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THE EFFECT OF ACETYLCHOLINE ON THE ELECTRICAL ACTIVITY OF INTESTINAL EPITHELIAL CELLS

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SUMMARY

1. The effect of the neurohumoral transmitter, acetylcholine, on the transepithelial electrical activity of rat jejunum was investigated both *in vitro* and *in vivo*.

2. Acetylcholine caused an increase in the transintestinal potential difference and this change was related to log dose, a sigmoid curve being obtained.

3. The increased potential difference reflected an alteration in net ion transport since no significant change in tissue resistance was observed.

4. The effect of acetylcholine was antagonised by atropine and enhanced by neostigmine, suggesting that acetylcholine is acting through the type of receptor usually associated with postjunctional cholinergic effector cells.

5. These observations suggest the possibility that intestinal transport activity may be under the control of the autonomic nervous system.

INTRODUCTION

There have been several reports in the literature which suggest that transport by the small intestine may be influenced by the autonomic nervous system[1–6]. Recent work[5,6] has concentrated on the effects of adrenergic activity on intestinal transport processes, while earlier reports[2–4] regarding the effects of cholinergic activity on intestinal function have received little attention. In order to investigate the role of this division of the autonomic nervous system in the control of intestinal transport, the effect of the neurohumoral transmitter, acetylcholine, on the transmural electrical activity of rat jejunum was studied. The electrical activity generated by this tissue reflects net ion movement across the epithelial cells[7] and therefore any modification in ion transport caused by acetylcholine would be observed as a change in the transintestinal potential difference, provided that the tissue resistance remained unchanged.

MATERIALS AND METHODS

White male rats bred in the Sheffield Field Laboratories and weighing between 230 and 250 g were used. Before experiment they were maintained on an unrestricted diet (diet 86, Oxoid, London) with free access to water. They were anaesthetised by an intraperitoneal injection of Nembutal (sodium pentobarbitone).

Abbreviation: PD, potential difference.

Experiments *in vitro* were carried out on everted sacs of rat jejunum incubated in Krebs bicarbonate saline[8]. The potential difference was measured as described by Barry *et al.*[9] and the resistance was determined using the method of Barry *et al.*[10].

In the *in vivo* preparation a 5-cm loop of intestine from the mid-region was isolated by tying off and was filled with 0.9% NaCl. The mucosal salt bridge was placed in the lumen of this segment and a wick electrode connected the serosal salt bridge to the peritoneal cavity which was kept moist with 0.9% NaCl. Drugs were injected intravenously through a cannula in the jugular vein. Preliminary experiments had shown that there was no difference in the response whether drugs were administered through the jugular or femoral veins.

The potential difference was measured by a Vibron electrometer (Electronic Instruments Ltd) and a visual record was obtained on a Telsec recorder (Telsec Instruments Ltd).

Acetylcholine chloride and neostigmine bromide were obtained from the Sigma Chemical Company and atropine sulphate from BDH Chemicals Ltd.

RESULTS AND DISCUSSION

The addition of acetylcholine to the serosal fluid caused a rise in the potential difference and the magnitude of this increase was related to the log of the dose, a sigmoid curve being obtained (Fig. 1). These results suggest that acetylcholine may stimulate electrogenic ion transport across the gut. However, it was possible that the rise in the potential difference was simply reflecting a change in tissue resistance rather than an alteration in ion movement. The resistance of the tissue was therefore measured in addition to the potential difference and the results are shown in Table I. The addition of a high concentration of acetylcholine (100 mM) to the serosal fluid caused the potential difference to increase but there was no significant change in tissue resistance. Since the small intestine behaves as an ohmic resistor[11] acetylcholine must have caused an alteration in the current generated by the gut. The fact that acetylcholine has caused a smaller change in the potential difference in these experiments is attributed to the presence of Ag^+ released from the Ag–AgCl electrodes

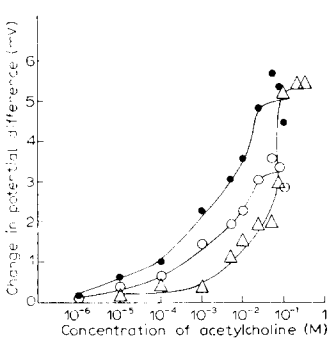

Fig. 1. Effect of varying concentrations of acetylcholine chloride in the serosal fluid on the potential difference across the wall of normal (△) and stripped (○) sacs of everted rat jejunum. The resistance of stripped sacs is 37% lower than that of normal sacs and therefore the values for the former have been corrected for this difference (●). The change in potential difference is plotted against log concentration and each point represents the mean of at least 6 determinations.

TABLE I

EFFECT OF 100 mM ACETYLCHOLINE CHLORIDE IN THE SEROSAL FLUID ON THE POTENTIAL DIFFERENCE AND RESISTANCE OF EVERTED SACS OF RAT JEJUNUM

Results are expressed as mean values ± S.E. of the mean with the number of observations in brackets.

	PD (*mV*)	*Resistance* (*Ω/8 cm sac*)
Initial	3.2±0.3 (8)	2.1±0.4 (8)
+acetylcholine	5.5±0.3 (8)	1.8±0.3 (8)

used to pass the current. It is considered that low concentrations of these ions have a deleterious effect on intestinal function[11].

Large concentrations of acetylcholine have been used in these experiments. However, the transmural electrical activity of the small intestine is generated by the mucosal epithelial cells (enterocytes) and so acetylcholine added to the serosal fluid presumably has to diffuse through all the submucosal layers before reaching its site of action in the direct vicinity of the receptors. This environment has been called the biophase[12]. Since the muscle layers contain considerable amounts of cholinesterase[13] it seems probable that the concentration of acetylcholine in the biophase is much lower than that in the bulk phase. Everted sacs of jejunum from which the muscle layers had been stripped were therefore prepared, using a method similar to that employed in the colon by Parsons and Paterson[14]. These sacs were found to give an enhanced response to acetylcholine at lower concentrations, although the maximum response obtained was less than in normal sacs (Fig. 1). However, the transmural resistance of stripped sacs (1.5±0.2 (7) Ω/8 cm sac) is 37.3% lower than that of normal sacs (2.3±0.2 (7) Ω/8 cm sac) and if this factor is taken into account the maximum response of stripped sacs to acetylcholine is very similar to that of normal sacs, but the curve for the stripped sacs has been shifted to the left (Fig. 1). These observations are consistent with the suggestion that removal of the muscle layers results in a loss of cholinesterase activity, with a subsequently greater concentration of acetylcholine in the biophase. If this interpretation is correct it would be expected that cholinesterase inhibitors should enhance the effect of acetylcholine in normal sacs. The presence of 25 mM neostigmine in the serosal fluid not only increased the initial rise in potential caused by 10 mM acetylcholine (Table II) but also prolonged the response. 20 min after adding acetylcholine the potential difference in control sacs had returned to normal, while in the presence of neostigmine the potential difference was still 2.4 mV greater than the resting level. The effect of neostigmine is complicated by the fact that neostigmine itself causes a small increase (0.5±0.1 (6) mV) in the transintestinal potential. The neostigmine molecule possesses a choline moiety which may enable it to interact with the receptor. Alternatively, neostigmine could enhance the effect of any acetylcholine that may be released spontaneously from intrinsic cholinergic fibres.

Atropine is known to competitively inhibit the interaction of acetylcholine with "muscarinic" receptors. Dose–response curves for acetylcholine were therefore

TABLE II

EFFECT OF 25 mM SEROSAL NEOSTIGMINE BROMIDE ON THE CHANGE IN POTENTIAL DIFFERENCE (ΔPD) ACROSS EVERTED SACS OF RAT JEJUNUM CAUSED BY 10 mM SEROSAL ACETYLCHOLINE CHLORIDE

Readings were taken at the peak of the response (ΔPD peak) and 20 min after the addition of the drug (ΔPD_{20}). The effect of neostigmine alone has been subtracted from the change in potential difference caused by acetylcholine in its presence. Results are expressed as mean values ± S. E. of the mean with the number of observations in brackets.

	ΔPD_{peak}	ΔPD_{20}
Neostigmine	0.5 ± 0.1 (6)	0.7 ± 0.3 (6)
Acetylcholine	2.0 ± 0.3 (11)	0.2 ± 0.5 (11)
Acetylcholine + neostigmine	3.2 ± 0.5 (11)	2.4 ± 0.9 (11)

determined in the absence and presence of 10 mM atropine in the serosal fluid. Atropine caused the curve to be shifted to the right, although the maximum response obtained was not altered (Fig. 2). Thus atropine seems to have competitively antagonised acetylcholine, without altering the intrinsic activity of the receptor. It therefore appears that acetylcholine is altering the electrical activity of the small intestine through an interaction with the type of receptor normally associated with postjunctional cholinergic effector cells (muscarinic sites).

The response of the transintestinal potential to acetylcholine has also been observed in an *in vivo* preparation. Injection of varying doses of acetylcholine into the jugular vein caused a transient rise in the potential difference, the relationship between log dose and the change in potential being sigmoid (Fig. 3). Acetylcholine was effective in increasing the potential difference at much lower concentrations *in vivo* than *in vitro*. This is probably due to the fact that *in vivo* acetylcholine is injected into the blood stream and will therefore be brought into closer proximity to its site of action. It must be appreciated however, that even under these circumstances the amount of acetylcholine reaching the biophase will be less than the dose given, due to the cholinesterase activity of the blood. Following the injection of $2 \cdot 10^{-6}$ g atropine the dose–response curve for acetylcholine was shifted to the right (Fig. 3) and this is consistent with competitive antagonism of acetylcholine by atropine. On the other hand, injection of 10^{-5} g of the cholinesterase inhibitor, neostigmine, caused an enhanced effect of acetylcholine and the dose–response curve was shifted to the left. The effect of neostigmine was observed at all doses of acetylcholine studied. The results obtained *in vivo* therefore confirm the physiological significance of those previously obtained *in vitro*.

Since acetylcholine is causing an alteration in active ion movement across the small intestine it is possible that ion transport by the intestinal epithelial cells may be influenced by the activity of the cholinergic division of the autonomic nervous system. The alteration in ion movement could be the result of a change in either the absorptive or secretory activity of the jejunum and further investigations are necessary to determine the exact nature of the changes in ion transport. It has been shown[2] that secretion of fluid by the small intestine is enhanced by vagal stimulation and

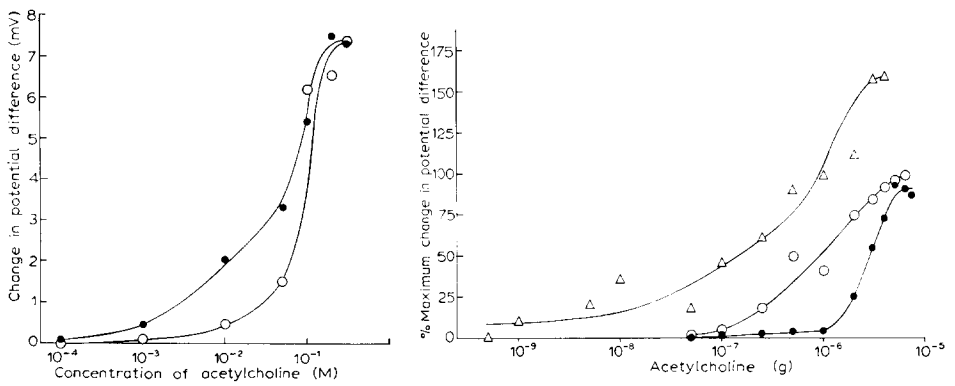

Fig. 2. Effect of 10 mM atropine sulphate in the serosal fluid on the change in the potential difference across everted sacs of rat jejunum caused by acetylcholine chloride. The change in potential difference in the absence (●) and presence (○) of atropine is plotted against log concentration and each point represents the mean of at least 4 determinations.

Fig. 3. Effect of acetylcholine chloride on the potential across rat jejunum *in vivo* (○). Drugs were injected into the jugular vein and washed in with 0.9% saline. The effects of 10^{-5} g neostigmine bromide (△) and $2 \cdot 10^{-6}$ g atropine sulphate (●) on the response to acetylcholine are shown. The change in potential difference is expressed as a percentage of the maximum change (3.8 ± 0.2 (10) mV) obtained under control conditions. This is plotted against log dose of acetylcholine.

administration of acetylcholine, while the presence of atropine is associated with an increased net absorption of Cl^- and water[3,4]. These findings are consistent with the concept that cholinergic activity can stimulate intestinal secretion.

It has been observed that there are cholinergic fibres in close proximity to the enterocytes[15] and the experiments described in this paper suggest a role for the acetylcholine released from these nerve endings in regulating the ionic transporting activity of intestinal epithelial cells.

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