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Involvement of calmodulin and protein kinase C in the regulation of K^+ transport by carbachol across the rat distal colon

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Abstract

The cholinergic agonist carbachol stimulates the apical H^+–K^+–ATPase and apical as well as basolateral K^+ channels in the rat distal colon. The effect of carbachol was tested in the presence of different inhibitors of the Ca^{2+} signaling pathway in order to characterize the intracellular mechanisms involved. Both carbachol-stimulated Rb^+-efflux as well as carbachol-stimulated mucosal Rb^+-uptake were dependent on the presence of serosal Ca^{2+}. The Ca^{2+}–calmodulin antagonist calmidazolium (10^{-7} mol l^{-1}) inhibited the stimulation of mucosal and serosal Rb^+ efflux by carbachol. A similar effect had KN-62 (10^{-5} mol l^{-1}), an inhibitor of the Ca^{2+}–calmodulin-dependent kinase II, suggesting the regulation of basolateral and apical K^+ channels by this kinase. Staurosporine (10^{-6} mol l^{-1}), which potently inhibits protein kinase C, did not alter the effect of carbachol on Rb^+ efflux, although the stimulation of apical Rb^+ efflux by carbachol seemed to be less prolonged, indicating that protein kinase C is not involved in the regulation of K^+ permeability. In contrast, mucosal Rb^+ uptake, which is determined by the ouabain- and vanadate-sensitive K^+ transport via the apical H^+–K^+–ATPase, was decreased to nearly one third of control values in the presence of calmidazolium. Both calmidazolium and staurosporine, but not KN-62, prevented the stimulatory action of carbachol on the H^+–K^+–ATPase, suggesting a synergistic control of this ion pump by both Ca^{2+}–calmodulin and protein kinase C.

Keywords: Ca^{2+}; Calmodulin; Carbachol; Electrolyte transport; K^+ transport; K^+ channel; H^+–K^+–ATPase; Protein kinase C; Colon rat

1. Introduction

One of the fundamental properties of the epithelium of the distal colon in different species is the ability to actively absorb and to secrete K^+. The K^+ ions to be absorbed enter the epithelium via a an apical H^+–K^+–ATPase; their basolateral exit may be mediated by basolateral K^+ channels. K^+ ions to be secreted are intracellularly accumulated by the basolateral Na^+–K^+-$2Cl^-$-cotransporter and the Na^+–K^+–ATPase; they pass into the colonic lumen via apical K^+ channels (for review, see Binder and Sandle, 1994).

One of the dominant intracellular second messengers, which regulate colonic K^+ transport, is Ca^{2+} (McCabe and Smith, 1985; DuVall and O'Grady, 1994). In the distal colon of the rat, an increase in the intracellular Ca^{2+} concentration induced by the cholinergic agonist, carbachol, has been shown to stimulate the H^+–K^+–ATPase as indicated by an increase in the ouabain- and vanadate-sensitive uptake of $^{86}Rb^+$, a marker for K^+, across the apical membrane, and to stimulate the basolateral and the apical K^+ permeability as shown by efflux experiments (Heinke et al., 1998).

The intracellular signaling cascades mediating these responses have not yet been identified. The dominant effects of intracellular Ca^{2+} on epithelial ion transport are often mediated either by Ca^{2+}–calmodulin-dependent protein kinase II, such as, e.g., the stimulation of apical Cl^- channels in the colonic tumor cell line, T84 (Worrell and Frizzell, 1991), or they are mediated by protein kinase(s) C, such as, e.g., the inhibition of the apical Na^+–H^+ exchanger in rabbit ileum (Cohen et al., 1991). Therefore, the effect of carbachol on K^+ transport across the rat distal colon was studied in the presence and absence of different inhibitors of the Ca^{2+}-signaling pathway, i.e., inhibitors of the Ca^{2+}–calmodulin and the protein kinase C pathway.

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2. Materials and methods

2.1. Solution

The standard buffer for the experiments was a modified Parsons solution containing (mmol l^{-1}): NaCl 107, RbCl 4.5, NaHCO3 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; pH was 7.4. The Ca^{2+}-free solution was prepared like normal bathing solution, omitting the $CaCl_2$ without addition of a calcium chelating agent.

2.2. Tissue preparation

Wistar rats were used with a weight of 180–380 g. The animals had free access to water and a standard rat diet (diet no. C1000, Altromin, Lange, Germany) until the day of the experiment. Animals were killed by a blow on the head followed by exsanguination (approved by Regierungspräsidium Gießen, Gießen, Germany). The serosa and muscularis propria were stripped away to obtain a mucosa–submucosa preparation of the distal colon. Briefly, the colon was placed on a small plastic rod with a diameter of 5 mm. A circular incision was made near the anal end with a blunt scalpel and the serosa together with the lamina propria were gently removed in a proximal direction.

2.3. Uptake experiments

The measurement of Rb^+ uptake was started after an equilibration period of 60 min in Lucite chambers with a volume of 2.5 ml on each side of the tissue. When the uptake was measured in the presence of the carbachol, the drug was administered 1 min prior to the addition of $^{86}Rb^+$ (22 kBq) to either the mucosal or the serosal side of the chamber. Putative inhibitors were added 30 min prior to administration of carbachol.

Five minutes after administration of $^{86}Rb^+$, standards were taken from the labeled side and the uptake was stopped by washing the chamber with 20 ml of fresh, unlabelled buffer solution on both sides. The tissue was removed from the chamber and blotted on filter paper. This procedure took 1–2 min. The tissue was solubilized in 1 ml 0.1 N HNO_3 for 20 h at 70°C (Venglarik et al., 1990). After neutralization with 0.1 ml 1 N NaOH, the radioactivity in the sample was determined in a liquid scintillation counter. Results were calculated as uptake per area mucosa (nmol cm^{-2}).

2.4. Efflux experiments

The tissue was loaded with $^{86}Rb^+$ (74 kBq at both sides of the chamber) for 90 min in 2.5 ml Parsons solution on each side of the tissue. Putative inhibitors were administered after 60 min. Thirty minutes later, the serosal and the mucosal compartments were washed twice at 5 min intervals with 20 ml buffer. These solutions contained putative inhibitors, when they were tested. Release of $^{86}Rb^+$ into the mucosal and the serosal compartment was determined simultaneously by taking $2\times$ 0.5 ml aliquots at 6 min intervals. All aliquots were replaced by unlabelled buffer solution, containing agonists and blockers in appropriate concentrations. Correction for this replacement volume was performed. Carbachol was added 1 min after the third sample. At the end of the experiment, the resting amount of $^{86}Rb^+$ in the tissue was determined as described for the uptake experiments. Release was expressed as efflux of the actual amount of radioactivity in the tissue per minute (Mandel et al., 1986).

2.5. Drugs

Calmidazolium (compound R24571), and 1[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]4-phenylpiperazine (KN-62) were dissolved in dimethylsulphoxide (DMSO; final concentration 0.25%, v/v). The solvent for indomethacin and staurosporine was ethanol (final maximal concentration 0.25%, v/v). Carbachol was dissolved in an aqueous stock solution diluted in salt buffer just before use. If not indicated differently, drugs were from Sigma, Deisenhofen, Germany. Radioisotopes were obtained from NEN, Dreieich, FRG. The initial activity of ^{86}Rb amounted to 411 GBq g^{-1}.

2.6. Statistics

Values are given as means $\pm$ one standard error of the mean (S.E.M.). When the means of several groups had to be compared, first an analysis of variances was performed. If the analysis of variances indicated significant differences between the groups investigated, further comparison was carried out by a Student's *t*-test or by the *U*-test. An *F*-test decided which test method was to be used. Both paired and unpaired two-tailed Student's *t*-tests were applied as indicated.

3. Results

3.1. Efflux experiments

In a first series of experiments the effect of carbachol on the efflux of Rb^+ in tissues preloaded with this tracer was investigated under control conditions. Carbachol (5×10^{-5} mol l^{-1} at the serosal side) induced a rise in the efflux to the serosal compartment from $2.8\pm0.25\%$ to $4.0\pm0.41\%$ min^{-1} ($P<0.05$, $n=6$; Fig. 1A). Efflux to the mucosal side also increased from $0.62\pm0.06\%$ to

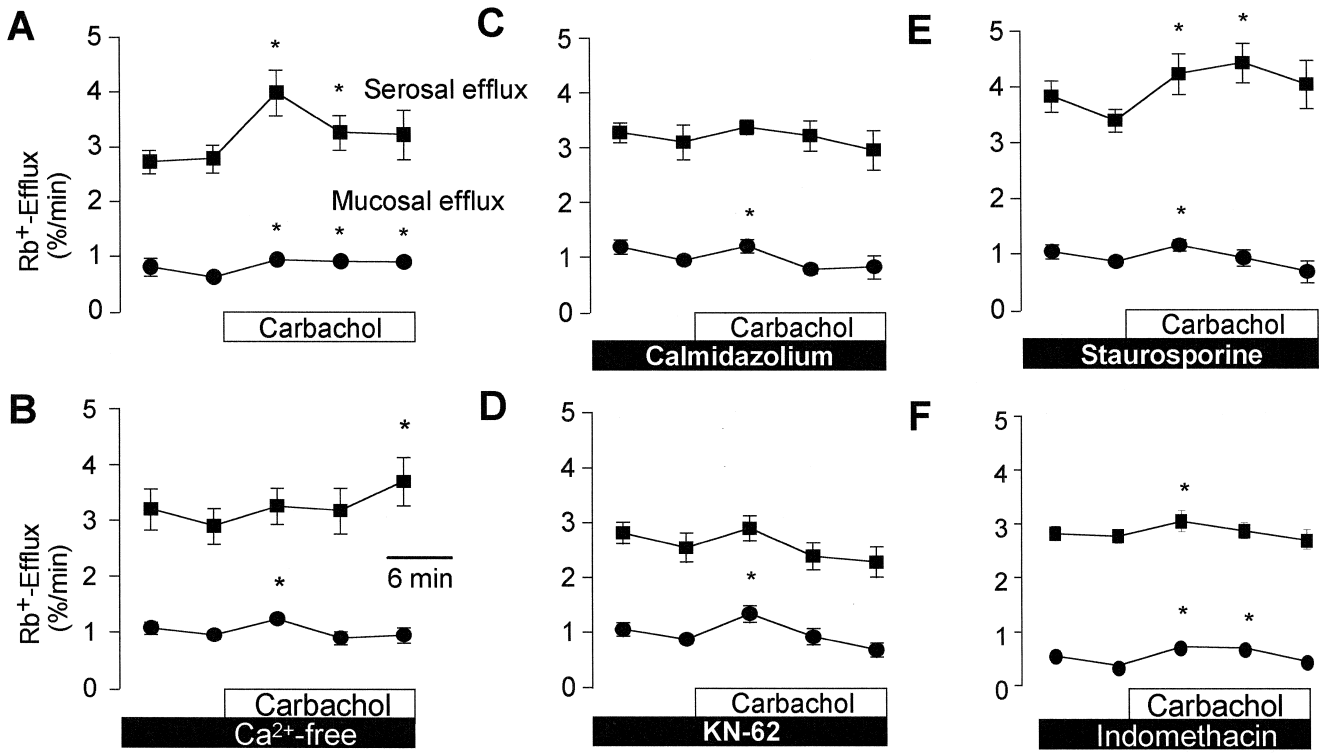

Fig. 1. Effect of carbachol (5×10^{-5} mol l^{-1} at the serosal side) on the efflux of Rb^+ on the mucosal (closed circles) and the serosal side (closed squares) under control conditions (A), in the absence of serosal Ca^{2+} (B), in the presence of calmidazolium (10^{-7} mol l^{-1} at the serosal side; C), in the presence of KN-62 (10^{-5} mol l^{-1} at the serosal side; D), in the presence of staurosporine (10^{-6} mol l^{-1} at the serosal side; E), or in the presence of indomethacin (10^{-6} mol l^{-1} at the mucosal and the serosal side; F). Values are means ± S.E.M., $n = 6–9$. * $P < 0.05$ vs. last control period (paired t-test).

$0.94 \pm 0.04\%$ min^{-1} ($P < 0.05$, $n = 6$). The stimulation of the serosal Rb^+ efflux was transient, returning close to basal levels within 12–15 min of addition despite the continued presence of the agonist, whereas the increase of the efflux into the mucosal compartment was constant over the complete duration of the experiment.

To determine whether the response to the cholinergic agonist was dependent on the presence of extracellular Ca^{2+}, the tissues were loaded with the tracer in Ca^{2+}-free Parsons solution on the serosal side. This experimental maneuver strongly inhibited the effect of carbachol on the serosal efflux and reduced its effect on the mucosal efflux of Rb^+ ($n = 8$; Fig. 1B). The small and transient increase of the mucosal efflux probably represents Ca^{2+} release from internal stores. Consequently external Ca^{2+} seems to be prerequisite for the carbachol-induced opening of the K^+ channels in the distal colon.

In order to investigate the role of Ca^{2+}–calmodulin pathway in the regulation of K^+ permeability, the effect of carbachol was tested in the presence of calmidazolium (10^{-7} mol l^{-1} at the serosal side), an inhibitor of the Ca^{2+}–calmodulin complex (see, e.g., Worrell and Frizzell, 1991). Preincubation with calmidazolium completely prevented the increase in serosal Rb^+ efflux induced by carbachol (Fig. 1C). The stimulation of mucosal efflux was reduced to a short-lived, small increase, which decayed completely after 6 min. A similar inhibition could be achieved with KN-62 (10^{-5} mol l^{-1} at the serosal side), a specific blocker of the multifunctional Ca^{2+}–calmodulin-dependent protein kinase II (Casnellie, 1991; Hidaka and Yokokura, 1996) ($n = 6$, Fig. 1D), suggesting a central role of the calmodulin pathway in the regulation of the epithelial K^+ permeability.

In contrast, staurosporine, a drug which potently inhibits protein kinase C (Tamaoki et al., 1986), was completely ineffective in inhibiting the stimulation of serosal Rb^+ efflux by carbachol (Fig. 1E, $n = 8$). The stimulation of basolateral Rb^+ efflux seemed to be even more pronounced in the presence of this inhibitor. The only inhibitory effect of staurosporine could be observed in the mucosal efflux, which was stimulated for a shorter period in the presence of staurosporine compared to its absence.

The effect of carbachol on Cl^- secretion is dependent on a prestimulation of the colonic epithelium by prostaglandins produced in the submucosal tissue (Strabel and Diener, 1995). Therefore, it seemed to be of interest to investigate whether indomethacin, a cyclooxygenase inhibitor, had an effect on the stimulation of K^+ efflux by carbachol. Indomethacin had nearly no effect on the stimulation of mucosal efflux by carbachol (Fig. 1F, $n = 9$). However, the cyclooxygenase inhibitor strongly reduced the stimulation of serosal efflux by the cholinergic agonist.

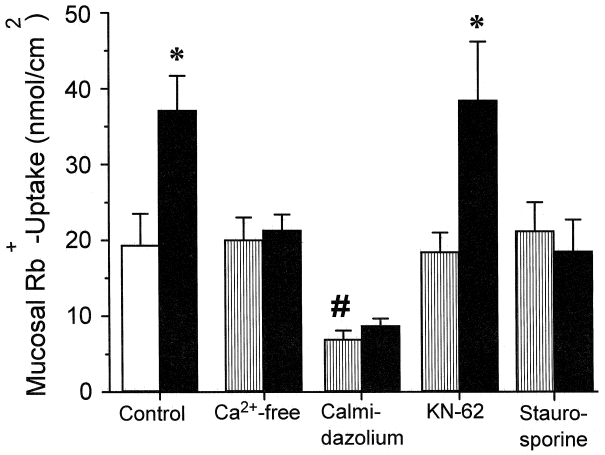

Fig. 2. Effect of carbachol (5×10^{-5} mol l^{-1} at the serosal side, black bars) on mucosal Rb^+ uptake in the distal colon under control conditions, in the absence of serosal Ca^{2+}, in the presence of calmidazolium (10^{-7} mol l^{-1} at the serosal side), KN-62 (10^{-5} mol l^{-1} at the serosal side), or staurosporine (10^{-6} mol l^{-1} at the serosal side). The white bar and the dashed bars, respectively, represent the mucosal uptake in the absence of carbachol under the individual conditions, the black bars represent the uptake in the presence of carbachol. Values are means + S.E.M., $n = 6–8$. * $P < 0.05$ vs. the uptake in the absence of carbachol (unpaired *t*-test); # $P < 0.05$ vs. uptake under control conditions (unpaired *t*-test).

3.2. Mucosal uptake

Carbachol (5×10^{-5} mol l^{-1} at the serosal side) caused a strong stimulation of mucosal Rb^+ uptake ($n = 6$, Fig. 2) representing the activation of the apical H^+–K^+–ATPase (Heinke et al., 1998). The effect of carbachol on mucosal Rb^+ uptake was completely dependent on the presence of serosal Ca^{2+} ($n = 8$). In addition, it was prevented by the calmodulin antagonist calmidazolium ($n = 6$). Calmidazolium not only suppressed the stimulation by carbachol but also diminished the basal uptake by more than 60% ($P < 0.05$). The inhibitor of the Ca^{2+}–calmodulin-dependent protein kinase II, KN-62, however, was ineffective, suggesting an effect of calmodulin on the H^+–K^+–ATPase not mediated by this kinase. Surprisingly, inhibition by calmidazolium was mimicked the protein kinase C inhibitor, staurosporine (Fig. 2).

4. Discussion

The cholinergic agonist carbachol is known to induce an increase in the intracellular Ca^{2+} concentration, which is mediated by a release of intracellularly stored Ca^{2+} followed by an influx of extracellular Ca^{2+} ions (see, e.g., Diener et al., 1991; Kerst et al., 1995). The consequence is an increase in the apical and the basolateral K^+ conductance as shown by electrical measurements using permeabilized mucosae (Schultheiß and Diener, 1997) and by efflux experiments (Heinke et al., 1998), and a stimulation of the apical H^+–K^+–ATPase (Heinke et al., 1998). As should be expected, both responses are strongly dependent on the presence of serosal Ca^{2+} (Figs. 1 and 2).

Different intracellular mechanisms seem to be involved in the mediation of these effects. The increase in apical and basolateral K^+ efflux represents the opening of K^+ channels in both cell poles. Ca^{2+}-dependent K^+ channels with single channel conductances of 16 and more than 200 pS, respectively, have been described in the basolateral (Bleich et al., 1996) and the apical membrane (Butterfield et al., 1997) of rat colonic epithelial cells, which, upon exposure to carbachol, open and thereby induce a hyperpolarization of the cell membrane (Böhme et al., 1991). Obviously, the opening of theses channels is controlled by the Ca^{2+}–calmodulin pathway as indicated by the inhibition by the specific calmodulin inhibitor, calmidazolium. A putative phosphorylation of the channel proteins in both membranes as final step of the regulation is suggested by the effect of KN-62, an inhibitor of the Ca^{2+}–calmodulin protein kinase II (Fig. 2D). In contrast, a role for protein kinase C can be excluded as a potent inhibitor of protein kinase C, staurosporine, is completely without effect (Fig. 2E).

Contrasting results regarding the regulation of K^+ conductances by intracellular Ca^{2+} have been reported from colonic tumor cells. In HT29-19A cells, only the basolateral K^+ permeability is increased by maneuvers affecting the intracellular Ca^{2+} concentration such as exposure of the cells to neurotensin (Fogg et al., 1994). The effect of this transmitter is blocked by calmodulin antagonists, suggesting a central role for this protein in the regulation of K^+ channels (Fogg et al., 1994). However, also the protein kinase C pathway has been shown to have a profound effect on basolateral K^+ channels in this cell line, as short-term exposure with protein kinase C activators such as phorbol esters induces a rise in basolateral K^+ efflux, whereas long-time exposure induces an obvious downregulation of basolateral K^+ channels (Vaandrager et al., 1991; Bajnath et al., 1992; van den Berghe et al., 1992). The expression of different isoformes of protein kinase C has been demonstrated along the crypt-surface axis of the rat colon immunohistochemically (Jiang et al., 1995). However, staurosporine, a potent protein kinase C inhibitor, had no effect on the carbachol-induced Rb^+ efflux (Fig. 1E). Also two activators of protein kinase C, phorbol 12-myristate 13-acetate (PMA; 10^{-7} mol l^{-1}) and the permeant diacylglycerol analogue 1-oleoyl-2-acetyl-*sn*-glycerol (OAG; 1.5×10^{-4} mol l^{-1}), had absolutely no effect on basolateral or apical K^+ efflux ($n = 6$ for each substance; data not shown), suggesting that in rat colonic epithelium the protein kinase C pathway does not play a pivotal role in the regulation of apical or basolateral K^+ conductance. The discrepancy to the results obtained with HT29 cells (see above) may be due to the fact that these cells possess properties which resemble more the proximal colon or the ileum (see Bajnath et al., 1992) or due to a species difference between man and rat.

Similar as it has been observed for the stimulation of Cl^- secretion by carbachol (Strabel and Diener, 1995), the stimulation of basolateral K^+ efflux was strongly reduced in the presence of the cyclooxygenase inhibitor, indomethacin (Fig. 1F). This could either mean that prostaglandins mediate, at least in part, the effect of the cholinergic agonist or that there is a permissive tone of prostaglandins in the intact mucosa leading to a basal production of cAMP, which brings the epithelium in a state, in which it can respond to the cholinergic agonist. The last hypothesis is quite improbable as most actions of cAMP are mediated by protein kinase A, which is potently inhibited by staurosporine that does not affect serosal efflux at all (Fig. 1E). On the other hand, several receptors of prostanoids, such as EP1-, EP_3-, FP- or TP-receptors, are positively coupled to the Ca^{2+}-signalling pathway (Coleman et al., 1994). Consequently, prostanoids released from the submucosal tissue after stimulation of phospholipase A_2 by carbachol (Craven and DeRubertis, 1981) may be involved in the control of basolateral K^+ conductance.

A different result was obtained for the stimulation of the apical Rb^+ uptake by carbachol, which represents the activation of the H^+–K^+–ATPase (Heinke et al., 1998). This stimulation was prevented by calmidazolium, but not by KN-62, suggesting an effect of calmodulin not mediated by the Ca^{2+}–calmodulin-dependent protein kinase II (Fig. 2). Surprisingly, the effect of calmidazolium was mimicked by staurosporine. To our knowledge, unspecific effects of staurosporine on other kinases, such as protein kinase A, are well known, but not unspecific effects on calmodulin (Casnellie, 1991). Experiments with activators of protein kinase C, phorbol 12-myristate 13-acetate (PMA; 10^{-7} mol l^{-1}, preincubated for 2 and 10 min or 2 h) and the permeant diacylglycerol analogue 1-oleoyl-2-acetyl-*sn*-glycerol (OAG; 1.5×10^{-4} mol l^{-1}), either alone, or in combination with the Ca^{2+} ionophore ionomycin (10^{-7} mol l^{-1}), did not reveal a significant stimulation of the apical K^+ uptake (data not shown, $n \geq 6$ for each series of experiments). However, also in rat colon, atypical protein kinase C-isoforms are present (Jiang et al., 1995), which are known to be independent from Ca^{2+} and which are not activated by diacylglycerol or phorbol esters (Wilkinson and Hallam, 1994), but are instead stimulated by unsaturated fatty acids or phosphatidylserine. Consequently, the most plausible explanation for this unexpected dual inhibition by calmidazolium and staurosporine may be a synergistic activation of the H^+–K^+–ATPase by both calmodulin and protein kinase C.

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References

Bajnath, R.B., van Hoeve, M.H., de Jonge, H.R., Groot, J.A., 1992. Regulation of apical Cl^- conductance and basolateral K^+ conductances by phorbol esters in HT-29cl.19A cells. Am. J. Physiol. 263, C759–66.

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

Bleich, M., Riedemann, N., Warth, R., Kerstan, D., Leipziger, J., Hor, M., van Driessche, W., Greger, R., 1996. Ca^{2+} regulated K^+ and non-selective cation channels in the basolateral membrane of rat colonic crypt base cells. Pflügers Arch. Eur. J. Physiol. 432, 1011–1022.

Böhme, M., Diener, M., Rummel, W., 1991. Calcium- and cyclic-AMP-mediated secretory responses in isolated colonic crypts. Pflügers Arch. Eur. J. Physiol. 419, 144–151.

Butterfield, I., Warhurst, G., Jones, M.N., Sandle, G.I., 1997. Characterization of apical potassium channels induced in rat distal colon during potassium adaptation. J. Physiol. 501, 537–547.

Casnellie, J.E., 1991. Protein kinase inhibitors: probes for the functions of protein phosphorylation. Adv. Pharmacol. 22, 167–205.

Cohen, M.E., Wesolek, J., McCullen, J., Rys-Sikora, K., Pandol, S., Rood, R.P., Sharp, G.W.G., Donowitz, M., 1991. Carbachol-induced and elevated Ca^{2+}-induced translocation of functionally active protein kinase C to the brush border of rabbit ileal Na^+ absorbing cells. J. Clin. Invest. 88, 855–863.

Coleman, R.A., Smith, W.L., Narumiya, S., 1994. VIII. International union of pharmacology. Classification of prostanoid receptors: properties, distributions, and structure of the receptors and their subtypes. Pharmacol. Rev. 46, 206–229.

Craven, P.A., DeRubertis, F.R., 1981. Stimulation of rat colonic mucosal prostaglandin synthesis by calcium and carbamylcholine: relationsship to alterations in cyclic nucleotide metabolism. Prostaglandins 21, 65–81.

Diener, M., Eglème, C., Rummel, W., 1991. Phospholipase C-induced anion secretion and its interaction with carbachol in the rat colonic mucosa. Eur. J. Pharmacol. 200, 267–276.

DuVall, M.D., O'Grady, S.M., 1994. cGMP and Ca^{2+} regulation of ion transport across the isolated porcine distal colon epithelium. Am. J. Physiol. 267, R1026–R1033.

Fogg, K.E., Higgs, N.B., Warhurst, G., 1994. Involvement of calmodulin in Ca^{2+}-activated K^+ efflux in human colonic cell line, HT29-19A. Biochim. Biophys. Acta 1221, 185–192.

Heinke, B., Hörger, S., Diener, M., 1998. Mechanisms of carbachol-induced alterations in K^+ transport across the rat colon. Eur. J. Pharmacol. 362, 199–206.

Hidaka, H., Yokokura, H., 1996. Molecular and cellular pharmacology of a calcium/calmodulin-dependent protein kinase II (CaM kinase II) inhibitor, KN-62, and proposal of CaM kinase phosphorylation cascades. Adv. Pharmacol. 36, 193–219.

Jiang, Y.H., Aukema, H.M., Davidson, L.A., Lupton, J.R., Chapkin, R.S., 1995. Localization of protein kinase C isozymes in rat colon. Cell Growth Differ. 69, 1381–1386.

Kerst, G., Fischer, K.G., Normann, C., Kramer, A., Leipziger, J., Greger, R., 1995. Ca^{2+} influx induced by store release and cytosolic Ca^{2+} chelation in HT29 colonic carcinoma cells. Pflügers Arch. Eur. J. Physiol. 430, 653–665.

Mandel, K.G., McRoberts, J.A., Beuerlein, G., Foster, E.S., Dharmsathaphorn, K., 1986. $Ba2^+$ inhibition of VIP- and A23187-stimulated Cl^- secretion by T84 cell monolayers. Am. J. Physiol. 250, C486–94.

McCabe, R.D., Smith, P.L., 1985. Colonic potassium and chloride secretion: role of cAMP and calcium. Am. J. Physiol. 248, C103–C109.

Schultheiß, G., Diener, M., 1997. Regulation of apical and basolateral K^+ conductances in the rat colon. Br. J. Pharmacol. 122, 87–94.

Strabel, D., Diener, M., 1995. Evidence against direct activation of

chloride secretion by carbachol in the rat distal colon. Eur. J. Pharmacol. 274, 181–191.

Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., Tomita, P., 1986. Staurosporine, a potent inhibitor of phospholipid/Ca^{2+} dependent protein kinase. Biochem. Biophys. Res. Commun. 135, 397–402.

Vaandrager, A.B., van den Berghe, N., Bot, A.G.M., de Jonge, H.R., 1991. Phorbol ester stimulate and inhibit Cl^- secretion by different mechanisms in a colonic cell line. Am. J. Physiol. 262, G249–G256.

van den Berghe, N., Vaandrager, A.B., Bot, A.G., Parker, P.J., de Jonge, H.R., 1992. Dual role for protein kinase Cα as a regulator of ion secretion in the HT29.cl19A human colonic cell line. Biochem. J. 285, 673–679.

Venglarik, C.J., Bridges, R.J., Frizzell, R.A., 1990. A simple assay for agonist-regulated Cl and K conductances in salt-secreting epithelial cells. Am. J. Physiol. 259, C358–C364.

Wilkinson, S.E., Hallam, T.J., 1994. Protein kinase C: is its pivotal role in cellular activation over-stated?. Trends Pharmacol. Sci. 15, 53–57.

Worrell, R.T., Frizzell, R.A., 1991. CaMKII mediates stimulation of chloride conductance by calcium in T84 cells. Am. J. Physiol. 260, C877–C882.