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Cholinergic activation of Cl^- secretion in rat colonic epithelia

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Abstract

Acetylcholine receptor agonists and antagonists were used in a pharmacological analysis to identify which muscarinic receptor(s) may be involved in cholinergic regulation of Cl^- secretion across rat colonic mucosa in vitro. A comparative ligand binding analysis for each of the antagonists was carried out in parallel. Both studies elicited identical rank order potencies (atropine ≥ 4-diphenyl-acetoxy-*N*-piperidine methiodide (4-DAMP) > pirenzepine > 11-[[2[(diethylamino)methyl]-1-pipiridinyl]acetyl[-5,11-dihydro-6*H*-pyrido[2,3-*b*]]1,4]benzodiazepine-6-one (AF-DX 116). Cholinomimetic-induced Cl^- secretion was predominantly mediated by activation of muscarinic receptors in rat isolated colonic mucosa, with only a modest contribution from nicotinic receptors. Short circuit current responses evoked by the selective muscarinic M_1 receptor agonist 4-[[(3-chlorophenyl)amino]carbonyl]-*N,N,N*-trimethyl-2-butyn-1-aminium chloride (McN-A-343) suggest that this receptor subtype, which is thought to be neuronally sited, also plays a minor role in regulation of intestinal ion transport. The principal epithelial cell receptors responsible for acetylcholine receptor-mediated Cl^- secretion appear to belong to the M_3 class.

Keywords: Cholinergic activation; Colonic; Epithelia; Ion transport; Muscarinic receptor; [^{3}H]*N*-Methylscopolamine

1. Introduction

Mammalian intestinal electrolyte absorption and secretion may be regulated by extrinsic or intrinsic neurons which supply epithelial cells. Many candidate neurotransmitter substances may contribute to intestinal function in health and disease (Wood, 1991). Of these, however, the cholinergic system is certainly involved (Goyal, 1989). Ion transport studies have shown that cholinomimetics promote mammalian intestinal electrolyte secretion in vivo and in vitro (Browning et al., 1978; Tapper et al., 1978; Zimmerman and Binder, 1983; Kuwahara et al., 1987a).

Although there are acetylcholine-sensitive nerves which are involved in regulation of epithelial function (Kilbinger and Nafziger, 1985; Ren and Harty, 1994), there is little doubt that acetylcholine also acts directly upon epithelial cells since cholinomimetics stimulate Cl^- secretion across epithelial monolayers in vitro (Dharmsathaphorn and Pandol, 1986; Dickinson et al., 1992). Any direct action of cholinomometics on intestinal epithelial cells appears to be mediated via muscarinic receptors since binding studies have supplied no evidence of the existence of nicotinic receptors on epithelial cells (Rimele et al., 1981) and muscarinic receptors are expressed on epithelial cells of guinea pig intestine (Buckley and Burnstock, 1984). Ligand binding studies using rat colon (Rimele et al., 1981), and human HT29 (Kopp et al., 1989) and T84 (Dickinson et al., 1992) colonic carcinoma cells have demonstrated the presence of muscarinic receptors on epithelial cells.

The objective of this study was to investigate the potential role of the cholinergic system in regulation of ion transport in rat colonic mucosa. Two methods were employed: (i) pharmacological analysis of acetylcholine receptor agonists and antagonists on electrogenic ion transport in isolated rat colon and (ii) a parallel, comparative ligand binding analysis for each of the antagonists. Both studies elicited identical rank order potencies (atropine ≥ 4-diphenyl-acetoxy-*N*-piperidine methiodide (4-DAMP) > pirenzepine > AF-DX 116).

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2. Materials and methods

Acetylcholine chloride, atropine sulphate, carbachol, DMPP (dimethylphenylpiperazinium), gallamine triethiodide, hexamethonium, methacholine chloride, pirenzepine dihydrochloride, scopolomine hydrochloride and tetrodotoxin were obtained from the Sigma Chemical Co., Poole, UK. 11-[[2[(Diethylamino) methyl]-1-pipiridinyl]acetyl[-5,11-di-hydro-6*H*-pyrido-[2,3-*b*]]1,4]benzodiazepine-6-one (AF-DX 116) was a gift from Boehringer Ingelheim Zentrale, Germany. [^{3}H]*N*-Methylscopolamine was obtained from Du Pont (UK), Stevenage, UK. 4-Diphenyl-acetoxy-*N*-piperidine methiodide (4-DAMP) and 4-[[(3-chlorophenyl)amino]carbonyl]-*N*,*N*,*N*-trimethyl-2-butyn-1-aminium chloride (McN-A-343) were obtained from Research Biochemicals Int., Natick, MA, USA. All other chemicals were of analytical grade.

2.1. Ion transport

Mucosal sheets (epithelium and attendant lamina propria) obtained from male Wistar rats (150–200 g) were prepared by stripping the underlying smooth muscle from segments of distal colon. Mucosae were then mounted in Ussing chambers (window area 0.63 cm^2), bathed on either side with 10 ml of Krebs-Hensleit solution (composition (in mM) 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$ and 11.1 D-glucose) gassed with 95% O_2/5% CO_2 and maintained at 37°C. Tissues were continuously voltage clamped to zero potential difference by the application of short circuit current using a DVC-1000 (World Precision Instruments). Transepithelial resistance was determined by altering the membrane potential stepwise ($\pm$4 mV) and applying the Ohmic relationship. Adjacent tissues from a single animal were used to permit paired design of experiments. Drugs were added either to the apical or the basolateral bathing solution.

2.2. Ligand binding

Segments of rat distal colon prepared as above were placed in ice-cold assay buffer consisting of (in mM) 100 NaCl, 10 $MgCl_2$, 20 Hepes acid at pH 7.4. Tissues were homogenised, filtered through muslin and centrifuged at 1000 $\times g$ for 10 min at 4°C. The pellet was discarded and the supernatant re-centrifuged at 20 000 $\times g$ for 30 min at 4°C. The resulting pellet was resuspended in assay buffer and protein content determined using the method of Lowry (Lowry et al., 1951). Membrane preparations were stored at −70°C until required. Saturation binding assays were carried out by incubating 0.5 mg protein with tritiated *N*-methylscopolamine (0.0625–2.0 nM) for 2 h at 25°C. Non-specific binding was determined by displacement with an excess (1 μM) of atropine. Bound and free ligand were separated by filtration using a Brandel cell harvester with Whatman GF/B filters, followed by washing with ice-cold assay buffer. Radioactivity retained on the washed filters was measured by liquid scintillation spectroscopy (LKB WALLAC 1217 Rackbeta counter). Displacement binding assays were carried out using a range of concentrations of each unlabelled ligand in the presence of 0.3 nM [^{3}H]*N*-methylscopolamine for 2 h at 25°C.

2.3. Data analysis

All results are expressed as mean $\pm$ S.E.M. for n independent experiments.

For ion transport studies, results are expressed as peak short circuit current response (μA/cm^2). Since data conformed to normal distribution, paired or unpaired Student's t-tests were used to compare test and control data. When indicated, analysis of variance was used to compare full concentration-response curves.

In the binding studies, each experiment was carried out in duplicate. Equilibrium dissociation constant (K_D), maximum number of specific binding sites (B_{max}) of [^{3}H]*N*-methylscopolamine and inhibition constant (K_i) of displacing agents were determined using the iterative, non-linear curve fitting programme LIGAND (Munson and Rodbard, 1980). Data from displacement experiments were fitted to one- and two-site models; a two-site model was adopted when an F-test indicated a significantly ($P < 0.05$) improved fit. Correlations were determined by simple linear regression.

3. Results

3.1. Concentration-response curves to cholinomimetics

Basal (non-stimulated) short circuit current and electrical resistance values for voltage clamped rat colon were 19.7 $\pm$ 1.1 μA/cm^2 and 152 $\pm$ 11 $\Omega \cdot$ cm^2 respectively (n = 130). Addition of a number of acetylcholine receptor agonists to the basolateral side of isolated colonic mucosae produced an increase in short circuit current which was, in each case, related to drug concentration. Examples of full concentration-response curves are shown in Fig. 1. Maximum short circuit current responses to McN-A-343 and DMPP were modest when compared with those of acetylcholine, carbachol or methacholine. These data indicate that rat colonic ion transport, in response to cholinomimetics, is predominantly mediated by activation of muscarinic receptors which includes a minor contribution from muscarinic M_1 receptors. Nicotinic receptor activation appears to make a moderate contribution.

Acetylcholine may promote intestinal Cl^- secretion

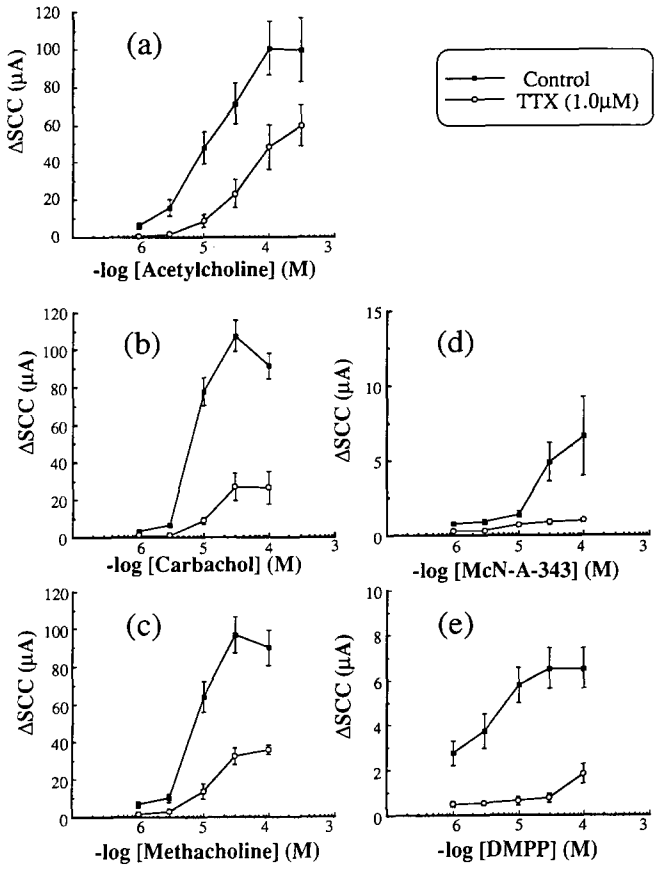

Fig. 1. Concentration-response curves to acetylcholine receptor agonists, (a) acetylcholine, (b) carbachol, (c) methacholine, (d) McN-A-343 and (e) DMPP, were constructed in the absence and presence of tetrodotoxin. Short circuit current responses in paired control and test tissue preparations were compared by analysis of variance (ANOVA). Tetrodotoxin (1 μM) reduced, but did not abolish, short circuit current responses to increasing concentrations (1–100, 300 μM) of acetylcholine ($P < 0.05$; $n = 9$), carbachol ($P < 0.005$; $n = 5$), methacholine ($P < 0.005$; $n = 6$), McN-A-343 ($P < 0.05$; $n = 6$) and DMPP ($P < 0.005$; $n = 6$). In each case, tetrodotoxin had no effect on short circuit current responses to prostaglandin E_2. These results suggest that acetylcholine receptor agonists induce ion transport responses by activating intrinsic neurons in addition to acting directly on epithelial cell receptors.

either by direct activation of epithelial cell receptors or indirectly via activation of nerves. Potential involvement of intrinsic neurons in cholinergic regulation of colonic ion transport was investigated using tetrodotoxin. Tetrodotoxin (1 μM) significantly reduced short circuit current responses to each of the acetylcholine receptor agonists tested (Fig. 1). Tetrodotoxin (1 μM) was without effect on the stimulation of short circuit current by other secretagogues. Responses to prostaglandin E_2 (10 μM) were 28.8 ± 4.4 μA in control preparations compared to 28.6 ± 3.9 μA ($n = 10$) in tetrodotoxin-treated rat colon; and responses to forskolin (10 μM) were 17.4 ± 6.0 μA in control preparations compared to 16.9 ± 2.9 μA ($n = 4$) in tetrodotoxin-treated rat colon.

3.2. Nature of charge carrying ion

A number of pharmacological agents which interfere with specific ion transport mechanisms were used to investigate basal and stimulated short circuit current in voltage clamped rat colon. Bumetanide (100 μM added basolaterally) significantly reduced basal short circuit current by 2.4 ± 0.5 μA ($P < 0.05$; $n = 6$) and also reduced short circuit current responses to 100 μM carbachol by $84.1 \pm 4.4\%$ from 61.2 ± 19.3 μA in control preparations to 9.0 ± 1.8 μA in the presence of bumetanide ($P < 0.05$; $n = 6$). Amiloride (100 μM added apically) or acetazolamide (100 μM added both apically and basolaterally) were without effect on short circuit current responses to carbachol. In Cl^--free conditions, basal short circuit current was reduced from 22.0 ± 3.1 μA to 4.9 ± 0.8 μA ($P < 0.005$; $n = 6$) and short circuit current responses to carbachol (1–100 μM) were virtually abolished.

3.3. Characterisation of cholinergic receptors mediating colonic ion transport

(i) Nicotinic receptors: The nicotinic receptor agonist DMPP stimulated short circuit current in a concentration dependent manner (Fig. 1) although the maximum change in short circuit current was much smaller than that obtained in response to muscarinic receptor agonists. Responses to a single concentration of DMPP (10 μM) were reduced from 6.0 ± 1.5 μA in

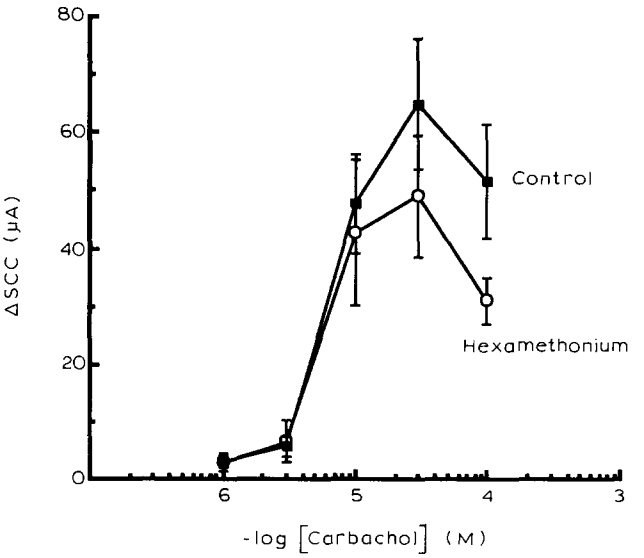

Fig. 2. Hexamethonium (1 μM) had no effect on short circuit current responses evoked by low concentrations of the non-selective acetylcholine receptor agonist carbachol (1–30 μM; $n = 6$); however, short circuit current responses to 100 μM carbachol were reduced by $39.1 \pm 8.6\%$ ($P < 0.05$; $n = 6$). These results suggest that at low concentrations (< 30 μM), carbachol acts predominantly as a muscarinic receptor agonist, while at high concentrations (> 30 μM) it also exhibits nicotinic properties.

Table 1
pA_2 values for different antagonists

Drug	pA_2	Slope	n
Atropine	8.84 (8.31–9.36)	0.77	6
4-DAMP	8.63 (8.11–8.94)	0.83	5
Pirenzepine	7.28 (6.58–7.78)	0.95	5
AF-DX 116	5.51 (5.17–5.96)	0.97	5

SCC responses to carbachol were examined in the presence of increasing concentrations of specific muscarinic antagonists. pA_2 values were estimated for each antagonist from individual Schild plots by the method of Arunlakshana and Schild (1958). In each case, tissues were bathed in Krebs-Henseleit solution containing tetrodotoxin (0.1 μM) to remove any involvement of intrinsic neurons.

control preparations to 0.6 ± 0.5 μA in the presence of the nicotinic receptor antagonist hexamethonium (1 μM), which had no effect on basal short circuit current. Short circuit current responses to low concentrations of carbachol (1–30 μM) were unaffected by hexamethonium. However, ion transport responses to 100 μM carbachol were significantly attenuated in the presence of 1 μM hexamethonium (Fig. 2). These results suggest that at low concentrations, carbachol acts predominantly via muscarinic receptors and at high concentrations exerts a nicotinic mediated effect.

(ii) Muscarinic receptors: From results in the previous section and the data in Fig. 1 it is clear that cholinomimetics stimulate ion transport in rat colon predominantly via muscarinic receptors. Atropine (1 μM) almost abolished effects of carbachol (100 μM), reducing the short circuit current responses by $93.0 \pm 10.0\%$ from 92.8 ± 10.9 μA in control preparations to 5.0 ± 3.0 μA in the presence of atropine ($P < 0.005$; $n = 6$).

A series of muscarinic receptor antagonists was used to quantitatively investigate colonic short circuit current responses to carbachol. pA_2 values were estimated for each antagonist from individual Schild plots by the method of Arunlakshana and Schild (1958). In each case, four concentrations of antagonist were used. The antagonists were atropine (non-selective), pirenzepine (M_1), AF-DX 116 (M_2) and 4-DAMP (M_3). For these experiments, tissues were bathed in Krebs-Henseleit solution containing tetrodotoxin (0.1 μM) to prevent any involvement of intrinsic neurons. Results are shown in Table 1. Gallamine (up to 10 μM; a non-competitive M_2 antagonist) was without effect on carbachol-evoked short circuit current responses. From estimated pA_2 values, the rank order of potency of antagonists was atropine $\geq$ 4-DAMP > pirenzepine > AF-DX 116.

Table 2
pK_i values for different ligands

Drug	K_i (nM)	pK_i ($-\log K_i$)
Scopolamine	0.32 ± 0.11	9.64 (9.25–10.09)
Atropine	0.87 ± 0.12	9.08 (8.90– 9.18)
4-DAMP	5.20 ± 0.47	8.29 (8.17– 8.36)
Pirenzepine	155 ± 33	6.87 (6.69– 7.21)
AF-DX 116	2189 ± 655	5.74 (5.37– 5.98)

Muscarinic receptor antagonists were examined for their ability to inhibit [^{3}H]*N*-methylscopolamine binding to rat colonic membranes. K_i (nM) values were calculated from individual competition curves using the iterative, non-linear curve-fitting program LIGAND (Munson and Rodbard, 1980). Results are mean ± S.E.M. of 4 experiments carried out in duplicate. The range of results is given in parentheses.

3.4. Radioligand binding

Membranes prepared from rat colonic mucosa were used in binding experiments using a range of concentrations of [^{3}H]*N*-methylscopolamine (0.0625–2.0 nM). Specific binding was calculated by subtracting non-specific binding which occurred in the presence of an excess of atropine (1 μM) from the total binding. Iterative curve fitting of data indicated the presence of a single population of muscarinic receptors with a calculated K_D of 0.27 ± 0.06 nM and a B_{max} of 153.5 ± 19.6 fmol/mg protein ($n = 5$; Scatchard slope = 0.92).

In displacement assays, a number of muscarinic receptor antagonists were examined for their capacity

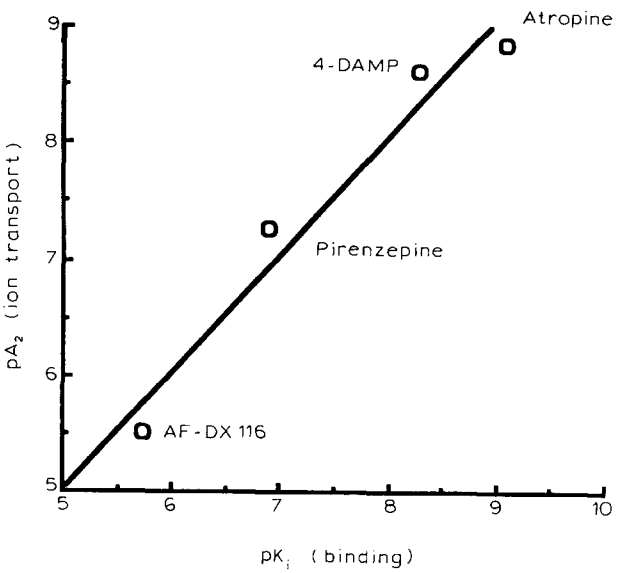

Fig. 3. pA_2 values were calculated from individual Schild plots constructed from ion transport studies, during which short circuit current responses to carbachol were examined in the presence of increasing concentrations of muscarinic receptor antagonists. pK_i values were calculated from individual competition curves constructed from ligand binding studies involving displacement of [^{3}H]*N*-methylscopolamine from colonic membrane receptors by increasing concentrations of muscarinic receptor antagonists. Although ion transport studies accounted for neuronal involvement (0.1 μM tetrodotoxin present throughout), there was a good correlation (coefficient of correlation $r = 0.97$) between pA_2 and pK_i values.

Table 3
Comparison of pK_i values

	Rat colon	T84 cells	HT-29 cells	Frog peptic cells	Rat pancreas	CHO cells (human m_3)
Scopolamine	9.6 (9.3–10.1)	–	–	–	–	–
Atropine	9.1 (8.9– 9.2)	9.3	9.1	9.3	–	9.8
4-DAMP	8.3 (8.2– 8.4)	9.1	–	9.0	9.1	–
Pirenzepine	6.9 (6.7– 7.2)	6.9	6.6	7.2	6.9	6.0
AF-DX 116	5.7 (5.4– 6.0)	5.4	5.1	6.2	5.8	6.1
r value	–	0.97	1.00	0.99	0.99	0.93

pK_i values (range shown in parentheses) obtained in rat colonic membrane preparations are compared with published values obtained in human T84 (Dickinson et al., 1992) and HT-29 (Kopp et al., 1989) colon carcinoma cells, frog peptic cells (Dickinson et al., 1988), rat pancreas (Waelbroeck et al., 1987) and Chinese hamster ovary (CHO) cells transfected with the human muscarinic m_3 receptor gene (Buckley et al., 1989). Comparison of pK_i values gave the indicated coefficients of correlation (*r*).

to displace the binding of [^{3}H]*N*-methylscopolamine (0.3 nM) from rat colonic membranes. Table 2 shows the K_i (nM) and pK_i values. From estimated K_i and pK_i values, the antagonist rank order of potency was scopolamine > atropine > 4-DAMP > pirenzepine > AF-DX 116.

3.5. Comparison of ion transport and binding data

From the previous results it is clear that the rank order of potency of antagonists for inhibition of short circuit current responses and radioligand binding was identical. A correlation coefficient of 0.97 was obtained when pA_2 and pK_i values were compared by simple linear regression (Fig. 3). Antagonist pK_i values, estimated using rat colonic membrane preparations, were also compared with previously published values using other tissues. The results (Table 3) show that there was good correlation between different tissue pK_i values and the rank order of antagonist potency in each case. Collectively, the results of our experiments using agonists and antagonists suggest that muscarinic receptors present on rat colonic epithelial cells are of the M_3 subtype.

4. Discussion

This study investigated the role of the cholinergic system in regulation of intestinal ion transport in rat colonic mucosa. Non-selective (carbachol), muscarinic (methacholine) and nicotinic (DMPP) receptor agonists each stimulated inward short circuit current responses. In our experiments, the change in short circuit current evoked by carbachol was reduced by bumetanide, a loop diuretic which inhibits the Na/K/Cl co-transporter necessary for electrogenic Cl^- secretion. Amiloride, which inhibits sodium absorption, and acetazolamide, which interferes with bicarbonate secretion by inhibition of carbonic anhydrase, were without effect upon carbachol-stimulated ion transport. These results, which suggest that chloride ions are the primary charge carrying species were confirmed by abolition of carbachol induced change in short circuit current in Cl^--free solutions. These data agree with the findings of others that Cl^- secretion in response to cholinomimetics is a feature in rat colon (Zimmerman and Binder, 1983), guinea pig colon (Kuwahara et al., 1987a,b) and T84 cells (Dharmsathaphorn and Pandol, 1986).

Stimulation of electrogenic ion transport by cholinomimetics was mediated principally by muscarinic receptors. There does, however, appear to be some nicotinic involvement. The nicotinic receptor agonist DMPP evoked a hexamethonium-sensitive increase in short circuit current which was abolished by tetrodotoxin (Tapper and Lewand, 1981) and the effects of carbachol, which, at high concentrations, acts at nicotinic as well as muscarinic receptors (Tapper et al., 1978) was partly sensitive to hexamethonium. Considering the information in Figs. 1 and 2, if effects of activation of different subtypes of cholinergic receptors are additive, then comparison of the absolute change in short circuit current values suggests that not more than 30% of cholinergic regulation of rat colonic ion transport is mediated by nicotinic receptors (accounted for by DMPP). Nicotinic receptors appear to be located primarily on intrinsic neurons (Buckley and Burnstock, 1984; Frieling et al., 1991). The remaining 70–75% of the short circuit current response which is mediated by muscarinic receptors (accounted for by methacholine) are present on neurons and epithelial cells (Rimele et al., 1981; Buckley and Burnstock, 1984; Kilbinger and Nafziger, 1985; Surprenant, 1986; Kachur et al., 1990; Dickinson et al., 1992). The M_1 selective muscarinic receptor agonist McN-A-343 produced a relatively small maximal change in short circuit current suggesting that muscarinic M_1 receptors make a minor contribution to cholinergic regulation of ion transport. It appears that muscarinic M_1 receptors are sited neu-

ronally (Surprenant, 1986; Przyborski et al., 1990, 1991) and function as an excitatory receptor (Kilbinger and Nafziger, 1985; Frieling et al., 1991) and this is supported by our data.

Use of acetylcholine receptor agonists enabled a preliminary investigation of the contribution(s) of nicotinic and muscarinic receptors to cholinergic regulation of intestinal electrolyte transport. However, further characterisation of muscarinic receptors by agonist studies is hindered by a lack of suitably selective compounds.

The direct actions of drugs on epithelial cholinergic receptors were studied by subtracting neuronal component(s) by carrying out ion transport experiments in the presence of tetrodotoxin. We confirmed that tetrodotoxin was without effect on directly acting secretagogues (O'Malley et al., 1993). pA_2 values calculated for each muscarinic receptor antagonist, as an estimation of potency to inhibit carbachol-evoked Cl^- secretion and thus an estimation of affinity for epithelial cell muscarinic receptors, showed the non-selective muscarinic receptor antagonist atropine and the muscarinic M_3 receptor antagonist 4-DAMP to display relatively high affinities for rat colonic epithelial receptors. Similar antagonist potencies for 4-DAMP and another muscarinic M_3 receptor antagonist, HHsiD, have been reported using other mammalian intestinal preparations (Kilbinger and Nafziger, 1985; Surprenant, 1986; Kachur et al., 1990). The muscarinic M_1 receptor antagonist pirenzepine displayed relatively low affinity in our functional assay, in common with similar values reported using guinea pig ileal smooth muscle and ileal mucosa (Kachur et al., 1990). Separate studies recording synaptic potentials in guinea pig intestine (Surprenant, 1986; Frieling et al., 1991) and acetylcholine release from guinea pig ileum myenteric plexus (Kilbinger and Nafziger, 1985) indicate that functional muscarinic M_1 receptors are located principally on neurons.

AF-DX 116, a muscarinic M_2 receptor antagonist (Micheletti et al., 1987) was only a weak antagonist of carbachol-evoked ion transport in rat colon, and in other non-cardiac tissue preparations such as guinea pig ileal smooth muscle and mucosa (Kachur et al., 1990). Gallamine, a non-competitive muscarinic M_2 receptor antagonist, had no effect on the short circuit current response to carbachol. These results taken together indicate that stimulation of Cl^- secretion in rat colon by cholinomimetics is mediated chiefly by receptors of the M_3 subtype. Muscarinic-receptor activation of epithelial Cl^- secretion in human colonic-derived cell lines appears to be mediated by elevation of intracellular Ca^{2+} (Kopp et al., 1989; Dickinson et al., 1992) although cAMP may also contribute to the overall response (MacVinish et al., 1993; Kachintorn et al., 1993).

Ligand binding studies were caried out in parallel using the same reagents to displace [^{3}H]*N*-methylscopolamine from rat colonic mucosal membranes. Although this preparation may be contaminated with neural elements and blood cells, they are unlikely to contribute more than a few per cent to the total tissue mass (Isaacs et al., 1982); therefore it seems reasonable to assume that ligand binding studies on mucosal membrane preparations reflect binding primarily to receptors from epithelial cells.

pK_i ($-$ log molar concentration required to inhibit [^{3}H]*N*-methylscopolamine binding by 50%) values estimated for each ligand reflect their potency to inhibit [^{3}H]*N*-methylscopolamine binding and thus reflect receptor affinity for each antagonist. [^{3}H]*N*-Methylscopolamine labelled a single population of high affinity binding sites on rat colonic membranes, which exhibited high affinity for the non-selective muscarinic receptor antagonists atropine and scopolamine and low affinity for the muscarinic M_1 receptor antagonist pirenzepine. The M_2 selective antagonist AF-DX 116 was the weakest inhibitor of [^{3}H]*N*-methylscopolamine binding. In contrast, 4-DAMP, the muscarinic M_3 receptor antagonist which showed potent inhibitory activity in binding studies performed on guinea pig oesophageal muscularis mucosae membrane preparations (Eglen and Whiting, 1988) and human-derived colonic cell lines, HT-29 (Kopp et al., 1989) and T84 (Dickinson et al., 1992), was the most potent antagonist found in our study.

A good correlation was observed between pA_2 and pK_i values estimated using electrophysiological and ligand-binding techniques. A series of correlations was observed when pK_i values from our binding study were compared with published values for the same antagonists obtained with membrane preparations from other tissues, all of which exhibited similar pharmacological profiles. Correlation with results obtained from experiments reported with the cloned human muscarinic m_3 receptor, stably expressed in CHO cells (Buckley et al., 1989) was less than for native muscarinic receptors with the pK_i value estimated for AF-DX 116 being slightly greater than that for pirenzepine. It has been suggested that the cellular environment in which these cloned receptors are placed may be responsible for the small differences in antagonist potencies.

In conclusion, cholinomimetic-induced Cl^- secretion is predominantly mediated by activation of muscarinic receptors in rat isolated colonic mucosa, with only a modest contribution from nicotinic receptors. Short circuit current responses evoked by the selective muscarinic M_1 receptor agonist McN-A-343 suggest that this receptor subtype, which is thought to be neuronally sited, also plays a minor role in cholinergic regulation of intestinal ion transport. Using a number

of subtype-selective antagonists in ion transport and ligand binding studies, we have found that epithelial cell receptors responsible for acetylcholine receptor-mediated Cl^- secretion are of the M_3 class.

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