Initial	10·2 ± 0·8	13·6 ± 1·0	0·77 ± 0·07
		+ACh	13·5 ± 1·2	9·6 ± 0·4	1·44 ± 0·16
		Response	3·3 ± 0·5	−4·0 ± 1·0	0·67 ± 0·13
Stripped sacs	8	Initial	11·0 ± 1·2	8·9 ± 0·7	1·26 ± 0·13
		+ACh	13·0 ± 1·2	6·5 ± 0·5	2·05 ± 0·18
		Response	2·0 ± 0·3	−2·4 ± 0·3	0·79 ± 0·11

It was possible that the muscle layers could have contributed to the electrical changes induced by acetylcholine. This was investigated by measuring the effect of acetylcholine on stripped sacs of rat colon from which the muscle layers had been removed. Again a very similar pattern of results was obtained (Table 1), acetylcholine causing an increased potential difference, a decreased resistance and hence a rise in the current generated by the tissue. Thus it seems unlikely that the muscle layers were making a major contribution to the changes observed.

TABLE 2. Effect of acetylcholine on the calculated current ($I_{calculated}$) generated by everted sacs of rat proximal colon. Acetylcholine was added to the serosal fluid to give a final concentration of 20 mM. In sodium-free medium sodium was replaced with Tris. In Cl^--free medium chloride was replaced with sulphate and isotonicity maintained with mannitol. The iodide salt of acetylcholine was used in chloride-free conditions. Bicarbonate-free experiments were carried out using a phosphate buffer. The potential difference was measured in paired sacs of colon and the resistance determined separately. The current was calculated from potential difference and resistance measurements using Ohm's Law and values are given as the mean ± 1 S.E. of the mean. A paired t test was used to calculate the level of significance.

	Number	Change in $I_{calculated}$ (mA/6 cm sac)	P
Control	6	0·51 ± 0·05	$0·05 > P > 0·01$
Na^+-free	6	0·32 ± 0·06	
Control	6	0·51 ± 0·11	$0·05 > P > 0·01$
Cl^--free	6	0·22 ± 0·10	
Control	10	0·46 ± 0·07	$P > 0·1$
HCO_3^--free	10	0·39 ± 0·06	
Control	6	0·36 ± 0·05	$0·01 > P > 0·001$
$Na^+ + Cl^-$-free	6	0·09 ± 0·04	

Ionic basis of the acetylcholine response. The fact that acetylcholine alters the current generated by the colon establishes that this transmitter is causing a change in net ion movement. To investigate the nature of the ions involved, the response to acetylcholine was measured in everted sacs of rat colon when the ionic composition of the medium was altered (Table 2). In the absence of sodium or chloride ions, the calculated current change elicited by acetylcholine was significantly reduced, although the elimination of bicarbonate ions was without significant effect. When both sodium and chloride were absent from the medium the response to acetylcholine was not significantly different from zero ($P > 0·05$). This suggested that changes in sodium and chloride movement might be responsible for the current change induced by acetylcholine.

This was confirmed by direct measurements of sodium and chloride

TABLE 3. Effect of acetylcholine (ACh) on unidirectional sodium and chloride fluxes and the short-circuit current (I_{sc}) in sheets of rat proximal colon. Acetylcholine was added to the serosal compartment to give a final concentration of 10 mM. The mucosal to serosal fluxes($J_{m \rightarrow s}$) and the serosal to mucosal fluxes ($J_{s \rightarrow m}$) were determined on adjacent segments of tissue and the net flux (J_{net}) calculated as the difference between the two unidirectional fluxes. The residual ion flux is taken as the difference between the short-circuit current and the sum of the net sodium and chloride fluxes. Each value represents the mean ± 1 S.E. of the mean

		Number	$J_{m \rightarrow s}$ (μmole/cm^2.hr)	$J_{s \rightarrow m}$ (μmole/cm^2.hr)	J_{net} (μmole/cm^2.hr)	I_{sc} (μmole/cm^2.hr)
Na	Control	6	5·69 ± 0·35	4·70 ± 0·42	0·97 ± 0·35	2·42 ± 0·25
	+ACh		5·39 ± 0·36	5·46 ± 0·40	−0·11 ± 0·24	3·09 ± 0·26
Cl	Control	7	8·11 ± 1·25	8·38 ± 0·88	−0·28 ± 1·55	3·00 ± 0·45
	+ACh		7·13 ± 0·85	9·15 ± 0·90	−2·02 ± 1·41	3·48 ± 0·46
Residual ion flux	Control				1·46	
	+ACh				1·38	

fluxes across sheets of rat colon. In the absence of acetylcholine there was active absorption of sodium with no net movement of chloride (Table 3). Acetylcholine abolished net sodium absorption ($0.02 > P > 0.01$) by causing a significant ($0.02 > P > 0.01$) increase in the serosal to mucosal flux of this ion, without significantly affecting its movement in the reverse direction ($P > 0.1$). Net chloride secretion was induced by acetylcholine and this was significantly different ($0.05 > P > 0.01$) from the situation observed in the absence of the drug. The net secretion of chloride resulted from a small decrease in the mucosal to serosal flux and a small increase in the serosal to mucosal flux. The increased short-circuit current obtained with acetylcholine was entirely accounted for by these changes in net sodium and chloride movement, the residual ion flux remaining unchanged.

Oxygen consumption. The increased electrical activity caused by the action of acetylcholine on the colon might be expected to alter the metabolic activity of the tissue. This was tested by measuring the oxygen consumption by rings of colon in the absence and presence of the drug. Neither 10 mM nor 50 mM-acetylcholine had any significant effect on the oxygen consumption. In the absence of the drug the oxygen consumption was 6.34 ± 0.95 μl. oxygen/mg dry weight.hr (6). In the presence of 10 mM-acetylcholine the oxygen consumption was 6.43 ± 0.95 μl. oxygen/mg dry weight.hr (6) and with 50 mM-acetylcholine it was 6.44 ± 0.89 μl. oxygen/mg dry weight.hr (4). Thus the changes in ion transport do not seem to be powered by an increase in aerobic metabolism. The fact that these high concentrations of acetylcholine do not reduce oxygen consumption suggests that they are not having any deleterious effect on the colon.

Demonstration of acetylcholinesterase. Acetylcholinesterase was observed in the submucous plexus of the muscularis mucosae (Pl. 1). From this region tracts staining specifically for the enzyme can be seen to run towards the mucosal epithelium.

DISCUSSION

The present study shows that ion transport in the colon can be influenced by acetylcholine. Thus cholinergic mechanisms may be involved in regulating transport processes in this tissue, as has been shown in the small intestine (Hardcastle & Eggenton, 1973; Hubel, 1976; Isaacs *et al.* 1976) and other epithelial tissues; the sweat gland (Foster, 1969), the gastric mucosa (Shoemaker, Makhlouf & Sachs, 1970) and the submaxillary gland duct (Martin, Frömter, Gebler, Knauff & Young, 1973).

The response of the potential difference to acetylcholine *in vivo* consists of two components, a rapid initial spike followed by a more gradual rise in the potential difference (Text-fig. 1). The first phase of this response does not appear *in vitro*, which suggests that it may be due to some

indirect action of this drug. Interestingly enough a rapid initial peak does not occur in the response of the jejunum to acetylcholine, either *in vivo* or *in vitro* (Hardcastle & Eggenton, 1973). When the action of acetylcholine on both jejunum and colon is monitored simultaneously (Aldous, Browning, Hardcastle & Hardcastle, 1976), then the initial spike in the colon occurs a little before both regions respond with a gradual rise in the potential difference. The second phase is consistent with the arrival of blood-borne acetylcholine, suggesting that the initial rise is not due to a direct action of this transmitter. The size of this initial peak is related to the dose of acetylcholine given and it is inhibited by atropine. However, the ganglion-blocking agents hexamethonium bromide and pentolinium do not significantly affect it and its origin remains obscure.

The sigmoid relationship between the change in potential difference and the logarithm of the dose of acetylcholine both *in vivo* and *in vitro* (Text-figs. 2 and 4) suggests that acetylcholine is bound to some saturable component of the tissue. The fact that the sensitivity of the colon to this drug is enhanced by neostigmine and reduced by atropine indicates that the receptor involved is of the muscarinic type.

Ganglion-blocking agents do not inhibit the response to acetylcholine, which must therefore be having a direct effect on the colon. This is confirmed by the finding that acetylcholine increases the potential difference of *in vitro* preparations of colon.

The changes in electrical activity produced by acetylcholine appear to be due primarily to an effect on the mucosa, since removal of the muscle layers does not alter the nature of the response (Table 1).

The colon is more sensitive to the action of acetylcholine *in vivo* than it is *in vitro*. There are probably several reasons for this. First, acetylcholine administered into the circulation is likely to be brought much closer to its site of action at the epithelial layer than it is when applied to the serosal fluid of an *in vitro* preparation. Here it has to cross the considerable diffusion barrier represented by the subepithelial tissue. In addition, during its passage across the muscle layer acetylcholine may be hydrolysed by the cholinesterase present there (Ambache, Freeman & Hobbiger, 1971). The relevant concentration is that at the receptor sites and this may be very much lower than the dose of acetylcholine administered into the bulk phase. The capacity of the tissue to respond to acetylcholine is similar *in vivo* and *in vitro*. The maximum response *in vivo* is $6 \cdot 5 \pm 0 \cdot 4$ mV (42) compared with $7 \cdot 6 \pm 0 \cdot 8$ mV (12) in everted sacs of rat colon.

The suggestion that a chloride diffusion potential could account for the increased potential difference observed with acetylcholine (Isaacs *et al.* 1976) can be ruled out in this study. 50 mM-sodium chloride in the serosal fluid produced the same decrease in potential difference as 50 mM-sodium

sulphate. Since the colon is much less permeable to sulphate than it is to chloride (Baillien & Schoffeniels, 1961) it appears that a chloride diffusion potential is not generated as a result of an increased serosal chloride concentration. In addition, the results *in vivo* cannot be explained in terms of a chloride diffusion potential. Here the doses of acetylcholine given were extremely small (less than 10 μg in a 250 g rat) and the rise in blood chloride concentration would therefore be minimal. Also it is unlikely that a chloride diffusion potential would be inhibited by atropine and enhanced by neostigmine, characteristics of the interaction of acetylcholine with a muscarinic type of receptor.

The increased potential difference caused by acetylcholine was accompanied by a decrease in resistance. Since the colon behaves as an ohmic resistor the current generated by the tissue rose in response to the transmitter (Table 1). This indicates that net ion movement has been altered and this change appears to originate from the mucosal layer since removal of the muscle layers did not reduce the response.

Ion replacement experiments suggested that changes in sodium and chloride movement were involved in the response to acetylcholine (Table 2). This was confirmed by direct measurements of the unidirectional fluxes of these ions which showed that acetylcholine abolished net sodium absorption and induced net chloride secretion (Table 3).

In view of these findings, it is perhaps surprising that the response to acetylcholine was lower in the sodium-free medium than it was under control conditions. However, in these experiments both mucosal and serosal fluids were modified and it has been shown that a decrease in the serosal sodium concentration causes a rise in the potential difference across the serosal membrane (Gilles-Baillien & Schoffeniels, 1967). Thus the movement of chloride into the cell from the serosal side will occur less readily as the electrical gradient across the serosal membrane will oppose its movement in this direction. A reduction in the entry of chloride into the cell from the serosal side may then result in its decreased secretion into the lumen across the mucosal border and this could explain the diminished response in sodium-free conditions.

Acetylcholine and other cholinergic drugs have been shown to influence ion transport in other regions of the intestinal tract. Pilocarpine alters ion transport in both rat jejunum and ileum *in vivo* (Hubel, 1976). In the jejunum the net absorption of sodium, potassium and water is decreased and chloride secretion is induced, while in the ileum chloride absorption is decreased, sodium and water secretion is induced and the secretion of potassium is enhanced. In the human ileum under *in vitro* conditions, acetylcholine applied to both mucosal and serosal fluids induced chloride secretion without affecting sodium absorption (Isaacs *et al.* 1976). Similarly,

Tidball (1961) has shown that bethanechol induces the secretion of fluid and chloride by dog jejunum *in vivo*. On the other hand, Tapper, Powell and Morris (1976) found that a high concentration of carbachol increased the potential difference and short-circuit current and enhanced the absorption of both sodium and chloride by rabbit ileum *in vitro*. Thus although there are several reports of an effect of cholinergic agents on ion transport by the small intestine the changes observed tend to differ. This rather confused situation may represent a species variation and the fact that the techniques and particular cholinomimetics used were different.

However, there are no reports concerning the action of cholinergic agents on ion transport by the colon. In the present study, the colon was found to actively absorb sodium while chloride moved passively under control conditions, which is in agreement with the findings of Curran & Schwartz (1960). The short-circuit current was greater than the net movement of sodium and the residual ion flux could be attributed to either cation movement from mucosa to serosa or anion movement from serosa to mucosa. A significant residual ion flux across rat colon has also been described by Binder & Rawlins (1973). Rat colon secretes bicarbonate (Edmonds, 1967) and this ion movement could account for the residual ion flux. In the rabbit short-circuited colon the residual ion flux is consistent with active bicarbonate secretion (Frizzell, Koch & Schultz 1976). Acetylcholine did not change the residual ion flux in rat colon (Table 3) and since bicarbonate was not involved in the acetylcholine response (Table 2), this supports the view that the residual ion flux is due to bicarbonate secretion. In this tissue the increased potential difference and short-circuit current caused by acetylcholine were due to the abolition of active sodium absorption and the induction of a chloride secretory process. These findings are similar to those observed in the small intestine by Tidball (1961), Hubel (1976) and Isaacs *et al.* (1976), who all find that chloride movement towards the lumen is induced by cholinergic drugs. In view of the different species and techniques employed, this suggests a general secretory effect of cholinergic stimulation in intestinal tissue.

The mechanism by which cholinergic stimulation causes intestinal secretion is still not fully understood. Brasitus, Field & Kimberg (1976) found a transient increase in cyclic GMP levels in rabbit ileal mucosa in the presence of carbachol and this was blocked by atropine. Tapper *et al.* (1976) demonstrated a similar effect of carbachol on ileal cyclic GMP levels, but their increased potential difference and short-circuit current resulted from enhanced sodium and chloride absorption, rather than from stimulation of a secretory process. Brasitus *et al.* (1976) attempted to relate the increased cyclic GMP levels to alterations in ion transport. However, they were unable to demonstrate any effect of exogenous cyclic GMP or

8-bromo-cyclic GMP on ion fluxes in rabbit ileum, although Field (1971) has shown that cyclic GMP increased the short-circuit current generated by this tissue.

Cyclic GMP is considered to be a mediator of the cholinergic response in several other tissues after its level has been found to rise following cholinergic stimulation (George, Polson, O'Toole & Goldberg, 1970; Yamashita & Field, 1972; Illiano, Tell, Siegel & Cuatrecasas, 1973; Eichorn, Salzman & Silen, 1974). It has been suggested that in the regulation of cell function cyclic GMP plays an antagonistic role to cyclic AMP (Goldberg, Haddox, Nicol, Glass, Sanford, Kuehl & Estensen, 1975), since agents which increase the level of cyclic GMP in intact cells do not usually elevate, and may even reduce, the level of cyclic AMP in the same cell. In support of the concept that cyclic GMP mediates the cholinergic response of the intestine it has been shown that carbachol has no effect on the levels of cyclic AMP in the rabbit ileum (Schwartz, Kimberg, Sheerin, Field & Said, 1974) and that acetylcholine does not alter the levels of this nucleotide in human ileum (Isaacs *et al.* 1976). Thus, although exogenous cyclic AMP and agents that enhance the endogenous levels of this nucleotide are known to have a secretory effect in both the small intestine (Field, 1971) and the colon (Frizzell, Koch & Schultz, 1976), it is doubtful whether the colonic secretion of chloride induced by acetylcholine is mediated by cyclic AMP. It is more likely that cyclic GMP is involved in this cholinergic response. We appear to have the situation where both cyclic AMP and cyclic GMP, rather than producing opposite physiological effects with regard to intestinal ion transport, both cause intestinal secretion, in spite of the fact that the adenylate cyclase system is located at the basolateral membrane, while the guanylate cyclase system is concentrated at the microvillous membrane of the epithelial cell (de Jonge, 1975). Both of these nucleotides have been shown to phosphorylate a specific microvillous protein from rat small intestine and this could be directly responsible for the observed change in ion transport (de Jonge, 1976).

Cholinergic stimulation has been shown to increase the mitotic rate in the crypts of Lieberkühn of rat jejunum (Tutton, 1975) and Trier (1964) noted changes in the morphology of undifferentiated crypt cells and Paneth cells in the human ileum after pilocarpine administration. These observations suggest that in the small intestine the crypts are primarily involved in the secretory processes induced by cholinergic stimulation as well as playing a secretory role in conditions such as cholera (Kimberg, Field, Gershon, Schooley & Henderson, 1973). In the colon a similar involvement of the crypts in cholinergically induced ion secretion could also exist.

This investigation has shown that acetylcholine has a direct effect on

the electrical activity of the colonic epithelium and that this results from changes in net sodium and chloride movement. Since acetylcholinesterase-staining tracts can be demonstrated in close proximity to the epithelial layer it seems possible that transport mechanisms in this tissue may be regulated by the autonomic nervous system.

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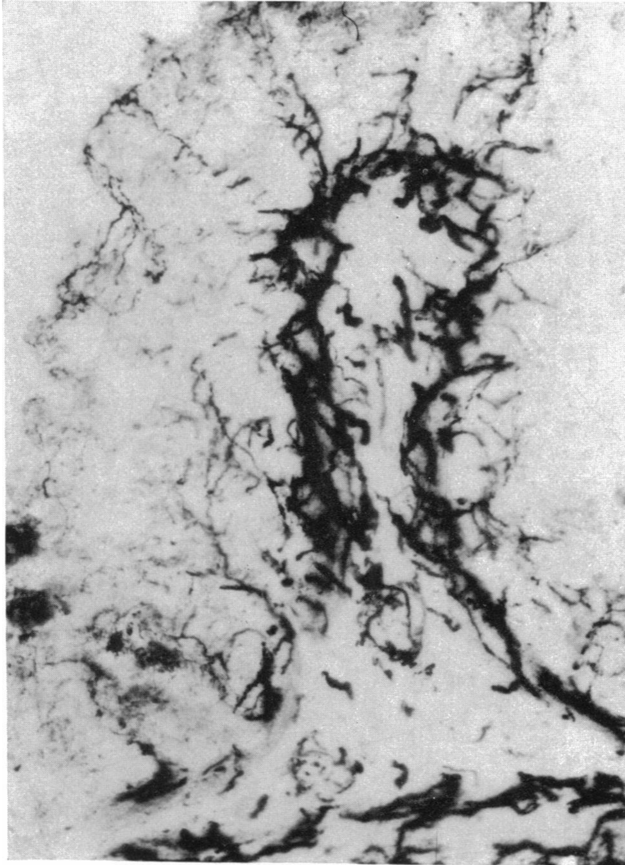
EXPLANATION OF PLATE

Localization of specific acetylcholinesterase activity in the mucosa of rat colon. Cholinesterase activity was determined by the Gomori (1952) method, pseudocholinesterase activity being specifically inhibited by iso-OMPA. A silver intensification process has been used to enhance the activity. The section thickness was approximately 50 μm and magnification × 100.



J. G. BROWNING AND OTHERS (*Facing p.* 754)