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Evidence against direct activation of chloride secretion by carbachol in the rat distal colon

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Abstract

Carbachol (5×10^{-5} mol · l^{-1}) induced a biphasic increase in short-circuit current (Isc) consisting of an initial peak phase followed by a long-lasting plateau. Complete dependence on the presence of Cl^- ions and sensitivity to bumetanide confirmed that carbachol induces Cl^- secretion. The plateau phase was blocked by indomethacin, and both the plateau and the peak phase were suppressed in the combined presence of indomethacin and tetrodotoxin. Inhibition of the carbachol response could be overcome by agonists of the cAMP pathway like prostaglandin E_2, forskolin or 8-(4-chlorophenylthio)-adenosine-3′,5′-cyclic monophosphate. The increase in Isc was inhibited by a blocker of cAMP-activated Cl^- channels, glibenclamide, but was resistant to an inhibitor of Ca^{2+}-activated Cl^- channels, 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid (DIDS). The K^+ channel blockers Ba^{2+} and charybdotoxin inhibited the first and suppressed the second phase of the carbachol response, whereas a less specific K^+ channel blocker, quinine, suppressed both phases. These results suggest that the dominant effect of carbachol in the intact colonic mucosa is an opening of Ca^{2+}-dependent, charybdotoxin- and Ba^{2+}-sensitive K^+ channels, which leads to hyperpolarization of the epithelial cells. This stimulates Cl^- secretion only if there are spontaneously open apical Cl^- channels which are basically stimulated by a continuous release of neurotransmitters and prostaglandins. Only during the first phase of the carbachol effect is there indirect evidence for activation of a Cl^- conductance synergistically with the cAMP pathway as shown by the increase in tissue conductance resistant to K^+ channel blockers.

Keywords: Colon, rat; Cl^- channel; K^+ channel, Ca^{2+}; Prostaglandin; Enteric nervous system

1. Introduction

The ability to secrete Cl^- is one of the basic properties of the intestinal epithelium. Chloride secretion is activated under physiologic conditions, e.g. after distension of the gut wall (see e.g. Diener and Rummel, 1990), and plays a prominent role under pathophysiologic conditions, i.e. during secretory diarrhoea (for recent review see Field and Semrad, 1993). The chloride ions to be secreted enter the epithelium by a basolateral bumetanide-sensitive Na^+-K^+-Cl^- cotransporter, which accumulates Cl^- ions in the cell above electrochemical equilibrium. They then leave the cell by Cl^- channels in the apical membrane. The final driving force for the exit of Cl^- ions is the membrane potential, because the intracellular Cl^- concentration is lower than the extracellular concentration. This system is under the control of intracellular second messengers like Ca^{2+}, cAMP and cGMP (for review see Binder and Sandle, 1987).

Controversy exists about the mode of activation of Cl^- secretion by intracellular Ca^{2+}. In airway epithelium (Frizzell et al., 1986) and in colonic tumour cell lines like T_{84} (Cliff and Frizzell, 1990) and HT29 cells (Kunzelmann et al., 1992; Morris et al., 1992), direct activation of apical Cl^- channels by intracellular Ca^{2+} has been demonstrated. Two types of Ca^{2+}-dependent Cl^- channels in HT29 cells have been described, a 15 pS channel with a linear current-voltage relationship (Morris and Frizzell, 1993), and a Cl^- channel with a very low single channel conductance (Kunzelmann et al., 1992). On the other hand, in native intestinal epithelia it has not yet been possible to demonstrate an increase in Cl^- conductance caused by an increase in the intracellular Ca^{2+} concentration. Neither in iso-

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lated colonic crypts from the rat (Böhme et al., 1991) or the rabbit (E. Lohrmann and R. Greger, personal communication) nor in isolated small intestinal crypts from the guinea-pig (Walters and Sepúlveda, 1991), has carbachol, a typical agonist of Ca^{2+}-mediated secretion, caused an increase in Cl^- current during whole-cell recordings using nystatin-permeabilized patches. The only effect of carbachol on these isolated crypts was an increase in Ca^{2+}-dependent K^+ conductance leading to hyperpolarization of the membrane. This will, however, increase the driving force for Cl^- secretion across apical Cl^- channels. This model has originally been proposed from radioisotope efflux studies by Dharmsathaphorn and Pandol (1986) for T_{84} cells, i.e. a cell line, in which evidence for direct activation of a Cl^- conductance by Ca^{2+} is controversial (Anderson and Welsh, 1991; Valverde et al., 1994).

In apparent contrast to these results with isolated crypts, which suggest an indirect effect of carbachol on Cl^- secretion due to hyperpolarization, studies with intact intestinal mucosa indicate that, e.g. in the colon, carbachol is one of the most efficient agonists to induce Cl^- secretion (Nobles et al., 1991). The question arises, therefore, of what the reason may be for this difference between the results from the same tissue, i.e. the rat distal colon, in which the isolated crypts respond only with an increase in K^+ conductance (Böhme et al., 1991), but the intact mucosa responds with a strong Cl^- secretion (see e.g. Diener et al., 1989). Two fundamental differences between the intact mucosa and the isolated crypt exist: (1) the presence of subepithelial connective tissue, producing eicosanoids like prostaglandins (Craven and DeRubertis, 1983), and (2) the presence of the enteric nervous system, spontaneously releasing neurotransmitters (Andres et al., 1985). Prostaglandins or neurotransmitters might either mediate the activation of Cl^- secretion by carbachol or, alternatively, they might create a facilitating influence on the epithelium, making the epithelium sensitive to stimulation of Cl^- secretion by carbachol. Consequently, the present study was designed to re-evaluate the effect of carbachol on the rat colonic mucosa with special attention to the possible influence of the eicosanoid and the neural system.

2. Materials and methods

2.1. Solutions

Most of the experiments were carried out in a bathing solution containing (mmol $\cdot l^{-1}$): NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1 and glucose 12. The solution was gassed with carbogen (5% CO_2 in 95% O_2) and kept at a temperature of 37°C; the pH was 7.4. For the Cl^--free buffer, NaCl was replaced by Na gluconate; the Ca^{2+} concentration in this solution was elevated to 5.8 mmol $\cdot l^{-1}$ in order to compensate for the Ca^{2+}-buffering properties of gluconate (Kenyon and Gibbons, 1977). The HCO_3^--free Tyrode solution consisted of (mmol $\cdot l^{-1}$): NaCl 140, KCl 5.4, $CaCl_2$ 1.25, $MgSO_4$ 1, Hepes (*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid) 10, glucose 12, and was gassed with O_2.

2.2. Tissue preparation

Female SIVZ-50 rats (Institut für Labortierkunde, Universität Zürich, Switzerland) were used with a weight of 180–220 g. The animals had free access to water and food until the day of the experiment. The animals were stunned by a blow on the head and killed by exsanguination. The serosa and muscularis propria were stripped away by hand to obtain the mucosa-submucosa preparation of the distal part of the colon descendens.

2.3. Short-circuit current measurement

The tissue was fixed in a modified Ussing chamber (Andres et al., 1985), bathed with a volume of 4 ml on each side of the mucosa and short-circuited by a voltage clamp (Aachen Microclamp, AC Copy Datentechnik, Aachen, Germany) with correction for solution resistance. The exposed surface of the tissue was 1 cm^2. Short-circuit current (Isc) was continuously recorded and tissue conductance (Gt) was measured every minute. For the figures, Isc and Gt were averaged every minute.

2.4. Data evaluation

The response to carbachol was tested in the absence and presence of putative antagonists. Antagonists were administered to the compartment indicated in the text and stabilization of Isc was waited for, which usually took 15–20 min. The baseline in Isc was determined as mean over 3 min just before the application of carbachol. In the tables, the maximal increase in Isc induced by carbachol and the plateau value in Isc, taken 10 min after administration of carbachol, are expressed as differences from the former baseline (delta Isc).

2.5. Drugs

4,4'-Diisothiocyanato-stilbene-2,2'-disulfonic acid (DIDS), glibenclamide (gift from Boehringer, Mannheim, Germany), and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB; gift from R. Greger, Physiologisches Institut, Freiburg, Germany) were dissolved in dimethyl sulfoxide (DMSO, final concentration 0.25%, v/v). Bumetanide, forskolin (gift from Hoechst, Frank-

furt, Germany), indomethacin, prostaglandin E_2, quinine, and staurosporine were added from ethanolic stock solutions (final maximal concentration 0.25%, v/v). Tetraethylammonium and Ba^{2+} (both from Fluka, Buchs, Switzerland) were administered as chloride salts. K^+ was added as K gluconate in the experiments with the increased extracellular K^+ concentration. Charybdotoxin (Peninsula, Belmont, CA, USA) was dissolved in an aqueous stock solution containing 0.1% (w/v) bovine serum albumin. All other drugs, including 8-(4-chlorophenylthio)-adenosine-3',5'-cyclic monophosphate (CPTcAMP) and tetrodotoxin, were applied from aqueous stock solutions. If not indicated differently, the drugs were from Sigma, Buchs, Switzerland.

2.6. Statistics

The results are given as means ± one standard error of the mean (S.E.). The significance of differences was tested by analysis of variances and, if indicated, by paired or unpaired two-tailed Student's *t*-test or a *U*-test, respectively. An *F*-test was applied to decide which test method was to be used. The quality of regressions was checked with the squared non-linear regression coefficient (r^2).

3. Results

3.1. Dependence of the carbachol effect on nerves and prostaglandins

Carbachol (5×10^{-5} mol · l^{-1}) induced a transient increase in Isc (Fig. 1a) and tissue conductance (Gt; Table 1). The short-circuit current rose quickly within 2–3 min to a peak of 8.5 ± 0.7 μEq · h^{-1} · cm^{-2} ($n = 6$) above baseline and then fell slowly with a biphasic time behaviour. The decay could be nearly perfectly described ($r^2 = 0.998$) by a bi-exponential function with time constants of 2.1 min and 14.4 min for the fast and the slow component, respectively (see inset of Fig. 1). The increase in Isc was completely dependent on the presence of Cl^- ions (Table 1). It was also completely blocked in the presence of a Cl^- channel blocker, NPPB (10^{-4} mol · l^{-1}), or in the presence of an inhibitor of the Na^+-K^+-Cl^- cotransporter, bumetanide (10^{-4} mol · l^{-1}), confirming previous observations that this response is caused by a transepithelial Cl^- secretion (Dharmsathaphorn and Pandol, 1986; Diener et al., 1989). In contrast, omission of HCO_3^- did not reduce but rather enhanced the increase in Isc induced by carbachol (Table 1). In accordance with previous

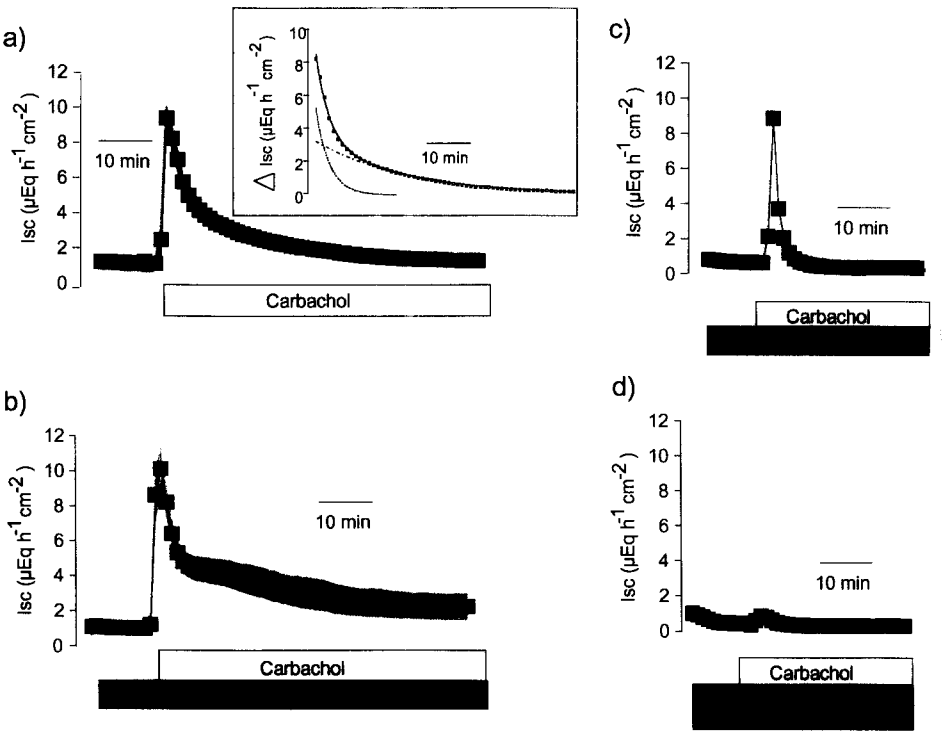

Fig. 1. Effect of carbachol (5×10^{-5} mol · l^{-1} administered on the serosal side) on Isc. (a) Effect of carbachol under control conditions. The inset shows the decay in the Isc response (rectangles = measured data points), which could be fitted by assuming a double exponential decay (solid line) composed of a fast (dotted line) and a slow (dashed) component. (b–d) Effect of carbachol in the presence of tetrodotoxin (10^{-6} mol · l^{-1} administered on the serosal side, Fig. 1b), indomethacin (10^{-6} mol · l^{-1} administered on the serosal and the mucosal side, Fig. 1c), or the combined presence of both inhibitors (Fig. 1d). All values are means (symbols) ± S.E. (shaded area).

Table 1
Response induced by carbachol

	Delta Isc (μEq · h^{-1} · cm^{-2})		Delta Gt (mS · cm^{-2})	
	Peak	Plateau	Peak	Plateau
Control	8.5 ± 0.7 [a]	2.6 ± 0.6 [a]	5.6 ± 0.6 [a]	2.7 ± 0.3 [a]
Tetrodotoxin	9.9 ± 0.9 [a]	3.4 ± 0.6 [a]	7.5 ± 1.7 [a]	5.0 ± 0.9 [a,b]
Indomethacin	8.5 ± 1.4 [a]	−0.2 ± 0.1 [b]	7.2 ± 1.3 [a]	2.7 ± 0.5 [a]
Tetrodotoxin + indomethacin	0.6 ± 0.2 [b]	−0.1 ± 0.1 [b]	2.4 ± 0.5 [a,b]	3.0 ± 0.4 [a]
Tetrodotoxin + indomethacin + Prostaglandin E_2	2.5 ± 0.3 [a,c]	−0.7 ± 0.2 [a]	2.8 ± 0.3 [a]	1.5 ± 0.2 [a,c]
+ Forskolin	6.8 ± 0.4 [a,c]	1.6 ± 0.3 [a,c]	5.4 ± 0.4 [a,c]	2.7 ± 0.4 [a]
+ CPTcAMP	5.9 ± 1.6 [a,c]	1.6 ± 0.5 [a,c]	4.8 ± 1.1 [a,c]	3.2 ± 0.6 [a]
Bumetanide	2.1 ± 0.4 [a,b]	0.7 ± 0.2 [a,b]	3.8 ± 0.7 [a]	5.1 ± 0.7 [a,b]
NPPB	2.6 ± 0.4 [a,b]	−0.9 ± 0.2 [a,b]	1.3 ± 0.8 [b]	0.8 ± 1.2
Glibenclamide	3.5 ± 0.8 [a,b]	−0.3 ± 0.3 [b]	2.9 ± 1.2	3.9 ± 2.4
DIDS	10.8 ± 0.8 [a,b]	2.3 ± 0.5 [a]	7.0 ± 1.3 [a]	3.5 ± 1.1 [a]
Ba^{2+}	6.1 ± 0.6 [a,d]	−0.4 ± 0.0 [a,d]	4.3 ± 0.6 [a,d]	−0.4 ± 0.5 [d]
Charybdotoxin	4.8 ± 1.2 [a,b]	−1.0 ± 0.6 [b]	3.7 ± 0.5 [a,b]	1.4 ± 0.3 [a,b]
Quinine	0.3 ± 0.1 [a,b]	−0.3 ± 0.1 [a,b]	0.9 ± 0.2 [a,b]	0.8 ± 0.2 [a,b]
Tetraethylammonium	7.0 ± 1.2 [a]	5.2 ± 0.7 [a,b]	4.1 ± 0.8 [a]	3.4 ± 0.5 [a]
Staurosporine	0.9 ± 0.4 [b]	0.0 ± 0.1 [b]	1.0 ± 0.5 [b]	0.9 ± 0.5 [b]
Cl^--free	1.8 ± 0.3 [a,b]	0.2 ± 0.3 [b]	1.8 ± 0.5 [a,b]	1.1 ± 0.5 [b]
HCO_3^--free	14.3 ± 1.4 [a]	3.2 ± 0.7 [a]	8.7 ± 1.1 [a]	3.6 ± 1.0 [a]
13.5 mmol · l^{-1} K^+	8.7 ± 1.3 [a]	−0.4 ± 0.3 [b]	10.4 ± 1.3 [a,b]	2.5 ± 1.3

Isc and Gt response induced by carbachol (5×10^{-5} mol · l^{-1} serosal) in the absence and presence of other drugs. $BaCl_2$ (10^{-2} mol · l^{-1} serosal, applied in HCO_3^--free buffer), bumetanide (10^{-4} mol · l^{-1} serosal), charybdotoxin (2×10^{-7} mol · l^{-1} serosal), CPTcAMP (2.5×10^{-5} mol · l^{-1} serosal), DIDS (10^{-3} mol · l^{-1} mucosal), forskolin (5×10^{-7} mol · l^{-1} mucosal and serosal), glibenclamide (5×10^{-4} mol · l^{-1} mucosal), indomethacin (10^{-6} mol · l^{-1} mucosal and serosal), NPPB (10^{-4} mol · l^{-1} mucosal), prostaglandin E_2 (5×10^{-8} mol · l^{-1} serosal), quinine (10^{-3} mol · l^{-1} serosal), staurosporine (10^{-6} mol · l^{-1} serosal), tetraethylammonium (5×10^{-3} mol · l^{-1} serosal), tetrodotoxin (10^{-6} mol · l^{-1} serosal).
[a] $P < 0.05$ versus baseline, [b] $P < 0.05$ versus carbachol alone, [c] $P < 0.05$ versus carbachol in the combined presence of tetrodotoxin and indomethacin, [d] $P < 0.05$ versus carbachol in the absence of HCO_3^-. Values are differences from the baseline just before carbachol administration and are means ± S.E., $n = 5$–7.

Table 2
Effect of drugs on baseline

	Delta Isc (μEq · h^{-1} · cm^{-2})	Delta Gt (mS · cm^{-2})
Tetrodotoxin	−0.3 ± 0.2	−0.5 ± 0.2 [a]
Indomethacin	−0.6 ± 0.3	−1.0 ± 0.5
Tetrodotoxin + indomethacin	−0.9 ± 0.3 [a]	−1.2 ± 0.3 [a]
+ Prostaglandin E_2	0.8 ± 0.2 [a]	1.8 ± 0.3 [a]
+ Forskolin	2.1 ± 0.3 [a]	1.8 ± 0.2 [a]
+ CPTcAMP	0.4 ± 0.3	0.9 ± 0.4
Bumetanide	−0.8 ± 0.2 [a]	0.3 ± 0.6
NPPB	−1.9 ± 0.7 [a]	2.4 ± 2.1
Glibenclamide	−0.3 ± 0.3	1.1 ± 0.5 [a]
DIDS	0.8 ± 0.2 [a]	2.2 ± 1.1
Ba^{2+}	−0.6 ± 0.2 [a]	0.8 ± 0.5
Charybdotoxin	0.0 ± 0.2	0.4 ± 0.3
Quinine	−0.5 ± 0.2	−0.4 ± 0.3
Tetraethylammonium	0.2 ± 0.2	0.1 ± 0.2
Staurosporine	−0.7 ± 0.2 [a]	−0.5 ± 0.4
13.5 mmol · l^{-1} K^+	0.7 ± 0.4	3.1 ± 1.0 [a]

Effect of drugs on baseline electrical parameters. $BaCl_2$ (10^{-2} mol · l^{-1} serosal, applied in HCO_3^--free buffer), bumetanide (10^{-4} mol · l^{-1} serosal), charybdotoxin (2×10^{-7} mol · l^{-1} serosal), CPTcAMP (2.5×10^{-5} mol · l^{-1} serosal), DIDS (10^{-3} mol · l^{-1} mucosal), forskolin (5×10^{-7} mol · l^{-1} mucosal and serosal), glibenclamide (5×10^{-4} mol · l^{-1} mucosal), indomethacin (10^{-6} mol · l^{-1} mucosal and serosal), NPPB (10^{-4} mol · l^{-1} mucosal), prostaglandin E_2 (5×10^{-8} mol · l^{-1} serosal), quinine (10^{-3} mol · l^{-1} serosal), staurosporine (10^{-6} mol · l^{-1} serosal), tetraethylammonium (5×10^{-3} mol · l^{-1} serosal), tetrodotoxin (10^{-6} mol · l^{-1} serosal).
[a] $P < 0.05$ versus baseline. Values are differences from the baseline just before administration of the drug and are means ± S.E., $n = 5$–7.

observations (Diener et al., 1989), the response to carbachol, used in the high concentration range (> 10^{-5} mol · l^{-1}) was not inhibited by the neurotoxin, tetrodotoxin (10^{-6} mol · l^{-1}; Fig. 1b). However, the cyclooxygenase inhibitor, indomethacin (10^{-6} mol · l^{-1}), completely abolished the second, slow phase of the Isc response evoked by carbachol (Fig. 1c). In the presence of indomethacin, the decay in Isc after administration of carbachol could be adequately described ($r^2 = 0.997$) by a mono-exponential function with a time constant of 0.8 min. Although tetrodotoxin alone had no effect on the response to carbachol (Fig. 1b), in the combined presence of indomethacin and tetrodotoxin, the effect of carbachol was completely abolished (Fig. 1d). Tetrodotoxin and indomethacin caused a decrease in baseline Isc, which was most pronounced when both blockers were combined (Table 2).

Surprisingly, none of these inhibitors had an effect on the increase in Gt induced by carbachol. Carbachol alone caused a biphasic increase in Gt, which transiently rose to a peak of 5.6 ± 0.6 mS · cm^{-2} above baseline ($n = 6$, $P < 0.05$) and then fell to a plateau of 2.7 ± 0.3 mS · cm^{-2} ($n = 6$, $P < 0.05$), which decreased only slowly over 60 min (Fig. 2a). This increase in Gt was resistant to tetrodotoxin (Fig. 2b). Although indomethacin suppressed the second, slow phase in the Isc response to carbachol (cf. Fig. 1c), it had no effect on the increase in Gt (Fig. 2c). Only the combined presence of tetrodotoxin and indomethacin blocked the first, fast increase in Gt induced by carbachol, but had no effect on the second, slow phase (Fig. 2d).

3.2. Restoration of the carbachol response

The sensitivity to tetrodotoxin and indomethacin suggests that the effect of carbachol is either mediated by nerves and prostaglandins or that it is dependent on the continuous release of neurotransmitters and prostaglandins, which brings the epithelium in a state in which Cl^- secretion can be induced by the acetylcholine receptor agonist. In order to distinguish between these two possibilities, it was attempted to 'restore' the baseline Isc which had been decreased by prior addition of tetrodotoxin and indomethacin (see inset of Fig. 3 for the time course of the experiments). Prostaglandin E_2 (5×10^{-8} mol · l^{-1}), administered in the presence of both inhibitors, increased the baseline again by 0.8 ± 0.2 μEq · h^{-1} · cm^{-2} ($n = 6$, $P < 0.05$, Table 2) to a stable plateau. When carbachol was applied subsequently, the inhibition by tetrodotoxin and indomethacin was partially overcome (Fig. 3). Under these conditions, carbachol increased Isc to a peak of 2.5 ± 0.3 μEq · h^{-1} · cm^{-2} ($n = 6$), which was 4 times higher than the response to carbachol in the presence of tetrodotoxin and indomethacin ($P < 0.05$, Table 1). However, prostaglandin E_2 did not restore the second, slow phase of the carbachol response (Fig. 3, Table 1).

Most of the effects of prostaglandins are mediated intracellularly by cAMP. Therefore, the effect of two agonists of the cAMP pathway, i.e. of the stimulator of the adenylate cyclase, forskolin, and of the membrane permeable cAMP analogue, CPTcAMP, was tested. Both forskolin (5×10^{-7} mol · l^{-1}) and CPTcAMP (2.5×10^{-5} mol · l^{-1}) increased the baseline Isc to a stable

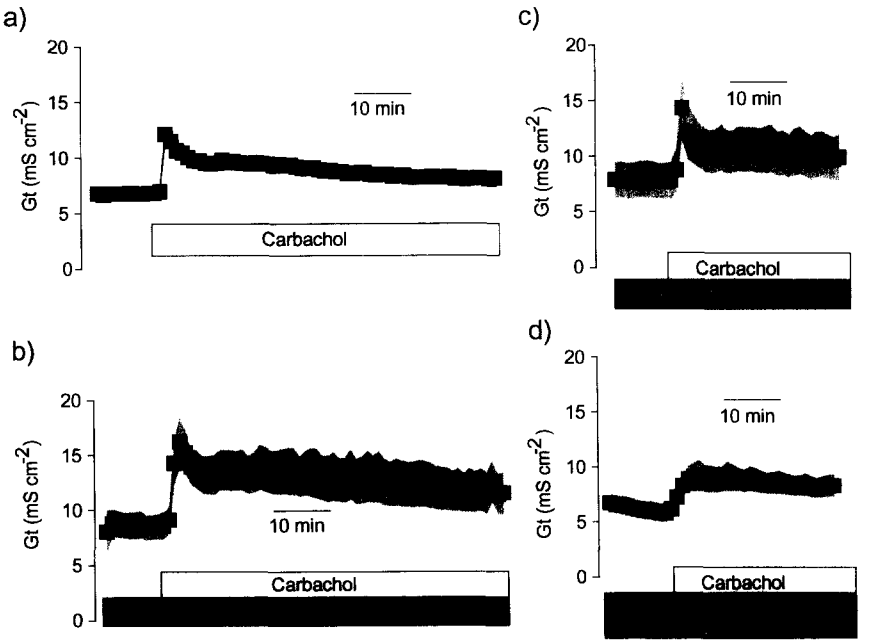

Fig. 2. Effect of carbachol (5×10^{-5} mol · l^{-1} administered on the serosal side) on Gt. (a–d) Effect of carbachol under control conditions (Fig. 2a), in the presence of tetrodotoxin (10^{-6} mol · l^{-1} administered on the serosal side, Fig. 2b), indomethacin (10^{-6} mol · l^{-1} administered on the serosal and the mucosal side, Fig. 2c), or the combined presence of both inhibitors (Fig. 2d). All values are means (symbols) ± S.E. (shaded area). $n = 6$.

plateau in the presence of tetrodotoxin and indomethacin (Table 2). When carbachol was added subsequently during this plateau, the inhibition by tetrodotoxin and indomethacin could be completely overcome. Like under control conditions, carbachol caused a strong increase in Isc (Fig. 3) with a biphasic time course. Both in the presence of forskolin and in the presence of CPTcAMP, the decay in Isc after administration of carbachol could again be adequately described ($r^2 \geq 2.996$) by a bi-exponential function. The complete restoration of the carbachol response in the presence of tetrodotoxin and indomethacin, i.e. under conditions where carbachol is not able to stimulate the release of neurotransmitters or prostaglandins, suggests strongly that the effect of the acetylcholine receptor agonist is not mediated by nerves or eicosanoids, but is dependent on continuous activity of the neural and the autocrine system, which induce an ongoing basal cAMP production and thereby contributes to the basal production of a secretory Isc.

3.3. Sensitivity to ion channel blockers

The above results suggest the hypothesis that Cl^- secretion induced by carbachol is driven by a Cl^- flux through cAMP-activated Cl^- channels. Therefore, the effect of glibenclamide, an inhibitor of the cystic fibrosis transmembrane regulator (CFTR) Cl^- channel (Sheppard and Welsh, 1992), which is thought to control cAMP-dependent secretion (Anderson et al., 1991), was tested. In a first step, it was tested whether glibenclamide inhibited the response to the activator of the cAMP pathway, forskolin. Glibenclamide (10^{-5}–10^{-3} mol·l^{-1}) concentration dependently inhibited the response to forskolin (Fig. 4, bottom). In contrast, DIDS (10^{-3} mol·l^{-1}), an inhibitor of Ca^{2+}-dependent Cl^- channels in the airway epithelium (Clarke et al., 1994), had only a small (and statistically not significant) effect on the response to forskolin (Fig. 4, top). In five experiments, forskolin (5×10^{-6} mol·l^{-1}) increased Isc by 6.2 ± 1.2 μEq·h^{-1}·cm^{-2}, and DIDS reduced this response only by 1.7 ± 0.9 μEq·h^{-1}·cm^{-2} ($n = 5$, difference not significant). A similar sensitivity to DIDS and glibenclamide was observed when carbachol was used as a secretagogue. Whereas DIDS was completely ineffective, glibenclamide caused a 60% inhibition of the peak response to carbachol and completely suppressed the second, slow phase of the carbachol response (Table 1).

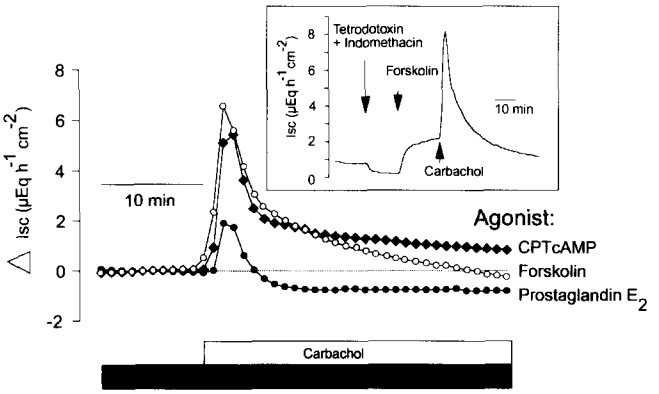

Fig. 3. Restoration of the carbachol response by prostaglandin E_2 (5×10^{-8} mol·l^{-1} administered on the serosal side; closed circles), forskolin (5×10^{-7} mol·l^{-1} administered on the mucosal and the serosal side; open circles), and CPTcAMP (2.5×10^{-5} mol·l^{-1} administered on the mucosal and the serosal side, filled diamonds). The agonists of the cAMP pathway were applied 10 min after administration of tetrodotoxin and indomethacin; carbachol (5×10^{-5} mol·l^{-1}) was administered, when Isc had risen to a stable plateau after administration of the agonist. The inset shows an original record of the series of experiments with forskolin. Values are means, $n = 6$–7. For statistics, see Table 1.

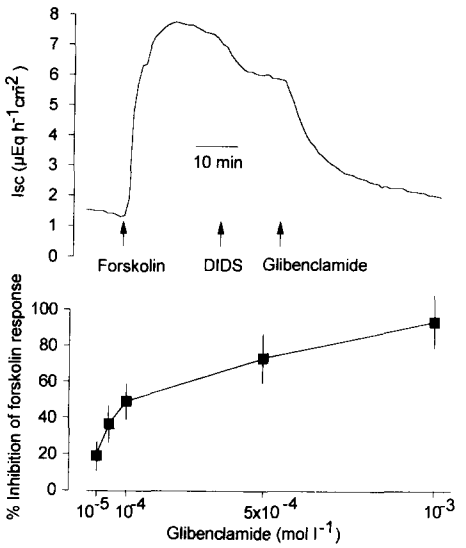

Fig. 4. Original record (top) demonstrating the effect of forskolin (5×10^{-6} mol·l^{-1} administered on the serosal and the mucosal side), the (insignificant) action of DIDS (10^{-3} mol·l^{-1} administered on the mucosal side), and the nearly complete inhibition by addition of glibenclamide (5×10^{-4} mol·l^{-1} administered on the mucosal side). Representative of 5 experiments with similar results. Bottom: Concentration-dependence of the inhibition of the response to forskolin by glibenclamide. Glibenclamide was added cumulatively to the mucosal compartment. The inhibition was statistically significant at glibenclamide concentrations $\geq 5 \times 10^{-5}$ mol·l^{-1}. Values are means ± S.E., $n = 5$.

The effect of carbachol was partially inhibited by the K^+ channel blocker, Ba^{2+}. This particular series of experiments was performed in HCO_3^--free buffer in order to avoid precipitation of Ba^{2+} as $BaCO_3$. Barium (10^{-2} mol·l^{-1}) reduced the peak in Isc by more than 55% (6.1 ± 0.6 μEq·h^{-1}·cm^{-2} in the presence and 14.3 ± 1.4 μEq·h^{-1}·cm^{-2} in the absence of Ba^{2+}; $n = 6$, $P < 0.05$) and completely suppressed the second phase of the Isc response (Fig. 5). A similar pattern of inhibition was observed with charybdotoxin (2×10^{-7} mol·l^{-1}; Table 1). In contrast, quinine (10^{-3} mol·l^{-1}), which inhibits K^+ channels and in addition Cl^- chan-

nels like e.g. the outward-rectifying Cl^- channel (Gögelein and Capek, 1990), suppressed both phases of the carbachol effect (Fig. 5). Another K^+ channel blocker tested, tetraethylammonium (5×10^{-3} mol · l^{-1}), was completely ineffective (Table 1).

3.4. Analysis of the action of carbachol on Gt

The inhibition of the Isc response by Ba^{2+}, charybdotoxin and quinine was parallelled by an inhibition of the Gt response induced by carbachol. Whereas Ba^{2+} and charybdotoxin reduced only the first peak phase and blocked the second, slow component of the carbachol response, quinine suppressed both phases (Fig. 6a, Table 1), suggesting that the increase in Gt, at least during the second phase, is caused by the opening of K^+ channels. However, the ion substitution experiments revealed that omission of Cl^- (but not of HCO_3^-; Fig. 6a) from the buffer not only prevented the effect of carbachol on Isc (see above), but also reduced the increase in Gt induced by carbachol (Fig. 6b). In order to find out whether this decrease in the carbachol-induced conductance represents a true chloride dependence of this conductance or whether it is caused by the membrane depolarization, which has to be expected when substituting Cl^- by an impermeant anion, the effect of partial depolarization of the epithelium was tested by increasing the extracellular K^+ concentration. Orientating experiments revealed that the response to carbachol could be concentration dependently inhibited, when the K^+ concentration in the buffer was elevated, and an intermediate concentration of K^+ (13.5 mmol · l^{-1}) was selected for the subsequent experiments. Increasing the extracellular K^+ concentration from 4.5 mmol · l^{-1} to 13.5 mmol · l^{-1} caused an increase of Isc by 0.7 ± 0.4 μEq · h^{-1} · cm^{-2} above baseline ($n = 6$, not significant) and of Gt by 3.1 ± 1.0 mS · cm^{-1} above baseline ($n = 6$, $P < 0.05$).

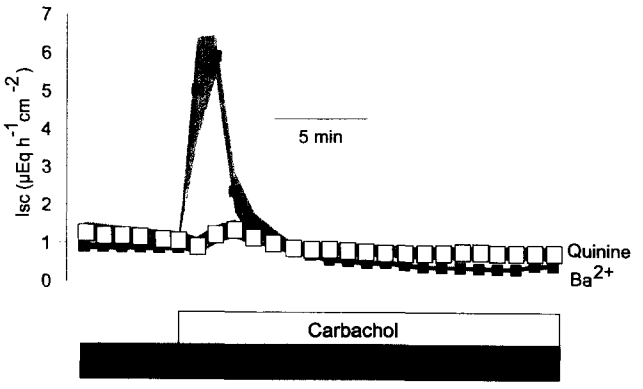

Fig. 5. Effect of carbachol (5×10^{-5} mol·l^{-1} administered on the serosal side) in the presence of serosal Ba^{2+} (10^{-2} mol·l^{-1}; filled squares) or quinine (10^{-3} mol·l^{-1}; open squares). Values are means (symbols) ± S.E. (shaded area), $n = 6$. The experiments with Ba^{2+} were performed in a HCO_3^--free buffer.

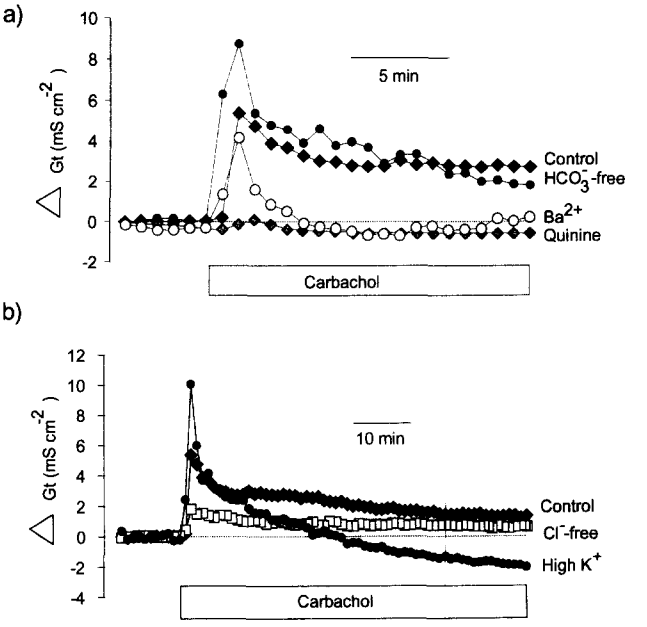

Fig. 6. Increase in Gt induced by carbachol (5×10^{-5} mol·l^{-1}). (a) Effect under control conditions (closed diamonds), in the absence of HCO_3^- ions (closed squares), and in the presence of Ba^{2+} (10^{-2} mol·l^{-1} administered on the serosal side under HCO_3^--free conditions; open circles) or quinine (10^{-3} mol·l^{-1} administered on the serosal side; dotted diamonds). (b) Effect under control conditions (closed diamonds), in the absence of Cl^- (open squares), and after increasing the extracellular K^+ concentration to 13.5 mmol·l^{-1} on both sides of the mucosa (closed circles). Values are means, $n = 6$. For statistics, see Table 1.

Carbachol, when administered under these conditions, caused only a short-lasting increase of Isc (Table 1), which was parallelled by a transient increase in Gt. Tissue conductance rose rapidly to a peak value of 10.4 ± 1.3 mS · cm^{-2} above baseline ($n = 6$, $P < 0.05$) but then fell soon below baseline (Fig. 6b). Consequently, the reduction of the plateau phase in Gt evoked by carbachol but not of the peak phase during the anion substitution experiments may be associated with the depolarization induced by Cl^- substitution.

In contrast to the K^+ channel blockers described above, staurosporine (10^{-6} mol · l^{-1}), a protein kinase C inhibitor (Tamaoki et al., 1986), blocked both the initial and the late phase of the Gt response evoked by carbachol. The biphasic increase in Isc evoked by the acetylcholine receptor agonist was also nearly suppressed (Table 1).

4. Discussion

Carbachol is an agonist of the Ca^{2+} pathway in the epithelium. The acetylcholine receptor agonist induces an increase in the cytosolic Ca^{2+} concentration (Dharmsathaphorn and Pandol, 1986). The Ca^{2+} response shows a biphasic time course in isolated rat

colonic crypts (Diener et al., 1991) consisting of an initial peak followed by a long-lasting plateau. This increase in the cytosolic Ca^{2+} concentration is paralleled by a biphasic increase in Isc (Fig. 1a). The complete dependence on the presence of Cl^- ions and the sensitivity to inhibitors of Cl^- secretion like the inhibitor of the Na^+-K^+-Cl^- cotransporter, bumetanide, or the Cl^- channel blocker, NPPB, confirm previous results (Zimmerman et al., 1981; Dharmsathaphorn and Pandol, 1986) that this increase in Isc represents a net Cl^- secretion. The increase in Isc was accompanied by an increase in Gt (Fig. 2a), which is in accordance with the assumption that carbachol induces the opening of epithelial ion channels.

The initial peak and the subsequent plateau phase differed in their sensitivity to inhibitors. The plateau phase was suppressed in the presence of cyclooxygenase inhibitor, indomethacin. In contrast, the peak response was only inhibited in the combined presence of tetrodotoxin and indomethacin (Fig. 1). At first glance, these results may suggest that the response to carbachol is mediated by enteric neurons and by prostaglandins. Indeed, carbachol has been shown to induce both a stimulation of secretomotor neurons (Zimmerman and Binder, 1983; Diener et al., 1989) as well as a release of prostaglandins (Craven and DeRubertis, 1981) in the rat colon. Alternatively, it could be discussed whether the carbachol response may be dependent on the continuous release of neurotransmitters and prostaglandins, 'sensitizing' the epithelium to the acetylcholine receptor agonist. In the rat colon in vitro, there is a spontaneous activity of submucosal secretomotor neurons responsible for the generation of basal Isc (Andres et al., 1985), and a spontaneous release of prostaglandins stimulating basal secretion directly at the epithelium and indirectly by stimulation of the secretomotor neurons (Diener et al., 1988). This basal activity was confirmed in the present experiments by the observation that in the combined presence of tetrodotoxin and indomethacin the basal Isc was reduced from 1.3 ± 0.2 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ to 0.5 ± 0.1 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ ($P < 0.05$; Table 2), i.e. to values near zero. Because prostaglandins (Craven and DeRubertis, 1981) and many neurotransmitters of the enteric nervous system like e.g. vasoactive intestinal peptide (Prieto et al., 1979) act by stimulating adenylate cyclase in the intestinal epithelium, it was tried whether restoring the basal Isc with low concentrations of forskolin or CPTcAMP had an effect on the inhibition of the carbachol response by tetrodotoxin and indomethacin. Indeed, inhibition by the combination of tetrodotoxin and indomethacin could be overcome by agonists of the cAMP pathway like forskolin or CPTcAMP (Fig. 3). In the presence of tetrodotoxin and indomethacin neither a release of prostaglandins nor stimulation of enteric secretomotor neurons by carbachol should be possible. Consequently, these results indicate that the Cl^- secretion induced by carbachol is *not mediated* by prostaglandins or neurotransmitters, *but is dependent* on both as activators of a continuous basal production of cAMP in the epithelium.

What might be the cellular basis for this interaction of the Ca^{2+} and the cAMP pathway ? Whole-cell patch-clamp experiments at isolated rat colonic crypts (Böhme et al., 1991) and at small intestinal crypts from the guinea-pig (Walters and Sepúlveda, 1991) have revealed that the only action of carbachol in these epithelia consists in the opening of a Ca^{2+}-sensitive (basolateral) K^+ conductance leading to a hyperpolarization of the enterocyte. Such a hyperpolarization increases the driving force for Cl^- secretion. This will, however, induce Cl^- secretion only if there are spontaneously open apical Cl^- channels. Due to a release of neurotransmitters and prostaglandins from the submucosal tissue, cAMP may be continuously produced in the epithelium, which is responsible for the opening of a fraction of the apical Cl^- channels by phosphorylation of e.g. the CFTR channel or the outward-rectifier Cl^- channel. These channels, which both seem to be involved in cAMP-mediated Cl^- secretion (Schwiebert et al., 1994), may provide the pathway for Cl^- efflux after the carbachol-induced hyperpolarization. Such a model has originally been proposed for the colonic epithelial cell line, the T_{84} cells, by Dharmsathaphorn and Pandol (1986) based on ^{36}Cl and ^{86}Rb (as a marker for K^+) efflux measurements. This model is supported by the present observation that although the combination of tetrodotoxin and indomethacin completely suppressed the increase in Isc induced by carbachol (Fig. 1d), it had no effect on the long-lasting increase in Gt during the second phase of the carbachol action (Fig. 2d). Only the first, fast peak phase of the increase in Gt was inhibited by these blockers (see below). In contrast, K^+ channel blockers like Ba^{2+}, charybdotoxin and quinine suppressed not only the increase in Isc but also that of Gt, at least during the second phase of the carbachol response (Fig. 6). Another K^+ channel blocker, tetraethylammonium, was ineffective, a pattern of sensitivity which is in accordance with observations on the action of carbachol on HT29.cl19A cells (Fogg et al., 1994), but contrasts with observations made on T_{84} cells (Devor and Frizzell, 1993).

Consequently, these results suggest that the main mechanism of carbachol-induced secretion is opening of Ca^{2+}-dependent K^+ channels, and not of Ca^{2+}-dependent Cl^- channels. This conclusion, i.e. the absence of intestinal Cl^- channels directly stimulated by intracellular Ca^{2+}, is supported by observations of Clarke et al. (1994), which showed that in Cftr(−/−) mice, i.e. animals with a defective CFTR channel, the secretion induced by the Ca^{2+} ionophore, ionomycin, is absent, and that in normal, i.e. Cftr(+/+) mice the

secretion induced by the ionophore is resistant to DIDS, which is an efficient inhibitor of the Ca^{2+}-dependent Cl^- channel in the murine airway epithelium (Clarke et al., 1994). Further indirect support for this conclusion arises from the present experiments with glibenclamide, a blocker of the CFTR channel (Sheppard and Welsh, 1992). As had to be expected, glibenclamide concentration dependently inhibited the response to the agonist of the cAMP pathway, forskolin, whereas DIDS, an inhibitor of both Ca^{2+}-activated Cl^- channels in the trachea (Clarke et al., 1994) and of the outwardly-rectifying Cl^- channel (Schwiebert et al., 1994), had only a minor effect (Fig. 4). The same pattern of sensitivity was observed for the carbachol-induced secretion, i.e. DIDS had no effect on the response to carbachol, whereas glibenclamide reduced the first phase of the carbachol response by 60% and suppressed the second phase completely (Table 1). However, as already observed by Hongre et al. (1994) in T_{84} cells, the concentrations of glibenclamide needed to inhibit Cl^- currents in intact epithelia were higher than those needed to inhibit Cl^- currents through recombinant CFTR (Sheppard and Welsh, 1992) and therefore effects of glibenclamide other than the inhibition of the CFTR channel like e.g. the well-known inhibitory action of the drug on ATP-sensitive K^+ channels cannot be excluded.

Two results are in obvious contradiction to the conclusion that the effect of carbachol may be exclusively explained by an increase in K^+ conductance, i.e. the partial inhibition of the carbachol-induced increase in Gt by the Cl^- channel blocker, NPPB, and by Cl^- substitution. Whereas the effect of NPPB may be easily explained by the well known K^+ channel-blocking effects of the drug (Illek et al., 1992), this does not hold true for the anion substitution experiments. However, many Ca^{2+}-dependent K^+ channels are also voltage-dependent, i.e. they are activated by depolarization (see e.g. Sheppard et al., 1988). Consequently, the depolarization induced by removing Cl^- ions from the buffer may already activate a part of the carbachol-induced (Ca^{2+}-sensitive) K^+ conductance and may thereby apparently diminish the carbachol response. Therefore, the concentration of K^+ ions in the extracellular medium was increased from 4.5 mmol $\cdot$ l^{-1} to 13.5 mmol $\cdot$ l^{-1}. Assuming an intracellular K^+ concentration of 130 mmol $\cdot$ l^{-1}, this will shift the K^+ equilibrium potential from -89 mV to -60 mV and thereby partially depolarize the epithelium. This manoeuvre induced – as expected – an increase in Isc and Gt (Table 2). Under these conditions, the effect of carbachol consisted only in a short-lasting, monophasic increase in Isc and Gt, which fell even below baseline after about 20 min. The peak response in Gt, however, was not affected by the K^+ depolarization (Fig. 6b). Consequently, the depolarization induced by Cl^- substitution may be responsible for the smaller increase in Gt during the plateau phase of the carbachol response but not for the observation that under Cl^--free conditions the increase in Gt during the peak phase was completely abolished (Fig. 6b; see below for discussion).

These results are in marked contrast with observations made on colonic tumour cell lines like T_{84} or HT29 cells (Cliff and Frizzell, 1990; Kunzelmann et al., 1992; Morris et al., 1992), where a direct activation of apical Cl^- channels by intracellular Ca^{2+} has been clearly demonstrated. However, it has been reported that the degree of cAMP- versus Ca^{2+}-induced Cl^- currents depends on the state of differentiation of the cells as judged from comparison of the less differentiated original HT29 cells with the more differentiated HT29.cl19A clone (Morris et al., 1992). In the unpolarized, undifferentiated HT29 cells the efflux of $^{125}J^-$ (a marker for Cl^-) induced by neurotensin, an agonist of the Ca^{2+} pathway, is about 3 times higher than in the polarized, more differentiated HT29.cl19A cells, and it decreases in both cell lines time dependently after seeding, i.e. after attaching to a firm support (Morris et al., 1992). In addition, no Ca^{2+}-activated Cl^- current across the apical membrane could be induced by Anderson and Welsh (1991) in T_{84}, HT29 and Caco-2 cells, when these cells had grown to confluence on a permeable filter support. Apparently, in the colonic epithelium – in contrast to the airway epithelium (Frizzell et al., 1986) – Ca^{2+}-activated Cl^- channels are only expressed (or active) in undifferentiated cells and play no major role under physiologic conditions.

In addition to the action of carbachol on Ca^{2+}-dependent K^+ channels, there is evidence for a second, synergistic interaction of the Ca^{2+} and the cAMP pathway in the rat colon. The fast, peak increase in Gt induced by carbachol was suppressed by the combination of tetrodotoxin and indomethacin (Fig. 2d), and this block could be overcome by forskolin or CPT-cAMP (Table 1). In other words, carbachol induces this conductance only in the presence of cAMP. This peak increase in Gt was completely dependent on the presence of Cl^- ions (Fig. 6b), an effect which – in contrast to the action of Cl^- substitution on the second phase of the carbachol response – cannot be explained by the depolarizing action of the Cl^--free solution. Consequently, these data suggest that carbachol activates a cellular Cl^- conductance during the first phase of its action, but this activation is only possible synergistically with the cAMP pathway. This behaviour has a parallel in results obtained on HT29.cl19A cells, where a Cl^- channel with a linear current-voltage relationship and a single channel conductance of 7.5 pS, i.e. with properties of the CFTR Cl^- channel, is synergistically activated by the cAMP and the protein kinase C pathway (Bajnath et al., 1993). In these cells phorbol 12,13-dibutyrate, a stimulator of the protein kinase C, when

given alone is ineffective, but it increases by a factor of 4 the probability of finding a cAMP-activated Cl^- channel in cell-attached patches (Bajnath et al., 1993). Because carbachol has been shown to activate protein kinase C (Cohn, 1990), one is tempted to speculate that during the initial, short-lasting phase of the carbachol response there is a synergistic activation of apical Cl^- channels by the cAMP/protein kinase A and the protein kinase C pathway. This conclusion is supported by the observation that staurosporine, a reported protein kinase C blocker (Tamaoki et al., 1986), suppressed the initial fast increase in Gt induced by carbachol, although the specificity of this drug is at least doubtful (Tamaoki et al., 1986; Diener et al., 1991).

In conclusion, these results suggest that the dominant effect of carbachol in the intact rat colonic epithelium is activation of basolateral Ca^{2+}-sensitive K^+ channels and possibly a synergistic activation of cAMP-sensitive apical Cl^- channels via the protein kinase C pathway during the initial peak phase. Both effects only lead to a net Cl^- secretion when there is a sufficient basal production of cAMP induced in the enterocytes by the spontaneous release of neurotransmitters and prostaglandins from the subepithelial tissue.

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References

Anderson, M.P. and M.J. Welsh, 1991, Calcium and cAMP activate different chloride channels in the apical membrane of normal and cystic fibrosis epithelia, Proc. Natl. Acad. Sci. USA 88, 6003.

Anderson, M.P., D.P. Rich, R.J. Gregory, A.E. Smith and M.J. Welsh, 1991, Generation of cAMP-activated chloride currents by expression of CFTR, Science 251, 679.

Andres, H., R. Bock, R.J. Bridges, W. Rummel and J. Schreiner, 1985, Submucosal plexus and electrolyte transport across rat colonic mucosa, J. Physiol. 364, 301.

Bajnath, R.B., J.A. Groot, H.R. DeJonge, M. Kansen and J. Bijman, 1993, Synergistic activation of non-rectifying small-conductance chloride channels by forskolin and phorbol esters in cell-attached patches of the human colon carcinoma cell line HT-29cl.19A, Pflüg. Arch. 425, 100.

Binder, H.J. and G.J. Sandle, 1987, Electrolyte absorption and secretion in the mammalian colon, in: Physiology of the Gastrointestinal Tract, Vol. 2, 2nd edn., ed. L.R. Johnson (Raven Press, New York) p. 1389.

Böhme, M., M. Diener and W. Rummel, 1991, Calcium- and cyclic-AMP-mediated secretory responses in isolated colonic crypts, Pflüg. Arch. 419, 144.

Clarke, L.L., B.R. Grubbe, J.J. Yankaskas, C.U. Cotton, A. McKenzie and R.C. Boucher, 1994, Relationship of a non-cystic fibrosis transmembrane conductance regulator-mediated chloride conductance to organ-level disease in *Cftr*(−/−) mice, Proc. Natl. Acad. Sci. USA 91, 479.

Cliff, W.H. and R.A. Frizzell, 1990, Separate Cl^- conductances activated by cAMP and Ca^{2+} in Cl^--secreting epithelial cells, Proc. Natl. Acad. Sci. USA 87, 4956.

Cohn, J.A., 1990, Protein kinase C mediates cholinergically regulated protein phosphorylation in a Cl^--secreting epithelium, Am. J. Physiol. 258, C227.

Craven, P.A. and F.R. DeRubertis, 1981, Stimulation of rat colonic mucosal prostaglandin synthesis by calcium and carbamylcholine: relationship to alterations in cyclic nucleotide metabolism, Prostaglandins 21, 65.

Craven, P.A. and F.R. DeRubertis, 1983, Patterns of prostaglandin synthesis and degradation in isolated superficial and proliferative colonic epithelial cells compared to residual colon, Prostaglandins 26, 583.

Devor, D.C. and R.A. Frizzell, 1993, Calcium-mediated agonists activate an inwardly rectified K^+ channel in colonic secretory cells, Am. J. Physiol. 265, C1271.

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

Diener, M. and W. Rummel, 1990, Distension-induced secretion in the rat colon: mediation by prostaglandins and submucosal neurons, Eur. J. Pharmacol. 178, 47.

Diener, M., R.J. Bridges, S.F. Knobloch and W. Rummel, 1988, Neuronally mediated and direct effects of prostaglandins on ion transport in rat colon descendens, Naunyn-Schmied. Arch. Pharmacol. 337, 74.

Diener, M., S.F. Knobloch, R.J. Bridges, T. Keilmann and W. Rummel, 1989, Cholinergic-mediated secretion in the rat colon: neuronal and epithelial muscarinic responses, Eur. J. Pharmacol. 168, 219.

Diener, M., C. Eglème and W. Rummel, 1991, Phospholipase C-induced anion secretion and its interaction with carbachol in the rat colonic mucosa, Eur. J. Pharmacol. 200, 267.

Field, M. and C.E. Semrad, 1993, Toxigenic diarrheas, congenital diarrheas, and cystic fibrosis: disorders of intestinal ion transport, Annu. Rev. Physiol. 55, 631.

Fogg, K.E., N.B. Higgs and G. Warhurst, 1994, Involvement of calmodulin in Ca^{2+}-activated K^+ efflux in human colonic cell line, HT29-19A, Biochim. Biophys. Acta 1221, 185.

Frizzell, R.A., D.R. Halm, G. Rechkemmer and R.L. Shoemaker, 1986, Chloride channel regulation in secretory epithelia, Fed. Proc. 45, 2727.

Gögelein, H. and K. Capek, 1990, Quinine inhibits chloride and nonselective cation channels in isolated rat distal colon cells, Biochim. Biophys. Acta 1027, 191.

Hongre, A.S., I. Baró, B. Berthon and D. Escande, 1994, Effects of sulphonylureas on cAMP-stimulated Cl^- transport via the cystic fibrosis gene product in human epithelial cells, Pflüg. Arch. 426, 284.

Illek, B., H. Fischer, K.M. Kreusel, U. Hegel and W. Clauss, 1992, Volume-sensitive basolateral K^+ channels in HT-29/B6 cells: block by lidocaine, quinidine, NPPB and Ba^{2+}, Am. J. Physiol. 263, C674.

Kenyon, J.L. and W.R. Gibbons, 1977, Effect of low-chloride solutions on action potentials of sheep cardiac Purkinje fibers, J. Gen. Physiol. 70, 635.

Kunzelmann, K., R. Kubitz, M. Grolik, R. Warth and R. Greger, 1992, Small-conductance Cl^- channels in HT_{29} cells: activation by Ca^{2+}, hypotonic cell swelling and 8-Br-cGMP, Pflüg. Arch. 412, 238.

Morris, A.P. and R.A. Frizzell, 1993, Ca^{2+}-dependent Cl^- channels

in undifferentiated human colonic cells (HT-29). I. Single-channel properties. Am. J. Physiol. 264, C968.

Morris, A.P., S.A. Cunnigham, D.J. Benos and R.A. Frizzell, 1992, Cellular differentiation is required for cAMP but not Ca^{2+}-dependent Cl^- secretion in colonic epithelial cells expressing high levels of cystic fibrosis transmembrane conductance regulator, J. Biol. Chem. 267, 5575.

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

Prieto, J.C., M. Laburthe and G. Rosselin, 1979, Interaction of vasoactive intestinal peptide with isolated intestinal epithelial cells from rat, Eur. J. Biochem. 96, 229.

Schwiebert, E.M., T. Flotte, G.R. Cutting and W.B. Guggino, 1994, Both CFTR and outwardly rectifying chloride channels contribute to cAMP-stimulated whole-cell chloride currents, Am. J. Physiol. 266, C1464.

Sheppard, D.N. and M.J. Welsh, 1992, Effect of ATP-sensitive K^+ channel regulators on cystic fibrosis transmembrane conductance regulator chloride currents, J. Gen. Physiol. 100, 573.

Sheppard, D.N., F. Giraldez and F.V. Sepúlveda, 1988, Kinetics of voltage- and Ca^{2+} activation and Ba^{2+} blockade of large-conductance K^+ channel from Necturus enterocytes, J. Membrane Biol. 105, 65.

Tamaoki, T., H. Nomoto, I. Takahashi, Y. Kato, M. Morimoto and F. Tomita, 1986, Staurosporine, a potent inhibitor of phospholipid/Ca^{2+} dependent protein kinase, Biochem. Biophys. Res. Commun. 135, 397.

Valverde, M.A., G.M. Mintenig and F.V. Sepúlveda, 1994, Cl^- currents of unstimulated T84 intestinal epithelial cells studied by intracellular recording, J. Membrane Biol. 137, 237.

Walters, R.J. and F.V. Sepúlveda, 1991, A basolateral K^+ conductance modulated by carbachol dominates the membrane potential of small intestinal crypts, Pflüg. Arch. 419, 537.

Zimmerman, T.W. and H.J. Binder, 1983, Effect of tetrodotoxin on cholinergic agonist-mediated colonic electrolyte transport, Am. J. Physiol. 244, G386.

Zimmerman, T.W., J.W. Dobbins and H.J. Binder, 1981, Mechanism of cholinergic stimulation of electrolyte transport in rat colon in vitro, Am. J. Physiol. 242, G116.