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The κ-opioid receptor agonist asimadoline inhibits epithelial transport in mouse trachea and colon

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Abstract

The potent κ-opioid receptor agonist *n*-methyl-*N*-[(1*S*)-1-phenyl-2-((3*S*)-3-hydroxypyrrolidin-1-yl)-ethyl]-2,2-diphenyl-acetamide hydrochloride (asimadoline, EMD 61753) was initially developed for the treatment of chronic pain. Because opioids are well known to reduce secretion and to cause constipation, we investigated the effects on epithelial transport in murine trachea and colon. In Ussing chamber experiments, asimadoline (100 μM) decreased short-circuit currents in airways and colon epithelium. The inhibition of I_{SC} was not blocked by naloxone (10 μM) or nor-binaltorphimine (10 μM), suggesting that the response was not mediated by κ-opioid receptors. The effect of asimadoline on I_{SC} was concentration-dependent with half-maximal inhibition of I_{SC} at 23.7 (9.5–49.3) μM and was sensitive to the K^+ channel blocker charybdotoxin (10 nM). The amiloride-sensitive Na^+ current was reduced by asimadoline, but not in cAMP stimulated tissues. Asimadoline strongly inhibited transient Ca^{2+}-dependent Cl^- secretion, activated by the muscarinic receptor agonist carbachol (100 μM) or the purinergic agonist ATP (100 μM). Thus, asimadoline inhibits epithelial transport independent of κ-opioid receptors, by inhibition of basolateral Ca^{2+}-activated and charybdotoxin-sensitive K^+ channels.

Keywords: Asimadoline; κ-Opiate receptor agonist; Epithelial transport; EMD 61753

1. Introduction

Active absorption of Na^+ and secretion of Cl^- across the intestinal epithelium are crucial for maintaining salt balance, digestive processes and defense against infections. Opioids are well known to reduce epithelial secretion and to promote salt and water absorption predominantly by activation of δ and μ receptors (Poonyachoti et al., 2001; Quito and Brown, 1991; Sheldon et al., 1990). These antisecretory actions of opioids take part in the known antidiarrheal and constipating effects in the intestine. *N*-Methyl-*N*-[(1*S*)-1-phenyl-2-((3*S*)-3-hydroxypyrrolidin-1-yl)-ethyl]-2,2-diphenyl-acetamide hydrochloride (asimadoline, EMD 61753) is a peripherally acting, potent selective κ-opioid receptor agonist developed for the treatment of pain of various origins (Barber et al., 1994; Gottschlich et al., 1995), and is still under clinical investigation (Phase II, Merck, Darmstadt, Germany). Beside specific effects of asimadoline on κ-opioid receptors, asimadoline has also non-κ-opioid receptor sites of action (Joshi et al., 2000; Ozaki et al., 2000; Sengupta et al., 1999; Su et al., 2000), which may contribute to its effects in animal (Ozaki et al., 2000; Su et al., 2000) and in human models (Delgado-Aros et al., 2003; Delvaux et al., 2003) of irritable bowl syndrome. Previous studies showed a non-opioid receptor-mediated action on Na^+ and K^+ channel function at higher doses of κ-opioid receptor agonists (Joshi et al., 2003; Pugsley, 2002). The aim of the present study was to investigate the mechanisms by which asimadoline acts on transepithelial transport in mouse trachea and colon and the possible κ-opioid receptors involved. To that end, transepithelial voltages and equivalent short circuit currents

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(I_{SC}) were measured in isolated epithelia from colon and trachea in a modified Ussing chamber set-up. The effects of asimadoline on basal and stimulated electrolyte transport were determined.

2. Materials and methods

2.1. Materials

All used compounds were of highest available grade of purity. Asimadoline (EMD 61753) was from Merck, Germany. ATP, carbachol, 3-isobutyl-1-methyl-xanthine (IBMX), forskolin and amiloride, naloxone and nor-binaltorphimine were from Sigma, Calbiochem and Boehringer.

2.2. Ussing chamber experiments and statistical analysis

For tissue preparations, 6–8 weeks old mice (BL/6-mice, Charles River, Germany) were sacrificed by cervical dislocation. After removal, tracheas were freed of connective tissue and opened by a longitudinal cut. Mouse distal colon was separated mechanically from submucosal tissues. The colonic mucosa was pre-incubated for 30 min in ice cold bath solution (mM: NaCl 145, KH_2PO_4 0.4, K_2HPO_4 1.6, D-glucose 6, $MgCl_2$ 1, Ca-gluconate 1.3, pH 7.4) containing amiloride (20 μM) and indomethacin (10 μM). The tissues were mounted into a modified Ussing chamber with a circular aperture of 0.785 mm^2 for tracheal tissues or 7.069 mm^2 for colonic tissues (Mall et al., 1998a). Luminal and basolateral sides of the epithelium were perfused continuously with bath solution at a rate of 10 ml/min. The bath solutions were heated to 37 °C using a water jacket. In experiments with colonic tissues, indomethacin (10 μM) was added to mucosal and serosal bath solutions to reduce intracellular cAMP levels and to de-stimulate the tissue (Mall et al., 2000a). Experiments were carried out under open circuit conditions. Data were collected continuously (PowerLab, AD-Instruments, Australia) and were analyzed by using the program chart (PowerLab, AD-Instruments). Values for transepithelial voltages (V_{te}) were referred to the serosal side of the epithelium. Transepithelial resistance (R_{te}) was determined by applying short (1s) current pulses (ΔI=0.5 μA). Voltage deflections obtained under conditions without the tissue presence in the chamber were subtracted from those obtained in the presence of the tissues. R_{te} and the equivalent short circuit current (I_{SC}) were calculated according to Ohm's law ($R_{te}=\Delta V_{te}/\Delta I$, $I_{SC}=V_{te}/R_{te}$). Amiloride is a specific inhibitor of the epithelial Na^+ channel. The Na^+ channel is responsible for the electrogenic uptake of Na^+ and generates a negative transepithelial voltage. Inhibition of the epithelial Na^+ channel by amiloride reduces transepithelial voltage and I_{SC}. These changes are directly related to Na^+ fluxes as shown in several studies (Friis and Nielsen, 2001; Matalon et al., 1993; Ropke et al., 1996). Thus, amiloride is a standard tool to examine electrogenic epithelial Na^+ absorption. Student's t-test P-values <0.05 were accepted to indicate statistical significance (n=number of animals).

3. Results

3.1. Asimadoline reduced basal electrolyte transport in the trachea and colon

Addition of the κ-opiate receptor agonist asimadoline (EMD 61753, 100 μM) to the mucosal side of tracheal epithelia significantly reduced the transepithelial voltage from −2.53±0.6 to −2.26±0.58 mV (n=10, Fig. 1A). The negative equivalent short circuit current was decreased when asimadoline was applied to either mucosal and serosal sides of nonstimulated tissues (ΔI_{SC}), incubated with the phosphodiesterase inhibitor indomethacin (Table 1). The effects of asimadoline on colonic epithelia were reduced when compared with that on tracheal epithelia (Table 1). Amiloride-sensitive Na^+ absorption in trachea as well as in colonic epithelia were inhibited ($\Delta I_{SC\text{-}Amil}$) by mucosal and serosal application of asimadoline (Table 1). Since asimadoline inhibited both basal electrolyte transport and amiloride-sensitive Na^+ absorption when applied from either side of the epithelium, it was further on applied to the mucosal side.

3.2. The effect of asimadoline was dose-dependent and sensitive to charybdotoxin, but was not mediated by κ-opiate receptors

Asimadoline (0.1–500 μM) inhibited basal I_{SC} dose-dependently (Fig. 1B). A half-maximal inhibition of I_{SC} was observed at 23.7 (9.5–49.3) μM. Inhibition of I_{SC} by

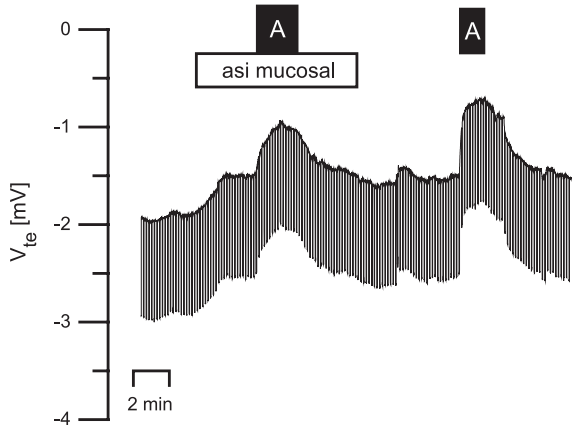

Fig. 1. Asimadoline reduced basal electrolyte transport in tracheal epithelia. Representative recording of changes of the transepithelial voltage (V_{te}) in mouse tracheal epithelium induced by amiloride (A, 20 μM) and mucosal application of asimadoline (asi, 100 μM) when applied to the mucosal side.

Table 1
Inhibitory effects of asimadoline on non-stimulated tissues. Inhibitory effects of mucosal or serosal application of asimadoline (100 μM) on basal short-circuit currents (ΔI_{SC}) and amiloride-sensitive short-circuit currents ($\Delta I_{SC\text{-}Amil}$)

Side of application	Inhibition of ion transport (ΔI_{SC}) in mouse trachea by asimadoline (μA/cm^2)	Inhibition of amiloride-sensitive Na^+ absorption ($\Delta I_{SC\text{-}Amil}$) in mouse trachea by asimadoline (μA/cm^2)
Mucosal	−23.27±5.08[a] (10)	−21.99±4.49[a] (10)
Serosal	−21.20±5.74[a] (10)	−7.58±2.29[a] (9)
Mucosal	−19.02±5.46[a] (5)	n.d.
Mucosal/+naloxone	−15.54±3.71[a] (5)	n.d.
Serosal	−13.65±3.04[a] (5)	n.d.
Serosal/+naloxone	−11.97±3.05[a] (5)	n.d.
Mucosal	−13.58±4.32[a] (4)	n.d.
Mucosal/+nor-binaltorphimine	−18.79±3.03[a] (4)	n.d.
Serosal	−7.93±1.65[a] (4)	n.d.
Serosal/+nor-binaltorphimine	−9.24±4.47[a] (4)	n.d.
Mucosal	−30.64±2.51[a] (6)	n.d.
Mucosal/+serosal charybdotoxin	−10.78±4.45[b] (6)	n.d.
	Inhibition of ion transport (ΔI_{SC}) in mouse colon by asimadoline (μA/cm^2)	Inhibition of amiloride-sensitive Na^+ absorption ($\Delta I_{SC\text{-}Amil}$) in mouse colon by asimadoline (μA/cm^2)
Mucosal	−3.33±0.64[a] (8)	−2.40±0.59* (7)
Serosal	−3.20±1.14[a] (8)	−1.19±0.36* (6)

The effects of asimadoline on I_{SC} were sensitive to serosal application of charybdotoxin (10 nM), but were insensitive to the opioid antagonists naloxone (10 μM) or nor-binaltorphimine (10 μM). Values are the means±S.E.M. (Number of animals.) n.d.: not determined.

[a] Indicates significant inhibition of transport (paired Student's *t*-test, $p \leq 0.05$).

[b] Indicates inhibition by charybdotoxin (paired Student's *t*-test, $p \leq 0.05$).

asimadoline applied to the mucosal or serosal side was not blocked by the opioid antagonist naloxone (10 μM), or by the selective κ-opiate receptor's antagonist nor-binaltorphimine (10 μM). This suggests that the response was not mediated by κ-opiate receptors (Table 1). In contrast, the effect of asimadoline was sensitive to serosal application of the blocker of Ca^{2+}-activated K^+ channels, charybdotoxin (10 nM) (Table 1). These experiments suggest inhibition of basolateral Ca^{2+}-activated K^+ channels by asimadoline.

3.3. Effects of asimadoline on cAMP induced I_{SC} in the trachea and colon

Increase of intracellular cAMP by treatment of the epithelia with the phosphodiesterase inhibitor IBMX (100 μM) and stimulation of adenylate cyclase by forskolin (2 μM) caused a significant increase of I_{SC} in tracheal and colonic epithelia (Table 2). Mucosal administration of asimadoline

Table 2
Inhibitory effects of asimadoline on stimulated tissues

Agonist	Stimulation of ion transport (ΔI_{SC}) by agonists in mouse trachea (μA/cm^2)	Ion transport inhibited by asimadoline (ΔI_{SC}) after stimulation of mouse trachea (μA/cm^2)
I/F	+111.37±16.73[a] (8)	−37.89±14.86[a] (8)
Carbachol	+340.92±78.21[a] (7)	−140.04±50.22[a] (7)
Carbachol in the presence of I/F	+163.56±34.78[a] (5)	−78.18±19.53[a] (5)
ATP	+284.08±46.66[a] (6)	−51.82±13.57[a] (6)
	Stimulation of ion transport (ΔI_{SC}) by agonists in mouse colon (μA/cm^2)	Ion transport inhibited by asimadoline (ΔI_{SC}) after stimulation of mouse colon (μA/cm^2)
I/F	+77.48±16.18[a] (11)	−22.99±9.03[a] (11)

Summary of the inhibitory effects of mucosal application of asimadoline (100 μM) on short-circuit currents (negative values), activated by IBMX/Forskolin (I/F, 100 μM/2 μM), carbachol (100 μM) and ATP (100 μM). Values are means±S.E.M. (Number of animals.).

[a] Indicates significant changes when compared to control (paired Student's *t*-test, $p \leq 0.05$).

(100 μM) significantly inhibited cAMP-activated I_{SC} in both tracheal and colonic epithelia. In cAMP stimulated tissues, amiloride-sensitive transport was 82.47±21.49 μA/cm^2 and was inhibited non-significantly by −8.67±12.73 μA/cm^2 (n=8). Thus, Na^+ absorption supported by basolateral cAMP-dependent K^+ channels was no longer dependent on Ca^{2+}-activated K^+ channels (Mall et al., 2000b).

3.4. Effects of asimadoline on carbachol and ATP induced I_{SC} in trachea

The muscarinic type 3-receptor agonist carbachol (100 μM) caused a significant increase in V_{te} from −1.99±0.68 to −5.46±1.13 mV (n=7) (Fig. 2) and thus a transient increase in I_{SC} (Table 2). Carbachol activates Cl^- secretion in epithelia by enhancing intracellular Ca^{2+} concentration, which activated basolateral SK4 K^+ channels (Greger et al.,

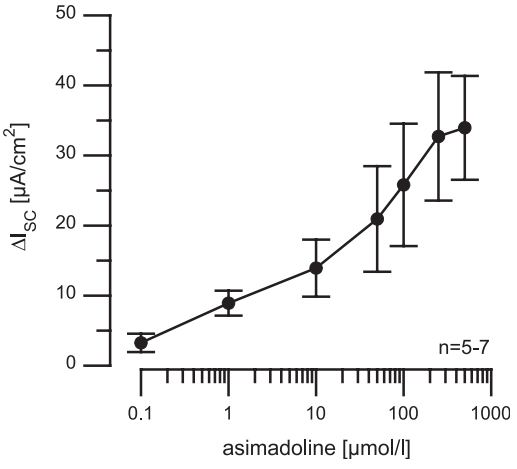

Fig. 2. Dose response curve for the inhibitory effects of asimadoline on I_{sc} (ΔI_{sc}). Values are means±S.E.M. n=number of animals.

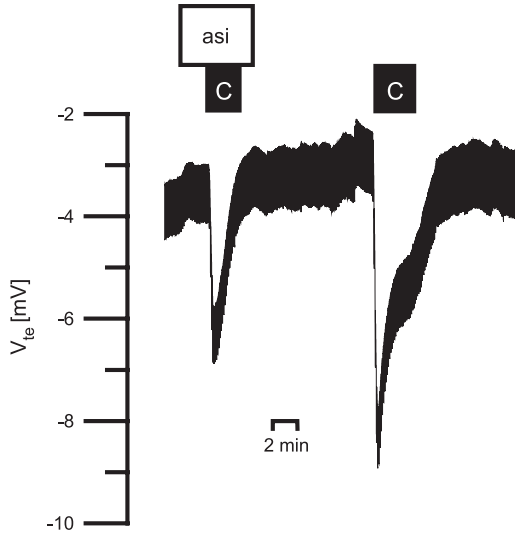

Fig. 3. Effect of asimadoline (asi) on carbachol (C) induced electrolyte transport in mouse trachea. Original recording of the effect of asimadoline (100 μM, mucosal) on carbachol (100 μmol, serosal) induced change of the transepithelial voltage (V_{te}).

1997; Mall et al., 2003; McCann and Welsh, 1990). In the presence of asimadoline (100 μM), carbachol induced I_{SC} was reduced significantly (Table 2, Fig. 2). These results further suggest that asimadoline inhibits basolateral Ca^{2+}-activated K^+ channels, but has little effects on cAMP transport. To investigate if asimadoline also inhibits ion transport activated by other agonist increasing intracellular Ca^{2+}, mouse tracheas were stimulated by mucosal application of ATP (100 μM). ATP, which binds to luminal $P2Y_2$ receptors, caused a transient increase in I_{SC} (Table 2). Asimadoline (100 μM) inhibited ATP induced transport significantly, although the inhibitory effect was reduced when compared to carbachol-activated transport (Table 2). The limited effect of asimadoline on ATP induced transport is probably due to the fact that ATP activates mainly luminal Ca^{2+}-dependent Cl^- channels (Clarke and Boucher, 1992; Leipziger, 2003), and only to a lesser degree basolateral Ca^{2+}-activated K^+ channels. Taken together, the data suggest an inhibitory effect of asimadoline on basolateral Ca^{2+}-dependent K^+ channels, which inhibits basal and Ca^{2+}-activated Cl^- secretion as well as electrogenic Na^+ absorption (Fig. 3).

4. Discussion

In the present study, we investigated possible κ-opioid receptor-dependent and independent effects of asimadoline (EMD 61753) on transepithelial transport in mouse trachea and colon. Asimadoline is a peripherally acting, potent selective κ-opioid receptor agonist (Barber et al., 1994; Gottschlich et al., 1994). The antisecretory actions of opioids take part in the known antidiarrheal and constipating effects in the intestine. Previous studies showed a non-opioid receptor-mediated action on Na^+ and K^+ channel function at higher doses of κ-opioid receptor agonists (Joshi et al., 2003; Pugsley, 2002). The aim of the present study was to investigate the mechanisms by which asimadoline acts on transepithelial transport in mouse trachea and colon, which may contribute to constipation. For that, transepithelial voltage and equivalent short circuit currents (I_{SC}) were measured in isolated epithelia from colon and trachea in a modified Ussing chamber (Mall et al., 1998a). The basal electrolyte transport was reduced by serosal and mucosal asimadoline by the same amount (Fig. 1, Table 1). Asimadoline contains a hydrophobic diphenylmethyl group in combination with a hydrophilic hydroxyl group (Barber et al., 1994). Because of the amphiphilic character asimadoline penetrates the cell membrane and may act from the intracellular side on both luminal and basolateral membranes. The effects of asimadoline on I_{SC} were neither influenced by the opioid antagonist naloxone nor by the selective κ-opioid receptor's antagonist nor-binaltorphimine suggesting that the response was not mediated by κ-opioid receptors (Table 1). Our results compare well to that obtain previously by other groups (Poonyachoti et al., 2001; Sheldon et al., 1990).

Endogenous PGE_2 has been shown to act as an agonist for cAMP-dependent Cl^- secretion (Brzuszczak et al., 1996; Calderaro et al., 1991). Thus, the cyclooxygenase inhibitor indomethacin antagonized Cl^- secretion in the colon (Brzuszczak et al., 1996; Calderaro et al., 1991). In the presence of indomethacin, asimadoline had only minor effects on colonic transport (Table 1). In contrast, asimadoline had a pronounced effect on both colonic and tracheal epithelia after increase of both intracellular cAMP and Ca^{2+} (Table 2), indicating the interference of asimadoline with epithelial electrolyte transport.

Under control condition the negative transepithelial voltage in trachea and colon epithelium is predominantly generated by Na^+ absorption through epithelial Na^+ channels and is maintained by the basolateral Na^+–K^+ ATPase as well as K^+ channels (Dawson, 1991; Koefoed-Johnson and Ussing, 1958). Asimadoline reduced amiloride-sensitive transport ($I_{SC\text{-}Amil}$), but had no effects on $I_{SC\text{-}Amil}$ in cAMP stimulated tissue. Increase of intracellular cAMP concentration elevates the driving force for apical Na^+ uptake by activating basolateral cAMP-sensitive KCNQ1 K^+ channels (Mall et al., 2000b). These channels do not seem to be inhibited by asimadoline. However, in the presence of the inhibitor of Ca^{2+}-activated K^+ channels charybdotoxin, the effects of asimadoline were largely reduced (Table 1). This strongly suggests that Ca^{2+}-mediated K^+ channels are inhibited by asimadoline (Ishii et al., 1997; Joiner et al., 1997; Logsdon et al., 1997; Syme et al., 2000). This is further supported by the pronounced inhibitory effects of asimadoline on carbachol induced Cl^- secretion, which is also supported by basolateral Ca^{2+}-activated SK4 K^+ channels (Bernard et al., 2003; Mall et al., 2003) and colon (von Hahn et al., 2001). However, unlike

carbachol-mediated transport purinergic stimulation by mucosal ATP primarily activates Ca^{2+}-dependent luminal Cl^- channels (Clarke and Boucher, 1992; Leipziger, 2003). This may explain why asimadoline inhibits ATP induced transport only by 18% (Table 2).

Previous studies showed other non-opioid receptor-mediated action of opioid agonists on ion channel function (Pugsley, 2002). Asimadoline inhibited tetrodotoxin-sensitive and insensitive Na^+ currents in colonic sensory neurons with an IC_{50} of around 1 μM (Joshi et al., 2003). In rat cardiac myocytes, the opioid agonist *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide, methane sulfonate, hydrate (U-50,488H) caused a concentration-dependent block of voltage-activated Na^+ and K^+ currents with an IC_{50} of around 15 and 50 μM, respectively (Pugsley et al., 1994). We obtained a similar IC_{50} value of around 24 μM for the inhibitory effects on Ca^{2+}-activated ion transport.

In summary, asimadoline reduced electrolyte secretion and amiloride-sensitive Na^+ absorption by attenuating the electrical driving force for electrolyte transport, probably by blocking Ca^{2+}-activated and charybdotoxin inhibited K^+ channels. Our results may provide an explanation for the previous observations that asimadoline elicits diuresis in rats (Barber et al., 1994) and in human (Kramer et al., 2000), probably also by interfering with basolateral Ca^{2+}-activated K^+ channels.

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References

Barber, A., Bartoszyk, G.D., Bender, H.M., Gottschlich, R., Greiner, H.E., Harting, J., Mauler, F., Minck, K.O., Murray, R.D., Simon, M., Seyfried, C.A., 1994. A pharmacological profile of the novel, peripherally-selective kappa-opioid receptor agonist, EMD 61753. Br. J. Pharmacol. 113, 1317–1327.

Bernard, K., Bogliolo, S., Soriani, O., Ehrenfeld, J., 2003. Modulation of calcium-dependent chloride secretion by basolateral SK4-like channels in a human bronchial cell line. J. Membr. Biol. 196, 15–31.

Brzuszczak, I.M., Zhao, J., Bell, C., Stiel, D., Fielding, I., Percy, J., Smith, R., O'Loughlin, E., 1996. Cyclic AMP-dependent anion secretion in human small and large intestine. J. Gastroenterol. Hepatol. 11, 804–810.

Calderaro, V., Giovane, A., De Simone, B., Camussi, G., Rosiello, R., Quagliuolo, L., Servillo, L., Taccone, W., Giordano, C., Balestrieri, C., 1991. Arachidonic acid metabolites and chloride secretion in rabbit distal colonic mucosa. Am. J. Physiol.: Gasterointest. Liver Physiol. 261, G443–G450.

Clarke, L.L., Boucher, R.C., 1992. Chloride secretory response to extracellular ATP in human normal and cystic fibrosis nasal epithelia. Am. J. Physiol. 263, C348–C356.

Dawson, D.C., 1991. Ion channels and colonic salt transport. Annu. Rev. Physiol. 53, 321–339.

Delgado-Aros, S., Chial, H.J., Camilleri, M., Szarka, L.A., Weber, F.T., Jacob, J., Ferber, I., McKinzie, S., Burton, D.D., Zinsmeister, A.R., 2003. Effects of a kappa-opioid agonist, asimadoline, on satiation and GI motor and sensory functions in humans. Am. J. Physiol.: Gasterointest. Liver Physiol. 284, G558–G566.

Delvaux, M., Jacob, J., Beck, A., Bouzamondo, H., Weber, F.T., Frexinos, J., 2003. Effect of asimadoline, a new agonist of peripheral kappa opiate receptors on pain induced by rectal distension in IBS patients. Gastroenterology 124, A-221.

Friis, S., Nielsen, R., 2001. Effect of the putative Ca^{2+}-receptor agonist Gd^{3+} on the active transepithelial Na+ transport in frog skin. J. Membr. Biol. 184, 291–297.

Gottschlich, R., Krug, M., Barber, A., Devant, R.M., 1994. Kappa-opioid activity of the four stereoisomers of the peripherally selective kappa-agonists, EMD 60,400 and EMD 61,753. Chirality 6, 685–689.

Gottschlich, R., Barber, A., Bartoszyk, G.D., Seyfried, C.A., 1995. The peripherally acting kappa-opiate agonist EMD 61753 and analogues: opioid activity versus peripheral selectivity. Drugs Exp. Clin. Res. 21, 171–174.

Greger, R., Bleich, M., Riedemann, N., van Driessche, W., Ecke, D., Warth, R., 1997. The role of K^+ channels in colonic Cl^- secretion. Comp. Biochem. Physiol., A Physiol. 118, 271–275.

Ishii, T.M., Silvia, C., Hirschberg, B., Bond, C.T., Adelman, J.P., Maylie, J., 1997. A human intermediate conductance calcium-activated potassium channel. Proc. Natl. Acad. Sci. U. S. A. 94, 11651–11656.

Joiner, W.J., Wang, L.Y., Tang, M.D., Kaczmarek, L.K., 1997. hSK4, a member of a novel subfamily of calcium-activated potassium channels. Proc. Natl. Acad. Sci. U. S. A. 94, 11013–11018.

Joshi, S.K., Su, X., Porreca, F., Gebhart, G.F., 2000. Kappa-opioid receptor agonists modulate visceral nociception at a novel, peripheral site of action. J. Neurosci. 20, 5874–5879.

Joshi, S.K., Lamb, K., Bielefeldt, K., Gebhart, G.F., 2003. Arylacetamide kappa-opioid receptor agonists produce a tonic- and use-dependent block of tetrodotoxin-sensitive and -resistant sodium currents in colon sensory neurons. J. Pharmacol. Exp. Ther. 307, 367–372.

Koefoed-Johnson, V., Ussing, H.H., 1958. The nature of the frog skin potential. Acta Physiol. Scand. 42, 298–308.

Kramer, H.J., Uhl, W., Ladstetter, B., Backer, A., 2000. Influence of asimadoline, a new kappa-opioid receptor agonist, on tubular water absorption and vasopressin secretion in man. Br. J. Pharmacol. 50, 227–235.

Leipziger, J., 2003. Control of epithelial transport via luminal P2 receptors. Am. J. Physiol., Renal. Physiol. 284, 419–432.

Logsdon, N.J., Kang, J., Togo, J.A., Christian, E.P., Aiyar, J., 1997. A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes. J. Biol. Chem. 272, 32723–32726.

Mall, M., Bleich, M., Greger, R., Schreiber, R., Kunzelmann, K., 1998a. The amiloride-inhibitable Na^+ conductance is reduced by the cystic fibrosis transmembrane conductance regulator in normal but not in cystic fibrosis airways. J. Clin. Invest. 102, 15–21.

Mall, M., Bleich, M., Schurlein, M., Kuhr, J., Seydewitz, H.H., Brandis, M., Greger, R., Kunzelmann, K., 1998b. Cholinergic ion secretion in human colon requires coactivation by cAMP. Am. J. Physiol. 275, G1274–G1281.

Mall, M., Gonska, T., Thomas, J., Schreiber, R., Seydewitz, H.H., Kuehr, J., Brandis, M., Kunzelmann, K., 2003. Modulation of Ca^{2+}-activated Cl^- secretion by basolateral K^+ channels in human normal and cystic fibrosis airway epithelia. Pediatr. Res. 53, 608–618.

Mall, M., Wissner, A., Gonska, T., Calenborn, D., Kuehr, J., Brandis, M., Kunzelmann, K., 2000a. Inhibition of amiloride-sensitive epithelial Na^+ absorption by extracellular nucleotides in human normal and cystic fibrosis airways. Am. J. Respir. Cell Mol. Biol. 23, 755–761.

Mall, M., Wissner, A., Schreiber, R., Kuehr, J., Seydewitz, H.H., Brandis, M., Greger, R., Kunzelmann, K., 2000b. Role of KvLQT1 in cyclic

adenosine monophosphate-mediated Cl^- secretion in human airway epithelia. Am. J. Respir. Cell Mol. Biol. 23, 283–289.

Matalon, S., Bauer, M.L., Benos, D.J., Kleyman, T.R., Lin, C., Cragoe Jr., E.J., O'Brodovich, H., 1993. Fetal lung epithelial cells contain two populations of amiloride-sensitive Na^+ channels. Am. J. Physiol. 264, L357–L364.

McCann, J.D., Welsh, M.J., 1990. Regulation of Cl^- and K^+ channels in airway epithelium. Annu. Rev. Physiol. 52, 115–135.

Ozaki, N., Sengupta, J.N., Gebhart, G.F., 2000. Differential effects of mu-, delta-, and kappa-opioid receptor agonists on mechanosensitive gastric vagal afferent fibers in the rat. J. Neurophysiol. 83, 2209–2216.

Poonyachoti, S., Portoghese, P.S., Brown, D.R., 2001. Pharmacological evidence for a 7-benzylidenenaltrexone-preferring opioid receptor mediating the inhibitory actions of peptidic delta- and mu-opioid agonists on neurogenic ion transport in porcine ileal mucosa. J. Pharmacol. Exp. Ther. 297, 672–679.

Pugsley, M.K., 2002. The diverse molecular mechanisms responsible for the actions of opioids on the cardiovascular system. Pharmacol. Ther. 93, 51–75.

Pugsley, M.K., Saint, D.A., Walker, M.J., 1994. An electrophysiological basis for the antiarrhythmic actions of the kappa-opioid receptor agonist U-50,488H. Eur. J. Pharmacol. 261, 303–309.

Quito, F.L., Brown, D.R., 1991. Neurohormonal regulation of ion transport in the porcine distal jejunum. Enhancement of sodium and chloride absorption by submucosal opiate receptors. J. Pharmacol. Exp. Ther. 256, 833–840.

Ropke, M., Carstens, S., Holm, M., Frederiksen, O., 1996. Ion transport mechanisms in native rabbit nasal airway epithelium. Am. J. Physiol. 271, L637–L645.

Sengupta, J.N., Snider, A., Su, X., Gebhart, G.F., 1999. Effects of kappa opioids in the inflamed rat colon. Pain 79, 175–185.

Sheldon, R.J., Riviere, P.J., Malarchik, M.E., Moseberg, H.I., Burks, T.F., Porreca, F., 1990. Opioid regulation of mucosal ion transport in the mouse isolated jejunum. J. Pharmacol. Exp. Ther. 253, 144–151.

Su, X., Julia, V., Gebhart, G.F., 2000. Effects of intracolonic opioid receptor agonists on polymodal pelvic nerve afferent fibers in the rat. J. Neurophysiol. 83, 963–970.

Syme, C.A., Gerlach, A.C., Singh, A.K., Devor, D.C., 2000. Pharmacological activation of cloned intermediate- and small-conductance $Ca2^+$-activated K^+ channels. Am. J. Physiol., Cell Physiol. 278, C570–C581.

von Hahn, T., Thiele, I., Zingaro, L., Hamm, K., Garcia-Alzamora, M., Kottgen, M., Bleich, M., Warth, R., 2001. Characterisation of the rat SK4/IK1 K^+ channel. Cell. Physiol. Biochem. 11, 219–230.