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Imidazolines inhibit secretory responses of rat colonic mucosa to calcium-dependent but not cyclic AMP-dependent secretagogues

Malcolm Anderson and David Burleigh

Abstract

The purpose of this study was to investigate whether imidazolines have an anti-secretory action on intestinal epithelial cells. Muscle-stripped preparations of rat colon and monolayers of T_{84} human colonic epithelial cells were set up in Ussing chambers for measurement of short-circuit current. In rat colon acetylcholine, histamine, vasoactive intestinal polypeptide and forskolin elicited secretory responses which were recorded as increases in short-circuit current. Secretory responses to acetylcholine were inhibited in a concentration-dependent manner by the imidazolines phentolamine, idazoxan and clonidine. The effect of clonidine was not reversed by pre-incubation of mucosal preparations with yohimbine. Secretory responses to vasoactive intestinal polypeptide were unaffected by the three imidazolines. Phentolamine reduced responses of colonic mucosa to histamine but had no effect on responses to forskolin. Responses to vasoactive intestinal polypeptide and forskolin were significantly reduced in the presence of barium. In T_{84} cell monolayers phentolamine significantly reduced responses to acetylcholine. Three imidazolines, two with alpha-adrenoceptor-antagonist properties and one with alpha-agonist properties, have anti-secretory effects in rat colonic mucosal preparations. The anti-secretory action appears to discriminate between calcium-dependent and cyclic AMP-dependent secretagogues, inhibiting the former but not the latter.

Introduction

Preliminary investigations have shown that the alpha-adrenoceptor antagonist phentolamine, an imidazoline, reduces secretory responses of rat colonic mucosa to acetylcholine (Darko et al 1997). This action probably results from inhibition of ATP-regulated potassium (K_{ATP}) channels (Dunne 1991) rather than antagonism of alpha adrenoceptors as it is generally considered that adrenergic nerves are pro-absorptive (Gaginella 1984). Furthermore, inhibition of potassium channels reduces secretion of chloride ions into the intestinal lumen (Hardcastle & Hardcastle 1987; Dawson & Richards 1990) thus producing anti-secretory effects (Sandle et al 1994) and K_{ATP} channels are present in the basolateral membrane of colonic epithelial cells (McNamara et al 1999). It has become apparent that, in general, compounds possessing a 2-substituted imidazoline group are capable of inhibiting K_{ATP} channels (Edwards & Weston 1993). Despite this, clonidine, a 2-substituted imidazoline and moxonidine, an imidazoline I_1/alpha$_2$ receptor agonist, have had their anti-secretory, pro-absorptive actions ascribed entirely to stimulation of alpha$_2$ adrenoceptors (Dharmsathaphorn 1986; Liu & Cooper 1997). Thus it is not

Molecular Pharmacology Section, Division of Biomedical Sciences, Queen Mary and Westfield College, University of London, London E1 4NS, UK

Malcolm Anderson, David Burleigh
Correspondence: D. E. Burleigh, Molecular Pharmacology Section, Division of Biomedical Sciences, Queen Mary and Westfield College, University of London, London E1 4NS, UK.

clear whether activation of imidazoline sites per-se results in an anti-secretory effect in the intestinal tract. Although there is debate about the diversity, tissue distribution and physiology of imidazoline sites (Eglen et al 1998), a putative I_3 site has been proposed to promote insulin secretion, possibly via inhibition of K_{ATP} channels (Chan et al 1994). In this study we have investigated the role such a site may play in modulating intestinal secretion by assessing the anti-secretory effects of three imidazoline compounds, phentolamine, idazoxan and clonidine, on secretory responses to calcium and cyclic AMP-dependent secretagogues.

Materials and Methods

Drugs

The drugs used were: acetylcholine chloride, barium chloride, clonidine hydrochloride, dimethylsulfoxide (DMSO), forskolin, histamine dihydrochloride, idazoxan hydrochloride, phentolamine hydrochloride, vasoactive intestinal polypeptide and yohimbine hydrochloride (all from Sigma). All other chemicals were of analytical grade. Aqueous stock solutions were prepared for all drugs except forskolin which was made up in DMSO (final bath concentration $\leqslant$ 0.1 % v/v). Krebs solution contained (mmol L^{-1}): NaCl 118, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.5.

Tissue preparation

Male Wistar rats (250–350 g) were killed by cervical dislocation and the large intestine removed. Taking care to exclude the proximal striated portion and distal lymph node (located at the pelvic brim), four to five muscle-stripped preparations could be obtained from each animal. Previous histological examination confirmed the presence of mucosa, muscularis mucosa and submucosa (Izmeth et al 1994). Although there is evidence showing regional heterogeneity in the rat colon (From & Hegel 1978; Hardcastle et al 1985; Nobles et al 1991), there are other instances where this does not appear to be the case (Hardcastle et al 1985; Burleigh & Kirkham 1993). As a precautionary measure the origin of each preparation was noted so that drug treatments were not restricted to a particular region. Preparations were mounted in Ussing chambers (0.64 cm^2 window area) containing modified Krebs solution at 37°C and gassed with 5 % CO_2 in oxygen. Each side of the membrane was bathed with 10 mL of fluid and drugs were added to the serosal (basolateral) side.

Cell preparation

T_{84} cells, a human colonic epithelial cell line, were obtained from the European Collection of Cell Cultures (ECACC). The methods for culturing the cells have been described previously (Burleigh et al 2000). Briefly, cells were grown as monolayers in a mixture of DMEM and Ham's F-12 media supplemented with fetal bovine serum, L-glutamine, penicillin and streptomycin. Following trypsinization, cells were seeded onto collagenized semi-permeable membranes (Costar Snapwell inserts, 12 mm diameter). After 9 or 11 days the insert with attached monolayer was placed into a modified Ussing chamber for continuous recording of short-circuit current.

Measurement of secretory response

Mucosal preparations and cell monolayers were clamped at zero potential by means of a high-impedance voltage clamp (DVC-1000, World Precision Instruments) and the transmucosal short-circuit current was measured and continuously recorded. At 5-min intervals the tissue was clamped at 2 mV for a period of 20 s. The resulting change in short-circuit current, together with the step in potential difference, was used to calculate the conductance of the tissue using Ohm's Law. Both current passing and voltage-detecting electrodes used a system of Ag/AgCl half-cells connected to large diameter agar bridges (4 % agar in modified Krebs solution minus Ca^{2+} and glucose). Compensation was made for fluid resistance between the tips of the voltage-detecting electrodes. All electrical values are quoted for an exposed membrane area of 1 cm^2.

Following 60 min equilibration, submaximal responses were obtained to acetylcholine, vasoactive intestinal polypeptide or forskolin. After washing out the secretagogue and allowing short-circuit current to return to baseline, control vehicle (0.1 mL H_2O or 0.01 mL DMSO for forskolin) or a single concentration of imidazoline was added for a 30-min contact time before exposing the tissue for a second time to the secretagogue. Because of desensitization, each preparation only received a single submaximal concentration of histamine given either in the absence or presence of imidazoline, or control vehicle. When preparations were exposed to a combination of clonidine plus yohimbine, yohimbine was given 10 min before clonidine. Contact times for agonists allowed peak responses to be achieved.

Statistical analysis

Statistical analysis was carried out using the Wilcoxon matched-pairs signed-ranks test and Mann-Whitney U test for paired and unpaired data, respectively. In both cases $P < 0.05$ was considered to represent a significant difference. Values quoted are mean $\pm$ s.e.m. and n equals number of animals or monolayers.

Results

After 60 min equilibration, the basal short-circuit current was $17 \pm 3\ \mu A\ cm^{-2}$ and tissue resistance was $111 \pm 27\ \Omega\ cm^{-2}$ (n = 29) for rat colon. Equivalent, respective, values for T_{84} cell monolayers were $1.8 \pm 0.5\ \mu A\ cm^{-2}$ and $487 \pm 44\ \Omega\ cm^{-2}$ (n = 12) after a 30-min equilibration period.

Serosal application of acetylcholine, histamine, vasoactive intestinal polypeptide and forskolin gave concentration-dependent increases in short-circuit current (Figure 1). Effects of imidazolines were assessed against the following concentrations of agonists: acetylcholine (1.4×10^{-5} M), vasoactive intestinal polypeptide (3.3×10^{-8} M), histamine (5×10^{-5} M) and forskolin (1.2×10^{-6} M), which were selected to produce submaximal increases in short-circuit current.

Imidazolines produced a concentration-dependent inhibition of responses to acetylcholine (Figure 2). Yohimbine did not inhibit the anti-secretory actions of clonidine (n = 5, $P > 0.05$). In the presence of yohimbine (10^{-5} M), clonidine (10^{-4} M) inhibited short-circuit current responses to acetylcholine (1.4×10^{-5} M) by $72 \pm 7\%$ (n = 5, $P < 0.05$). In the absence of yohimbine, clonidine (10^{-4} M) inhibited short-circuit current responses to acetylcholine (1.4×10^{-5} M) by $65 \pm 3\%$ (n = 5, $P < 0.05$). Clonidine had no significant effect on

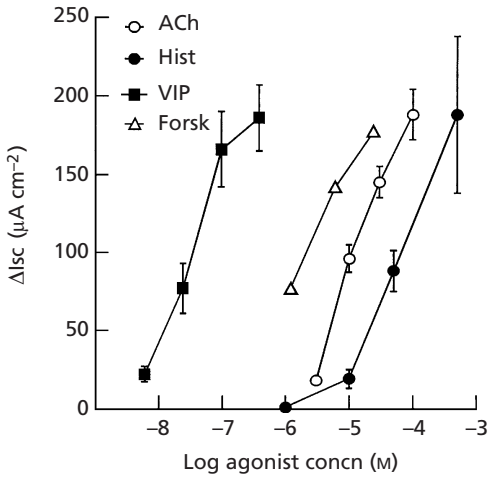

Figure 1 Concentration-dependent secretory effects of acetylcholine (ACh), histamine (Hist), vasoactive intestinal peptide (VIP) and forskolin (Forsk) on rat colonic mucosal preparations. Secretory effects were measured as an increase in short-circuit current (△Isc). Each value represents mean $\pm$ s.e.m. of results from at least four experiments, except for forskolin (n = 2).

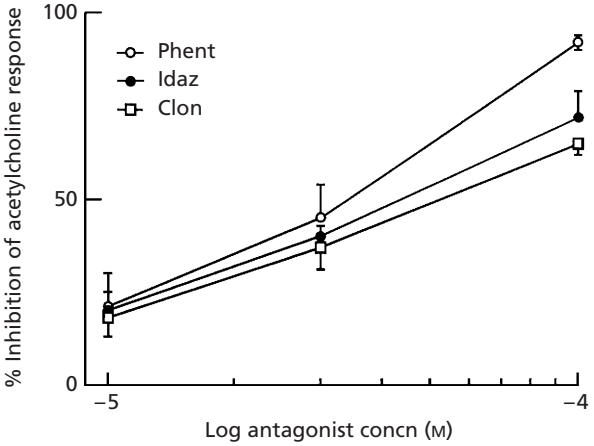

Figure 2 Concentration-dependent inhibition by phentolamine (Phent), idazoxan (Idaz) and clonidine (Clon) of short-circuit current (Isc) responses of rat colonic mucosa to acetylcholine (1.4×10^{-5} M). Each value is the mean $\pm$ s.e.m. of results from five or more experiments.

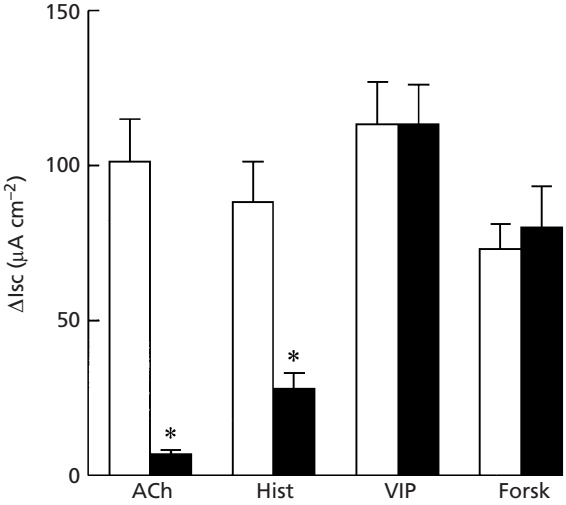

Figure 3 Secretory responses of colonic mucosa to acetylcholine (ACh, 1.4×10^{-5} M) histamine (Hist, 5×10^{-5} M), vasoactive intestinal polypeptide (VIP, 3.3×10^{-8} M) and forskolin (Forsk, 1.2×10^{-6} M) in the absence (open columns) or presence (solid columns) of phentolamine (10^{-4} M). Each value represents the mean $\pm$ s.e.m. increase in short-circuit current (△Isc) from five or more experiments. *$P < 0.05$, compared with responses in absence of phentolamine.

basal short-circuit current of rat colonic mucosal preparations. After 30 min the basal short-circuit current had decreased by 2 ± 2 μA cm^{-2} in the presence of clonidine (10^{-4} M, n = 9) and by 2 ± 1 μA cm^{-2} in the presence of control vehicle (0.1 mL H_2O, n = 7, $P > 0.05$).

Increases in short-circuit current to vasoactive intestinal peptide (3.3×10^{-8} M) were not inhibited by phentolamine (10^{-4} M, n = 5, $P > 0.05$; Figure 3), idazoxan (10^{-4} M, n = 4, $P > 0.05$) or clonidine (10^{-4} M, n = 4, $P > 0.05$) but were reduced by 91 ± 5% by barium (5×10^{-3} M, n = 5, $P < 0.05$).

The anti-secretory action of phentolamine (10^{-4} M) was investigated further by determining its effect on secretory responses to the calcium-dependent secretagogue histamine (5×10^{-5} M) and the cyclic AMP-dependent secretagogue forskolin (1.2×10^{-6} M). Responses to histamine were reduced and those to forskolin were unaffected (Figure 3). Responses to forskolin were reduced by 75 ± 7% by barium (5×10^{-3} M, n = 4, $P < 0.05$).

Finally, short-circuit current responses of T_{84} cell monolayers to acetylcholine (1.4×10^{-5} M) were reduced from 7.5 ± 0.3 μA cm^{-2} (n = 6) to 2.5 ± 0.4 μA cm^{-2} (n = 6, $P < 0.05$) in the presence of phentolamine (10^{-4} M).

Discussion

In secretory diarrhoea the primary event driving fluid secretion is a transcellular, serosal-to-mucosal electrogenic transport of chloride ions. The electrical driving force for such transport requires the maintenance of an electrically negative cell membrane voltage. This is achieved through a basolateral outward leakage of potassium ions (Hardcastle & Hardcastle 1987; Dawson & Richards 1990). It has previously been shown that inhibition of ATP-activated potassium (K_{ATP}) channels by glibenclamide and phentolamine produces anti-secretory effects in the rat colon (Darko et al 1997). The aim of this study was to determine whether the anti-secretory actions of phentolamine are related to its imidazoline structure, as imidazolines inhibit K_{ATP} channels (Edwards & Weston 1993) and thereby promote insulin release (Chan et al 1994).

Both calcium-dependent (acetylcholine and histamine) and cAMP-dependent (vasoactive intestinal polypeptide and forskolin) secretagogues produce increases in short-circuit current of rat colonic mucosal preparations. Secretory responses to acetylcholine were reduced in a concentration-dependent manner by three imidazolines: two with alpha-adrenoceptor antagonist properties (phentolamine and idazoxan) and one with alpha-adrenoceptor agonist properties (clonidine). Secretory responses of T_{84} human colonic epithelial cell monolayers to acetylcholine were also inhibited by phentolamine. T_{84} monolayers are independent of hormonal and neuronal influences thus indicating that the anti-secretory effect of phentolamine occurs at the level of the epithelial cell.

The anti-secretory effects of clonidine are well established and have been ascribed solely to stimulation of alpha adrenoceptors leading to increased intestinal transit time and an increase in net fluid absorption (Chang et al 1982; Dharmsathaphorn 1986). However the insensitivity to yohimbine indicates that clonidine possesses additional actions which contribute towards its anti-secretory effects. In rabbit ileum clonidine produced a concentration-dependent, yohimbine sensitive, decrease in basal short-circuit current (Chang et al 1982). In rat colon, the basal short-circuit current was low (Darko et al 1997) compared with rabbit ileum and this may explain why clonidine did not cause further decrease.

Secretory responses to vasoactive intestinal polypeptide were unaffected by the three imidazolines. Phentolamine was also without effect against responses to forskolin although it inhibited responses to histamine. The anti-secretory action of imidazolines appears to be selective for calcium-dependent secretagogues. Responses to vasoactive intestinal polypeptide and forskolin were reduced by the non-selective potassium-channel blocker barium indicating the general requirement, by secretagogues, of functioning basolateral potassium channels. This selectivity of action of imidazolines can be explained by the observation that basolateral potassium channels represent a heterogeneous population. For instance, chromanol derivatives have been shown to inhibit potassium channels associated with cAMP- but not calcium-dependent chloride secretion (Lohrmann et al 1995). Furthermore, in rat colon acetylcholine-induced chloride secretion is associated with activation of a 16-ps calcium-dependent channel while cAMP-dependent secretagogues activate a small conductance basolateral potassium channel while simultaneously inhibiting the calcium-dependent channel (Greger et al 1997).

In a recent review of imidazoline sites (Eglen et al 1998) the following potency order was proposed for binding at I_1 and I_2 sites: I_1 – phentolamine > clonidine > idazoxan; I_2 – idazoxan > clonidine > phentolamine. For both sites the potency difference between phentolamine and idazoxan was reported to be greater than two

orders of magnitude. Clearly, no such discrimination exists between the anti-secretory effects of these three compounds in rat colonic mucosa. This may indicate that they are acting at a putative imidazoline I_3 site, especially as there is good evidence that such a site modulates the opening of K_{ATP} channels (Chan et al 1994; Eglen et al 1998).

In conclusion, the results show that two imidazolines with alpha-adrenoceptor antagonist properties (phentolamine and idazoxan) and one with alpha-adrenoceptor agonist properties (clonidine) all produce anti-secretory effects in muscle-stripped rat colon. The anti-secretory effects are selective in that calcium-dependent secretagogues are inhibited while those compounds utilizing cAMP as a second messenger are unaffected.

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