J. Pharm. Pharmacol. 1997, 49: 985–987
Received April 17, 1997
Accepted July 11, 1997

Inhibition of Potassium (K_{ATP}) Channels Reduces the Short-circuit Current Response of Rat Colonic Mucosa to Acetylcholine

S. K. A. DARKO, N. D. PALMERLEY AND D. E. BURLEIGH

Department of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK

Abstract

Intestinal secretion depends upon electrogenic chloride transport into the gut lumen, which requires maintenance of an electrically negative cell-membrane voltage. We have investigated whether secretory responses of rat colonic mucosa to acetylcholine were sensitive to inhibition of potassium channels and whether selective inhibition could indicate the nature of the channel involved.

Rat colonic mucosa was set up in Ussing chambers, short-circuit current responses obtained to acetylcholine, and the sensitivity of such responses to inhibition of potassium channels was investigated. Non-selective potassium-channel blockade by barium induced concentration-dependent inhibition of responses to acetylcholine. Similar inhibitory effects were obtained using 4-aminopyridine and glibenclamide. 5-Hydroxydecanoate and phentolamine also inhibited the increase in short-circuit current. However, a combination of charybdotoxin plus apamin was without effect.

We conclude that a basolateral outward movement of potassium ions is required for the secretory action of acetylcholine on rat colonic mucosa. The potassium channel involved seems to be ATP-dependent and calcium-insensitive.

Intestinal secretion depends upon electrogenic chloride transport into the gut lumen; such transport requires the maintenance of an electrically negative cell membrane voltage. It has been suggested that this is achieved by basolateral outward movement of potassium ions (Dawson & Richards 1990). Preliminary evidence for a secretagogue-activated basolateral potassium channel in mammalian intestinal epithelial cells (enterocytes) came initially from work on a cultured colonic epithelial cell line—T_{84} cells (Dharmsathaphorn & Pandol 1986) and intact mucosal sheets of rat ileum (Hardcastle & Hardcastle 1986). Direct evidence of potassium channels on basolateral membranes of colonic enterocytes has been obtained from patch-clamping T_{84} cells (Devor & Frizzell 1993) and isolated colonic intestinal crypts from rabbit, rat and man (Loo & Kaunitz 1989; Burckhardt & Gögelein 1992; Lomax et al 1996). In the colon in man the potassium channel associated with chloride secretion appears to be a cyclic AMP- and calcium-activated low-conductance channel (23 pS) which is also activated by hyperpolarization (Lomax et al 1996). In contrast the predominant channel found in basolateral membranes of rabbit distal colonic crypt enterocytes is high-conductance (130 pS) and activated by depolarization (Loo & Kaunitz 1989). In the rat the dominant channel was low-conductance (12 pS) and thought to be responsible for maintaining the membrane resting potential under unstimulated conditions (Burckhardt & Gögelein 1992). The aim of the present investigation was to determine whether secretory responses of rat colonic mucosa to acetylcholine were sensitive to inhibition of potassium channels and whether selective inhibition could indicate the nature of the channel involved. A preliminary account of this investigation has been reported to the British Pharmacological Society (Darko et al 1997).

Correspondence: D. E. Burleigh, Department of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK.

Materials and Methods

Muscle-stripped preparations of rat (Wistar, male, 200–300 g) large intestine (excluding striated portion) were mounted in Ussing chambers (0·64 cm^2 window area) containing Krebs solution at 37°C and gassed with 5% CO_2 in O_2; each side of the membrane was bathed with 5 mL fluid. Four preparations could be obtained from each colon (Burleigh & Kirkham 1993) and their origin was noted. Animals were killed by cervical dislocation. The tissue was clamped at zero potential by means of a high-impedance voltage clamp (DVC-1000, World Precision Instruments) and transmucosal short-circuit current (I_{sc}) was measured and continuously recorded on a pen recorder. At 5-min intervals the tissue was clamped at 2 mV (rather than 0 mV) and conductance calculated from the resulting change in I_{sc}. Both current-passing and voltage-detecting electrodes used a system of Ag/AgCl half-cells connected to large-diameter agar bridges (4% agar in Krebs solution minus calcium and glucose). To prevent precipitation of barium sulphate, magnesium sulphate was replaced by magnesium chloride in the Krebs solution.

The drugs used were: acetylcholine chloride, barium chloride, glibenclamide, charybdotoxin, apamin and phentolamine HCl (all from Sigma) and 5-hydroxydecanoic acid sodium salt and 4-aminopyridine (both from Research Biochemicals International). With the exception of glibenclamide, fresh aqueous stock solutions were prepared and dilutions made using Krebs solution. Glibenclamide was dissolved in DMSO, the maximum final concentration of DMSO was 0·25% (v/v). Drugs were added to the serosal (basolateral) side of the membrane.

Statistical analysis was performed by means of the Mann-Whitney U test for unpaired data, with $P < 0.05$ being taken as indicative of a significant difference. Values are quoted as mean ± s.e.m.

Results

After 60 min equilibration, basal I_{sc} was $15 \pm 3\,\mu A\,cm^{-2}$ and tissue conductance was $9 \pm 4\,mS\,cm^{-2}$ (n = 27). Serosal application of acetylcholine ($10^{-6} - 10^{-3}$ M) gave a concentration-dependent increase in I_{sc} with an EC50 value of $14 \pm 1\,\mu M$ and a maximum response of $188 \pm 16\,\mu A\,cm^{-2}$ (n = 4). There were no significant differences among the magnitudes of responses to acetylcholine (14 μM, n = 24) obtained from the most proximal and distal preparations. Using a 30-min contact time, potassium-channel inhibitors were tested against I_{sc} responses to acetylcholine (14 μM).

The effect of K^+-channel inhibitors on basal electrical activity is shown in Table 1. Barium (1–20 mM, n = 4) produced a concentration-dependent inhibition of responder to acetylcholine of 38 ± 13 to 90 ± 5%, the values for 4-aminopyridine (1–5 mM, n = 5) and glibenclamide (100–500 μM, n = 5) were 9 ± 5 to 66 ± 5% and 29 ± 6 to 95 ± 1% inhibition, respectively (Fig. 1). Responses to acetylcholine were also reduced by the K_{ATP}-channel inhibitors 5-hydroxydecanoate (10 μM, 26 ± 6%, n = 6, $P < 0.05$) and phentolamine (100 μM, 92 ± 2%, n = 5, $P < 0.05$). A combination of charybdotoxin (0·3 μM) plus apamin (0·3 μM) did not reduce responses to acetylcholine (n = 8, $P > 0.05$). Parallel time-matched control experiments showed no significant change in responses to acetylcholine after exposure to aqueous or DMSO vehicles ($P > 0.05$).

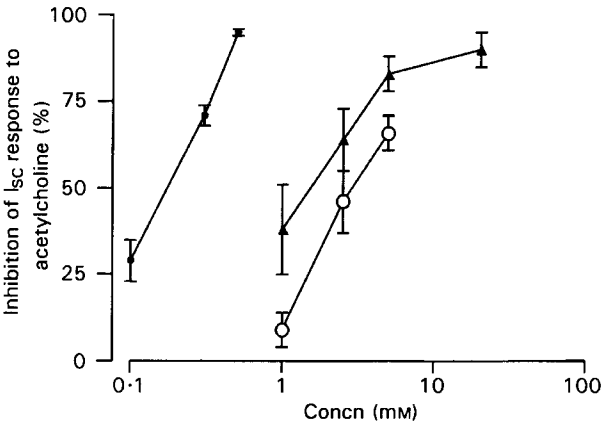

FIG. 1. Concentration-dependent inhibition of short-circuit current (I_{sc}) responses of rat colonic mucosa to 14μM acetylcholine. Effects of the potassium channel inhibitors are expressed as % inhibition of the response to acetylcholine. ● Glibenclamide, ▲ barium, ○ 4-aminopyridine. Each value is the mean ± s.e.m. of results from 4 or 5 experiments.

Discussion

Although there is evidence showing regional heterogeneity in the rat colon (Fromm & Hegel 1978; Hardcastle et al 1985; Nobles et al 1991), the response to acetylcholine was found to be region-independent. A lack of regional heterogeneity has also been observed for other secretagogues (Hardcastle et al 1985; Burleigh & Kirkham 1993) and for electrical properties and net Na fluxes (Binder & Rawlins 1973). Despite these observations it should be noted that administration of a K^+-channel inhibitor was never restricted to a particular region of the colon.

The increase in I_{sc} induced by Ba^{2+} has been observed previously in muscle-stripped sheets of rat colon (Hardcastle et al 1985). These authors concluded that the effect seemed to represent a direct action on the transporting cells resulting from release of intracellular calcium. Such an action would not explain the inhibition of acetylcholine responses observed in the current study, as increasing concentrations of Ba^{2+} induced greater inhibition of acetylcholine responses but had less effect on basal electrical activity. We also believe phentolamine and glibenclamide were acting selectively on K^+ channels despite their respective inhibition of α-adrenoceptors and lumenal chloride channels. For instance, although phentolamine antagonizes α_1- and α_2-adrenoceptors it would be difficult to explain its inhibition of acetylcholine responses by such an action. It is generally considered that adrenergic nerves are pro-absorptive and that α_2-agonists are effective anti-secretory agents (Dharmsathaphorn 1986). For glibenclamide, blockade of K_{ATP} channels rather than lumenal chloride channels is considered the most likely explanation of its reduction of acetylcholine responses because: other K_{ATP}-channel inhibitor drugs reduced I_{sc} responses to acetylcholine; it seems to be a general observation that compounds do not move freely across muscle-stripped intestinal preparations, the evidence being that many compounds produce 'sided' responses, i.e. responses that are only elicited when the compound is applied to the appropriate side of the preparation; and unpublished observations have shown that mucosally applied glibenclamide reduced responses to acetylcholine (albeit to a lesser extent than serosally applied glibenclamide) in the rat colon but not in the rat ileum. Such results cannot be explained by lumenal blockade of chloride channels because properties of apical chloride

Table 1. Effect of K^+-channel inhibitors on basal electrical activity.

Barium (n = 4)	1 mM	2·5 mM	5 mM	20 mM
	+54 ± 7	+24 ± 6	+13 ± 3	0 ± 7
4-Aminopyridine (n = 5)	1 mM	2·5 mM	5 mM	
	−1 ± 2	−2 ± 5	+5 ± 3	
Glibenclamide (n = 5)	100 μM	300 μM	500 μM	
	−5 ± 4	+5 ± 3	−9 ± 10	
Charybdotoxin + apamin (n = 8)	0·3 μM			
	+6 ± 2			
5-Hydroxydecanoate (n = 6)	10 mM			
	−6 ± 3			
Phentolamine (n = 5)	100 μM			
	0 ± 2			

Results are mean ± s.e.m. + = increase, − = decrease in basal electrical activity ($\mu A\,cm^{-2}$).

channels are broadly similar in a variety of chloride-secreting intestinal epithelia (Binder & Sandle 1994).

Preliminary experiments with the non-selective potassium-channel blocker barium indicated that a basolateral outward movement of potassium ions is required for the I_{sc} response of rat colonic mucosa to acetylcholine. Inhibition of the response to acetylcholine by 4-aminopyridine would indicate an effect on voltage-dependent potassium channels, because with very few exceptions this compound has been shown to be without effect on calcium-dependent potassium (K_{Ca}) channels (Cook & Quast 1990; Trends Pharmacol. Sci. receptor supplement 1995). Lack of involvement of K_{Ca} channels was confirmed by the failure of a combination of charybdotoxin plus apamin to inhibit the secretory effect of acetylcholine. A similar insensitivity to charybdotoxin and apamin was seen in patch-clamped cells from the base of rat colonic crypts, even though such cells were activated by elevated cytosolic calcium concentration (Burckhardt & Gögelein 1992). In the current investigation charybdotoxin, an inhibitor of high- and intermediate-conductance K_{Ca} channels, and apamin, an inhibitor of low-conductance K_{Ca} channels (Brewster & Strong 1992) were used in combination as this has been shown to be more effective than giving the compounds individually (Zygmunt & Högestätt 1996).

In the rat small intestine acetylcholine was reported to enhance basolateral K^+ conductance by activating K_{Ca} channels (Hardcastle & Hardcastle 1986). The main evidence for this observation was the inhibitory effect of quinine on increases in short-circuit current and ^{86}Rb efflux induced by acetylcholine. However, as the authors themselves point out, quinine is not a specific inhibitor of K_{Ca} channels and it has subsequently been shown to close K_{ATP} channels (Findlay et al 1985).

4-Aminopyridine can inhibit K_{ATP} channels (Trends Pharmacol. Sci. receptor supplement 1995) and the inhibitory effect of glibenclamide, a more selective K_{ATP}-channel inhibitor (Ashford 1990), indicated the involvement of such channels. Glibenclamide has been reported to inhibit apical chloride channels (Rabe et al 1995) an effect which would also inhibit short-circuit current responses to acetylcholine; however, it is unlikely that glibenclamide diffused across to the apical domain of the membrane. Involvement of K_{ATP} channels in the secretory response to acetylcholine was confirmed by the inhibitory effects of 5-hydroxydecanoate and phentolamine, both of which are inhibitors of K_{ATP} channels (McCullough et al 1991; Ibbotson et al 1993).

In conclusion, the particular potassium channel involved in the secretory response of rat colonic mucosa to acetylcholine appears to be ATP-dependent and calcium insensitive. ATP-regulated channels are also known to be active in the basolateral membrane of colonic epithelial cells in man (Cuffe et al 1995).

Acknowledgements

N. D. Palmerley gratefully acknowledges financial support from The Nuffield Foundation.

References

Ashford, M. L. J. (1990) Potassium channels and modulation of insulin secretion. In: Cook, N. S. (ed.) Potassium Channels—Structure, Classification, Function and Therapeutic Potential. Ellis Horwood, Chichester, pp 300–325

Binder, H. J., Rawlins, C. L. (1973) Electrolyte transport across isolated large intestinal mucosa. Am. J. Physiol. 225: 1232–1239

Binder, H. J., Sandle, G. I. (1994) Electrolyte transport in the mammalian colon. In: Johnson, L. J. (ed.) Physiology of the Gastrointestinal Tract. 3rd edn, Raven Press, New York, pp 2133–2171

Brewster, B. S., Strong, P. N. (1992) Naturally-occurring potassium-channel blockers. In: Weston, A. H., Hamilton, T. C. (eds) Potassium-Channel Modulators. Blackwell, Oxford, pp 272–303

Burckhardt, B. C., Gögelein, H. (1992) Small and maxi K^+ channels in the basolateral membrane of isolated crypts from rat distal colon: single channel and slow whole-cell recordings. Pflügers Arch. 420: 54–60

Burleigh, D. E., Kirkham, S. E. (1993) Lack of effect of three putative vasoactive intestinal peptide receptor antagonists on vasoactive intestinal peptide-induced secretory responses in rat colon. Eur. J. Pharmacol. 249: 239–242

Cook, N. S., Quast, U. (1990) Potassium-channel pharmacology. In: Cook, N. S. (ed.) Potassium Channels—Structure, Classification, Function and Therapeutic Potential. E. Horwood, Chichester, pp 181–255

Cuffe, J., McNamara, B., O'Sullivan, G., Harvey, B. (1995) Non-genomic activation of K_{ATP} channels in human colon by oestradiol. J. Physiol. 489: 122P–123P

Darko, S. K. A., Palmerley, N. D., Burleigh, D. E. (1997) Inhibition of potassium (K_{ATP}) channels reduces short-circuit current response of rat colonic mucosa to acetylcholine. Br. J. Pharmacol. 120: 195P

Dawson, D. C., Richards, N. W. (1990) Basolateral K conductance: role in regulation of NaCl absorption and secretion. Am. J. Physiol. 259: C181–C195

Devor, D. C., Frizzell, R. A. (1993) Calcium-mediated agonists activate an inwardly rectified K channel in colonic secretory cells. Am. J. Physiol. 265: C1271–C1280

Dharmsathaphorn, K. (1986) α_2-Adrenergic agonists: a newer class of antidiarrheal drug. Gastroenterology 91: 769–770

Dharmsathaphorn, K., Pandol, S. J. (1986) Mechanism of chloride secretion induced by carbachol in a colonic epithelial cell line. J. Clin. Invest. 77: 348–354

Findlay, L., Dunne, M. J., Ullrich, S., Wollheim, C. B., Petersen, O. H. (1985) Quinine inhibits Ca^2-independent K^+ channels whereas tetraethylammonium inhibits Ca^2-activated K^+ channels in insulin-secreting cells. FEBS Lett. 185: 4–8

Fromm, M., Hegel, U. (1978) Segmental heterogeneity of epithelial transport in rat large intestine. Pflügers Arch. 378: 71–83

Hardcastle, J., Hardcastle, P. T. (1986) The involvement of basolateral potassium channels in the intestinal response to secretagogues in the rat. J. Physiol. 379: 331–345

Hardcastle, J., Hardcastle, P. T., Noble, J. M. (1985) The secretory action of barium chloride in the rat colon. J. Physiol. 361: 19–33

Ibbotson, T., Edwards, G., Noack, Th., Weston, A. H. (1993) Effects of P1060 and aprikalim on whole-cell currents in rat portal vein: inhibition by glibenclamide and phentolamine. Br. J. Pharmacol. 108: 991–998

Lomax, R. B., Warhurst, G., Sandle, G. I. (1996) Characteristics of two basolateral potassium-channel populations in human colonic crypts. Gut 38: 243–247

Loo, P. D. F., Kaunitz, J. D. (1989) Ca^2 and cAMP activate K^+ channels in the basolateral membrane of crypt cells isolated from rabbit distal colon. J. Membrane Biol. 110: 19–28

McCullough, J. R., Normandin, D. E., Conder, M. L., Sleph, P. G., Dzwonczyk, S., Grover, G. J. (1991) Specific block of the anti-ischaemic actions of cromokalin by sodium 5-hydroxydecanoate. Circ. Res. 69: 949–958

Nobles, M., Diener, M., Mestres, P., Rummel, W. (1991) Segmental heterogeneity of the rat colon in response to activators of secretion on the cAMP-, the cGMP and the Ca^2 pathway. Acta Physiol. Scand. 142: 375–386

Rabe, A., Disser, J., Fromter, E. (1995) Cl^- channel inhibition by glibenclamide is not specific for the CFTR-type Cl^- channel. Eur. J. Physiol. 429: 659–662

Trends in Pharmacological Sciences (1995) Receptor and ion-channel nomenclature supplement. 6th edn, Trends Pharmacol. Sci. 16: 66–99

Zygmunt, P. M., Högestätt, E. D. (1996) Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. Br. J. Pharmacol. 117: 1600–1606