Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 512 (2005) 67–71

www.elsevier.com/locate/ejphar

The epidermal growth factor-pathway is not involved in down-regulation of Ca^{2+}-induced Cl^- secretion in rat distal colon

Gerhard Schultheiss, Martin Diener*

Institut für Veterinär-Physiologie, Justus-Liebig-Universität Gießen, Frankfurter Str. 100, D-35392 Gießen, Germany

Received 25 November 2004; received in revised form 11 February 2005; accepted 18 February 2005
Available online 17 March 2005

Abstract

Ca^{2+}-dependent secretagogues such as carbachol induce a transient Cl^- secretion followed by long-lasting inhibition (run-down) of secretion. In the colonic tumour cell line, T84, epidermal growth factor (EGF) inhibits Ca^{2+}-dependent secretion, whereas antagonists of the EGF-signalling pathway slow down its run-down. The aim of the present study was to investigate whether a similar mechanism underlies the down-regulation of carbachol-induced Cl^- secretion measured as change in short-circuit current (I_{sc}) in a native intestinal epithelium, i.e. rat distal colon.

In contrast to the colonic tumour cell line, EGF (1–100 μg/l) induced a transient secretory I_{sc} and did not interfere with a subsequent administration of carbachol. Pretreatment with inhibitors of enzymes involved in the signalling cascade induced by EGF, i.e. tyrphostin AG1478, an inhibitor of the EGF receptor protein tyrosine kinase, PD 98059, an inhibitor of MAP kinase, and wortmannin, a blocker of the phosphatidylinositol-3-kinase, did also not affect the action of carbachol on transepithelial I_{sc}. In order to investigate potential effects of these inhibitors on apical Cl^- channels, the basolateral membrane was depolarized and a Cl^- current across the apical membrane was driven by a Cl^- gradient. Under these conditions, carbachol evoked a transient increase in I_{sc}, caused by the stimulation of Ca^{2+}-dependent Cl^- channels, followed by a long-lasting down-regulation of apical Cl^- conductance leading to a decrease in I_{sc}. All blockers of the EGF-signalling pathway tested did not interfere with the action of carbachol at the apical membrane. Consequently, the EGF-pathway seems not to be involved in the down-regulation of Ca^{2+}-dependent Cl^- secretion across rat colon.

Keywords: Carbachol; Cl^- channel; Rat colon

1. Introduction

An increase in the intracellular Ca^{2+} concentration, induced e.g. by the stable acetylcholine derivative carbachol, in colonic epithelial cells evokes an anion secretion with a characteristic time course: a strong increase in anion secretion, e.g. measured as increase in short-circuit current (I_{sc}) under voltage-clamp conditions, is followed by long-lasting decrease (see e.g. Strabel and Diener, 1995). The secondary decrease in I_{sc} is not just only a run-down of the secretory response, but also seems to involve an active, antisecretory mechanism induced by carbachol or other Ca^{2+}-dependent secretagogues. This has been shown by the ability of carbachol to inhibit the long-lasting secretagogue-induced increase in I_{sc} when this drug is applied during the plateau phase of the Cl^- secretion induced e.g. by a clostridial phospholipase C in rat distal colon (Diener et al., 1991), by prostaglandin E_2 in T84 cells (Warhurst et al., 1991), or by forskolin in colony 1 epithelial cells (Holliday and Cox, 1999).

For T84 cells, an interesting model has been developed to explain this long-lasting inhibition of anion secretion by carbachol. In this colonic tumour cell line, carbachol has been demonstrated to cause a so-called transactivation of the receptor for epidermal growth factor (EGF) and the consecutive stimulation of the extracellular signal-regulated kinase (ERK) isoforms of mitogen-activated protein (MAP) kinase (Keely et al., 1998). Tyrphostin AG1478, an inhibitor of the EGF receptor protein tyrosine kinase, and PD 98059,

* Corresponding author. Tel.: +49 641 99 38161; fax: +49 641 99 38159.
E-mail address: martin.diener@vetmed.uni-giessen.de (M. Diener).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.02.025

an inhibitor of MAP kinase, potentiated the secretory response evoked by carbachol and slowed down its inactivation (Keely et al., 1998), suggesting a role of this pathway in the mediation of the antisecretory action of carbachol in T84 cells. For the inhibitory action of EGF itself on Ca^{2+}-dependent secretion, the wortmannin-sensitive phosphatidylinositol (PI) 3-kinase, leading to the production of 3-phosphorylated lipids, has been observed to play a central role in T84 cells (Chow et al., 2000). There are no data available whether this pathway is also involved in the down-regulation of Ca^{2+}-dependent anion secretion in native colonic epithelium. Therefore, in the present study, EGF and typical inhibitors of the signalling cascade induced by EGF-receptor activation were tested for their ability to interfere with Ca^{2+}-dependent Cl^- secretion evoked by carbachol in rat distal colon.

2. Materials and methods

2.1. Solutions

The Ussing-chamber experiments were carried out in a bathing solution containing (in mM): 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 a gas mixture of 5% CO_2 and 95% O_2 (v/v); the pH was 7.4. For the depolarization of the basolateral membrane, a 111.5 mM KCl solution was used, in which NaCl was equimolarly replaced by KCl. In order to apply a serosally to mucosally directed Cl^- gradient, NaCl at the apical side was equimolarly substituted by K gluconate using a mucosal buffer solution containing (in mM): K gluconate 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 5.75, $MgSO_4$ 1, and glucose 12.

2.2. Tissue preparation

Wistar rats were used with a weight of 180–220 g. The animals had free access to water and food until the day of the experiment. Animals were stunned by a blow on the head and killed by exsanguination (approved by Regierungspräsidium Gießen, Gießen, Germany). The serosa and muscularis propria were stripped away by hand to obtain the mucosa–submucosa preparation of the colon descendens. Two segments of the distal colon of each rat were prepared; in general, one was treated with drugs acting on the EGF-pathway, the other served as control treated only with the solvent for the drug under investigation.

2.3. Short-circuit current measurement

The tissue was mounted in a modified Ussing-chamber, bathed with a volume of 3.5 ml on each side of the mucosa, and short-circuited by a voltage clamp (Ing. Buero Mußler, Aachen, Germany) with correction for solution resistance. The exposed surface of the tissue was 1 cm^2. Short-circuit current (I_{sc}) was continuously recorded and tissue conductance (Gt) was measured every minute. I_{sc} is expressed as $\mu Eq/h/cm^2$, i.e. the flux of a monovalent ion per time and area with 1 $\mu Eq/h/cm^2 = 26.9\ \mu A/cm^2$. Tissues were left for about 1 h to stabilize I_{sc}, before the effect of drugs was studied. The baseline in electrical parameters was determined as mean over 3 min just before administration of a drug and effects of drugs were given as difference to this baseline (ΔI_{sc}). In the case of carbachol, which causes a strong, but transient anion secretion, the maximum increase in I_{sc} (peak) above baseline and the ΔI_{sc} 10 min after administration of the cholinergic agonist is given in Tables 1 and 2.

2.4. Drugs

PD 98059 (2′-amino-3′-methoxyflavone; Calbiochem, Bad Soden, Germany), tyrphostin AG 1478 (4-(3-chloranilino)-6,7-dimethoxyquinazoline; Calbiochem, Bad Soden, Germany), and wortmannin (Alomone Labs, Jerusalem, Israel) were dissolved in dimethylsulfoxide (DMSO; final maximal concentration 1 ml/l). Carbachol was dissolved in aqueous stock solution and diluted in salt buffer just before use. Epidermal growth factor (human recombinant) was

Table 1
Effect of drugs acting on the EGF-signalling pathway on basal and carbachol-stimulated I_{sc}

Drug	With drug ΔI_{sc} ($\mu Eq/h/cm^2$)	Without drug ΔI_{sc} ($\mu Eq/h/cm^2$)	*n*
EGF 1 μg/l	0.2 ± 0.1^a	–	8
EGF 5 μg/l	0.3 ± 0.1^a	–	8
EGF 10 μg/l	0.5 ± 0.2^a	–	8
EGF 50 μg/l	0.8 ± 0.2^a	–	8
EGF 100 μg/l	0.8 ± 0.2^a	–	8
Carbachol peak	7.7 ± 0.9^a	10.8 ± 1.4^a	8
Carbachol 10 min	3.0 ± 0.9^a	3.0 ± 0.7^a	8
Tyrphostin 10^{-7} M	0.1 ± 0.1	–	7
Tyrphostin $5 \cdot 10^{-7}$ M	0.1 ± 0.1	–	7
Tyrphostin 10^{-6} M	0.1 ± 0.1	–	7
Carbachol peak	10.7 ± 0.6^a	10.8 ± 1.4^a	7–8
Carbachol 10 min	3.5 ± 0.9^a	3.0 ± 0.7^a	7–8
PD 98059 10^{-6} M	-0.3 ± 0.1^a	–	5
PD 98059 10^{-5} M	-0.4 ± 0.2	–	5
PD 98059 $2.5 \cdot 10^{-5}$ M	0.0 ± 0.3	–	5
PD 98059 $5 \cdot 10^{-5}$ M	-0.5 ± 0.3	–	5
Carbachol peak	4.5 ± 0.5^a	5.0 ± 0.6^a	5–6
Carbachol 10 min	1.4 ± 0.3^a	2.4 ± 0.6^a	5–6
Wortmannin 10^{-7} M	-0.1 ± 0.3	–	8
Carbachol peak	9.5 ± 1.3^a	11.7 ± 1.1^a	6–8
Carbachol 10 min	0.3 ± 0.7	1.4 ± 0.7^a	6–8

EGF, PD 98059, tyrphostin AG1478, or wortmannin were applied to the mucosal and the serosal side, then carbachol was administered at a concentration of $5 \cdot 10^{-5}$ M at the serosal side. For carbachol, the maximal increase in I_{sc} (peak) and the current 10 min after administration of the cholinergic agonist are given. Values are presented as difference to the baseline prior drug administration (ΔI_{sc}) and are means ± S.E.M.

[a] $P < 0.05$ versus baseline.

Table 2
Effects of inhibitors of the EGF signalling pathway on carbachol-induced changes in apical Cl^- conductance

Inhibitor	With inhibitor ΔI_{sc} (μEq/h/cm^2)	Without inhibitor ΔI_{sc} (μEq/h/cm^2)	*n*
Tyrphostin 10^{-6} M:			
Carbachol peak	0.3 ± 0.1^a	0.2 ± 0.0^a	9–10
Carbachol 10 min	-1.3 ± 0.2^a	-0.9 ± 0.1^a	9–10
PD 98059 $2.5 \cdot 10^{-5}$ M:			
Carbachol peak	0.4 ± 0.1^a	0.3 ± 0.1^a	6–7
Carbachol 10 min	-0.9 ± 0.2^a	-0.8 ± 0.2^a	6–7
Wortmannin 10^{-7} M:			
Carbachol peak	0.2 ± 0.0^a	0.2 ± 0.1^a	7–8
Carbachol 10 min	-1.2 ± 0.2^a	-0.9 ± 0.1^a	7–8

Response to carbachol ($5 \cdot 10^{-5}$ M at the serosal side), in basolaterally depolarized tissue in the presence of a Cl^- gradient (111.5 mM KCl at the serosal side, 107 mM K gluconate/4.5 mM KCl at the mucosal side). The maximal increase in I_{sc} (peak) and the current 10 min after administration of the cholinergic agonist is given in the presence (middle column) and the absence (right column) of putative inhibitors (indicated in the left column), which all were administered at the mucosal and the serosal side. Values are given as difference to the baseline prior to drug administration (ΔI_{sc}) and are means ± S.E.M.

[a] $P < 0.05$ versus baseline.

dissolved in 10 mM acetic acid with 1 g/l bovine serum albumin (BSA). If not indicated differently, drugs were from Sigma, Deisenhofen, Germany.

2.5. Statistics

Results are given as means ± 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 (paired or unpaired as appropriate) or by the Mann Whitney *U*-test. An *F*-test was applied to decide which test method was to be used. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Action of EGF on basal and carbachol-stimulated secretion

In a first set of experiments, EGF (1–100 μg/l) was cumulatively applied to the mucosal and the serosal side. In contrast to the antisecretory effect observed in colonic tumour cells, EGF did not induce a decrease in I_{sc} across rat distal colon. In contrast, EGF induced a concentration-dependent increase in I_{sc}, which was maximal at a concentration between 50 and 100 μg/l (Fig. 1A; Table 1). When subsequently carbachol was added, the cholinergic agonist induced a strong increase in I_{sc}, which was not different from a time-dependent control (Fig. 1A; Table 1).

Also when EGF (50 μg/l at both sides) was administered during the decaying phase of the I_{sc} response evoked by carbachol, the growth factor stimulated a further increase in I_{sc} (Fig. 1B).

3.2. Blockers of the EGF-signalling pathway

Different inhibitors of the EGF-signalling pathway were tested for their ability to alter basal or carbachol-stimulated I_{sc}. Neither pretreatment with tyrphostin AG1478, an inhibitor of the EGF receptor protein tyrosine kinase (Keely et al., 1998), nor PD 98059, an inhibitor of MAP kinase of the ERK form (Keely and Barrett, 2003), nor wortmannin, a PI3-kinase blocker (Duronio et al., 1998), did enhance the effect of a subsequent administration of carbachol (Table 1). In contrast, in the case of wortmannin, the run-down of Ca^{2+}-induced secretion was even accelerated. In the wortmannin-pretreated tissue, carbachol stimulated an I_{sc} which 10 min after administration had fallen to a value no more distinguishable from the baseline (Table 1). With the exception of PD 98059, inducing a slight decrease in I_{sc} (but not the expected increase when assuming an antisecretory

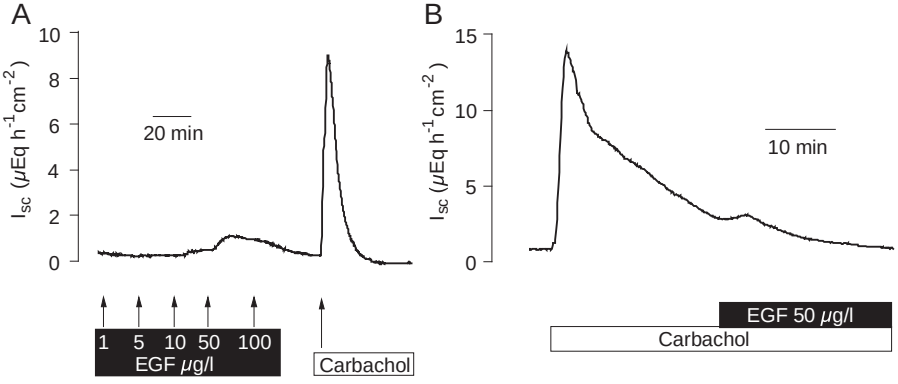

Fig. 1. (A) Concentration-dependent effect of EGF (cumulatively administered at the mucosal and the serosal side) on I_{sc} followed by the administration of carbachol ($5 \cdot 10^{-5}$ M at the serosal side). The response to carbachol was unaltered by pretreatment with EGF when compared with an untreated control (Table 1). (B) Also when applied during the decaying phase of the I_{sc} induced by carbachol ($5 \cdot 10^{-5}$ M at the serosal side), EGF (50 μg/l at both sides) did not lead to a decrease in I_{sc} but rather stimulated a transient increase in current, which in average amounted to 0.8 ± 0.2 μEq/h/cm^2 ($P < 0.05$, $n = 8$). Typical tracings for 8 experiments under each condition.

action of MAP kinase(s)), none of the inhibitors tested had a pronounced effect on baseline I_{sc} (Table 1).

3.3. Effects on apical Cl^- conductance

The dominant action of carbachol, mainly responsible for the increase in transepithelial I_{sc}, is the opening of basolateral K^+ channels leading to the hyperpolarization of the membrane and thereby increasing the driving force for apical Cl^- exit via anion channels (Strabel and Diener, 1995). However, when the action of carbachol on K^+ conductance is suppressed (see below), an additional action site of carbachol is unmasked, i.e. the transient activation of a Ca^{2+}-dependent Cl^- conductance followed by a long-lasting inhibition of Cl^- currents across the apical membrane (Schultheiss et al., 2003). In order to investigate potential effects of the EGF pathway on apical Cl^- channels, the basolateral membrane was depolarized by exchanging equimolarly the NaCl of the standard buffer solution by KCl (111.5 mM KCl buffer solution at the serosal side). This maneuver electrically eliminates the basolateral membrane (Schultheiss et al., 2003). In order to drive a Cl^- current across the apical membrane, a Cl^- gradient was applied, i.e. NaCl in the mucosal compartment was equimolarly substituted by K gluconate (107 mM K gluconate/4.5 mM KCl buffer on the mucosal side). As the only concentration gradient at the apical membrane is then a Cl^- gradient (the K^+ concentration being equal at the mucosal and the serosal side), this procedure allows to measure changes in the apical anion conductance. Under these conditions, carbachol induced a biphasic change in I_{sc}: a transient increase representing the activation of Ca^{2+}-dependent Cl^- channels (Schultheiss and Diener, 2004) followed by a long-lasting inhibition of I_{sc} (Fig. 2). Pretreatment with tyrphostin AG1478, PD 98059, or wortmannin did not change the action of a subsequent administration of carbachol on apical Cl^- conductance (Table 2).

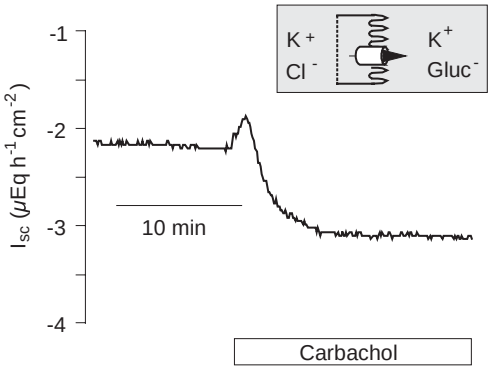

Fig. 2. Carbachol ($5 \cdot 10^{-5}$ M at the serosal side) transiently stimulates a Cl^- current across the apical membrane (indicated by an increase in I_{sc}) followed by a long-lasting inhibition. Tissues were basolaterally depolarized in the presence of a Cl^- gradient (111.5 mM KCl at the serosal side; 107 mM K gluconate/4.5 mM KCl at the mucosal side as indicated by the schematic inset). Typical tracing from 23 experiments; for statistics, see Table 2.

4. Discussion

Epidermal growth factor is a mitogenic polypeptide mediating its actions via a specific EGF receptor (Carpenter and Cohen, 1990). This receptor possesses an intrinsic protein tyrosine kinase activity, leading to autophosporylation of the receptor after EGF binding and dimerization of receptor molecules (for review see Herbst, 2004). Several enzymatic pathways are activated in parallel after receptor phosphorylation. A central one consists in the stimulation of the small GTP-binding protein Ras via an adapter molecule (GRB2) and a guanine nucleotide exchange factor (mSOS) leading to the activation of the MAPK pathway (Seger and Krebs, 1995), which essentially consists in the sequential phosphorylation of different proteins via kinases such as MAP3K, MAP2K, and MAPK. Among the latter are extracellular signal-regulated kinase(s) (ERK), which in T84 cells are phosphorylated after exposure to EGF (Keely et al., 1998). Other signalling pathways initiated upon EGF receptor stimulation are a phospholipase-γ responsible for the production of inositol-1,4,5-trisphospate (IP_3), or the PI3-kinase phosphorylating the 3-OH position of the inositol ring of different phosphoinositides lipids leading to the production of e.g. phosphatidylinositol-(3,4)-bisphosphate or phosphatidylinositol-(3,4,5)-trisphosphate able to stimulate different types of protein kinase C (Duronio et al., 1998).

This EGF signalling cascade can also be transactivated by different agonists acting at G-protein-coupled receptors, although the mechanism(s) are not yet fully understood (Filardo, 2002; Wu and Cunnick, 2002). For T84 cells, calmodulin has been proposed to be involved in the phosphorylation of the EGF-receptor following muscarinic M_3-receptor activation by carbachol (Keely and Barrett, 2003). The consequences are e.g. an enhanced phosphorylation of ERK and of the EGF receptor itself (Keely et al., 1998).

In the colonic epithelial tumour cell line, T84, the EGF-cascade and the transactivation of the EGF receptor by carbachol have been unequivocally shown to be involved in the down-regulation of Ca^{2+}-dependent Cl^- secretion. This is supported by functional and biochemical studies, in which tyrphostin AG1478, an inhibitor of the EGF receptor protein tyrosine kinase, and PD 98059, an inhibitor of ERK-type MAP kinase, potentiated the secretory response evoked by carbachol and slowed down its inactivation (Keely et al., 1998), whereas EGF itself inhibited Ca^{2+}-dependent anion secretion via a wortmannin-sensitive PI3-kinase (Chow et al., 2000). However, none of these inhibitors showed the expected action on carbachol-induced I_{sc}, when these drugs were tested at a native colonic epithelium, i.e. rat distal colon (Fig. 1; Table 1).

One part of the long-lasting inhibition of Cl^- secretion by carbachol seems to take place at the apical membrane, because in basolaterally depolarized rat colonic epithelium carbachol suppresses the Cl^- current induced by forskolin,

which is carried by the CFTR (cystic fibrosis transmembrane regulator) Cl^- channel (Schultheiss et al., 2001). In order to investigate potential effects of antagonists on apical Cl^- channels, the basolateral membrane was depolarized and a Cl^- current across the apical membrane was driven by a Cl^- gradient. Under these conditions, carbachol evoked a transient increase in I_{sc} (Fig. 2), caused by the stimulation of Ca^{2+}-dependent Cl^- channels (Schultheiss and Diener, 2004), followed by a long-lasting down-regulation of apical Cl^- conductance leading to a decrease in I_{sc}. Again, none of these responses was altered by the inhibitors of the EGF-signalling cascade tested (Table 2). These data strongly suggest that, in a native colonic epithelium, mechanisms other than enzymes involved in EGF signalling must be responsible for the down-regulation of Ca^{2+}-dependent anion secretion.

The nature of the antisecretory pathway in rat colonic can only be speculated about. One candidate are fatty acids, produced after the stimulation of phospholipase A_2 caused by the carbachol-induced increase in the intracellular Ca^{2+} concentration (Schultheiss et al., 2001). Another candidate might be inositol-3,4,5,6-tetrakisphosphate (Vajanaphanich et al., 1994). However, this metabolite does not block CFTR (Shears, 1998), which is the predominant apical Cl^- channel in rat colonic epithelium (Greger, 2000).

Consequently, other mechanisms, e.g. direct protein–protein interaction between the Ca^{2+}-dependent Cl^- channel in the apical membrane and the CFTR or others must be responsible for the down-regulation of anion secretion by carbachol in rat distal colon.

Acknowledgment

The diligent care of Mrs. B. Brück, E. Haas, A. Metternich, and B. Schmidt is a pleasure to acknowledge. Supported by Deutsche Forschungsgemeinschaft, grant Di 388/6-3.

References

Carpenter, G., Cohen, S., 1990. Epidermal growth factor. J. Biol. Chem. 265, 7709–7712.

Chow, J.Y.C., Uribe, J.M., Barrett, K.E., 2000. A role for protein kinase C epsilon in the inhibitory effect of epidermal growth factor on calcium-stimulated chloride secretion in human colonic epithelial cells. J. Biol. Chem. 275, 21169–21176.

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.

Duronio, V., Scheid, M.P., Ettinger, S., 1998. Downstream signalling events regulated by phosphatidylinositol 3-kinase activity. Cell. Signal. 10, 233–239.

Filardo, E.J., 2002. Epidermal growth factor receptor (EGFR) transactivation by estrogen via the G-protein-coupled receptor, GPR30: a novel signaling pathway with potential significance for breast cancer. J. Steroid Biochem. Mol. Biol. 80, 231–238.

Greger, R., 2000. Role of CFTR in the colon. Annu. Rev. Physiol. 62, 467–491.

Herbst, R.S., 2004. Review of epidermal growth factor receptor biology. Int. J. Radiat. Oncol. Biol. Phys. (59 Suppl.), S21–S26.

Holliday, N.D., Cox, H.M., 1999. Modulation of chloride, potassium and bicarbonate transport by muscarinic receptors in a human adenocarcinoma cell line. Brit. J. Pharmacol. 126, 269–279.

Keely, S.J., Barrett, K.E., 2003. p38 mitogen-activated protein kinase inhibits calcium-dependent chloride secretion in T84 colonic epithelial cells. Am. J. Physiol., Cell Physiol. 284, C339–C348.

Keely, S.J., Uribe, J.M., Barrett, K.E., 1998. Carbachol stimulates transactivation of epidermal growth factor receptor and mitogen-activated protein kinase in T84 cells: implications for carbachol-stimulated chloride secretion. J. Biol. Chem. 273, 27111–27117.

Schultheiss, G., Diener, M., 2004. Ca^{2+}-activated apical Cl^--conductance in rat colon in vitro. J. Physiol. Biochem. 60, 142 (Abstract; full length paper in preparation).

Schultheiss, G., Ribeiro, R., Diener, M., 2001. Fatty acids inhibit anion secretion in rat colon: apical and basolateral action sites. Pflügers Arch. Eur. J. Physiol. 442, 603–613.

Schultheiss, G., Ribeiro, R., Schäfer, K.H., Diener, M., 2003. Activation of apical K^+-conductances by muscarinic receptor stimulation in rat distal colon: fast and slow components. J. Membr. Biol. 195, 183–196.

Seger, R., Krebs, E.G., 1995. The MAPK signaling cascade. FASEB J. 9, 726–735.

Shears, S.S.B., 1998. The versatility of inositol phosphates as cellular signals. Biochim. Biophys. Acta 1436, 49–67.

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.

Vajanaphanich, M., Schultz, C., Rudolf, M.T., Wasserman, M., Enyedi, P., Craxton, A., Shears, S.B., Tsien, R.Y., Barrett, K.E., Traynor-Kaplan, A., 1994. Long-term uncoupling of chloride secretion from intracellular calcium levels by $Ins(3,4,5,6)IP_4$. Nature 371, 711–714.

Warhurst, G., Higgs, N.B., Tonge, A., Turnberg, L.A., 1991. Stimulatory and inhibitory actions of carbachol on chloride secretory responses in human colonic cell line T84. Am. J. Physiol.: Gasterointest. Liver Physiol. 261, G220–G228.

Wu, J., Cunnick, J.M., 2002. Trans-regulation of epidermal growth factor receptor by lysophosphatidic acid and G protein-coupled receptors. Biochim. Biophys. Acta, Mol. Cell Biol. Lipids 1582, 100–106.