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Cloning and Function of the Rat Colonic Epithelial K^+ Channel K_VLQT1

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Abstract. K_VLQT1 (KCNQ1) is a voltage-gated K^+ channel essential for repolarization of the heart action potential that is defective in cardiac arrhythmia. The channel is inhibited by the chromanol 293B, a compound that blocks cAMP-dependent electrolyte secretion in rat and human colon, therefore suggesting expression of a similar type of K^+ channel in the colonic epithelium. We now report cloning and expression of K_VLQT1 from rat colon. Overlapping clones identified by cDNA-library screening were combined to a full length cDNA that shares high sequence homology to K_VLQT1 cloned from other species. RT-PCR analysis of rat colonic musoca demonstrated expression of K_VLQT1 in crypt cells and surface epithelium. Expression of rK_VLQT1 in *Xenopus* oocytes induced a typical delayed activated K^+ current, that was further activated by increase of intracellular cAMP but not Ca^{2+} and that was blocked by the chromanol 293B. The same compound blocked a basolateral cAMP-activated K^+ conductance in the colonic mucosal epithelium and inhibited whole cell K^+ currents in patch-clamp experiments on isolated colonic crypts. We conclude that K_VLQT1 is forming an important component of the basolateral cAMP-activated K^+ conductance in the colonic epithelium and plays a crucial role in diseases like secretory diarrhea and cystic fibrosis.

Key words: rK_VLQT1 — rKCNQ1 — Rat colon — K^+ channel — Epithelium — Electrolyte secretion — Ion transport — *Xenopus* oocytes — Expression — Cloning — Ussing chamber

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Introduction

cAMP-dependent stimulation of ion secretion by the colonic mucosa of mice, rat and humans activates luminal Cl^- channels. Several studies indicate that this is paralleled by activation of basolateral K^+ channels [7, 10, 18]. Parallel activation of a basolateral K^+ conductive pathway is essential in order to maintain the electrical driving force for luminal Cl^- exit. There is clear evidence that the cystic fibrosis transmembrane conductance regulator (CFTR) is responsible for luminal Cl^- exit. It is obviously the only luminal Cl^- conductance that is activated during secretory stimulation of the colonic epithelium. This is supported by the notion that inhibition of CFTR or mutations of CFTR as in cystic fibrosis abolish Cl^- secretion [19, 29].

The molecular identity of the basolateral cAMP-activated K^+ channel is unclear, although, it has been characterized in patch-clamp experiments as a very low conductance K^+ channel [32]. Among other properties this K^+ channel is characterized by its sensitivity towards the inhibitory chromanol compound 293B that blocks cAMP-activated colonic Cl^- secretion with an IC_{50} around and even below 1 μM [15]. The very same compound has been found to block K_VLQT1 K^+ channels expressed in *Xenopus* oocytes [2, 28]. Meanwhile several hundred compounds have been tested for their inhibitory effects on colonic ion secretion and on K_VLQT1 expressed in oocytes. In fact, the pharmacological profiles of both colonic cAMP-activated K^+ conductance

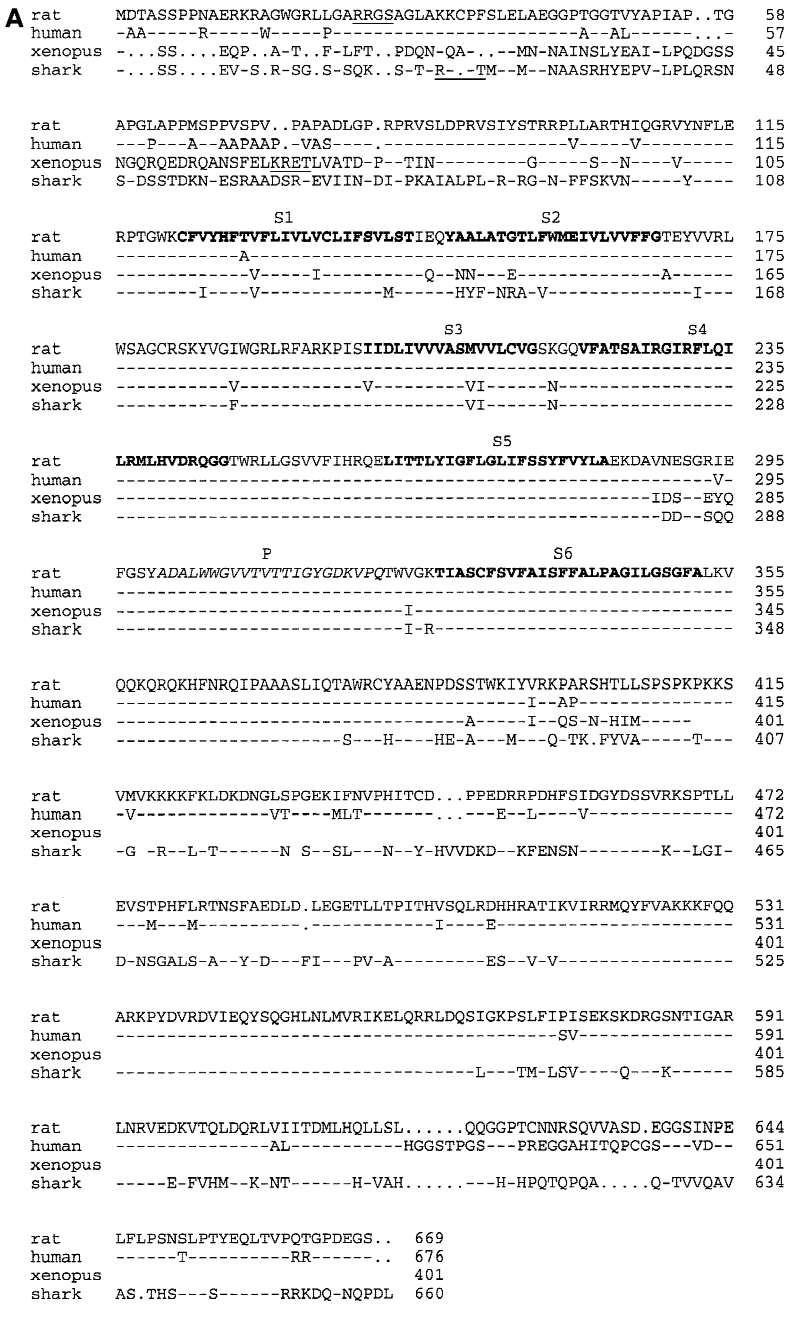

Fig. 1. (*A*) Sequence alignment of K_VLQT1 cloned from rat colon (rK_VLQT1) and K_VLQT1 cloned from human heart, *Xenopus laevis* and *Squalus acanthias* rectal gland. S1–S6 indicate putative transmembrane-spanning domains and P the pore-associated domain. The protein kinase A phosphorylation sites are underlined. For human, *Xenopus laevis* and *Squalus acanthias* only amino acids that differ to that of the rat sequence are given. (*B*) RT-PCR-analysis of mRNA isolated from epithelial surface and basis cells of rat colonic crypts. The band at 631 bp indicates expression of K_VLQT1 in both epithelial cells isolated from the surface and the basis of rat colonic crypts.

and expressed K_VLQT1 match quite well [31]. Although the affinity of the K^+ channel for 293B may be influenced by other proteins forming a heteropolymeric complex with K_VLQT1 such as IsK (minK), K_VLQT1 seems to be target for the 293B [3, 17]. Based on these results, it was suggested that the effects of 293B observed in Ussing chamber and patch-clamp experiments are caused by inhibition of K_VLQT1 and that this protein forms the cAMP-activated K^+ channel. Now, we report cloning of K_VLQT1 from rat colonic epithelial cells, thereby providing further evidence for K_VLQT1 forming the basolateral cAMP-activated K^+ channel together with the β subunit KCNE3 in rat colon [25].

Materials and Methods

Cloning of rK_VLQT1 and RT-PCR of rKCNE3

Total RNA was extracted from distal colon of rats (Wistar 10-week-old, males) that received three daily injections of dexamethasone (6 mg/Kg). Poly A+ mRNA was selected by two passes through oligo-dT

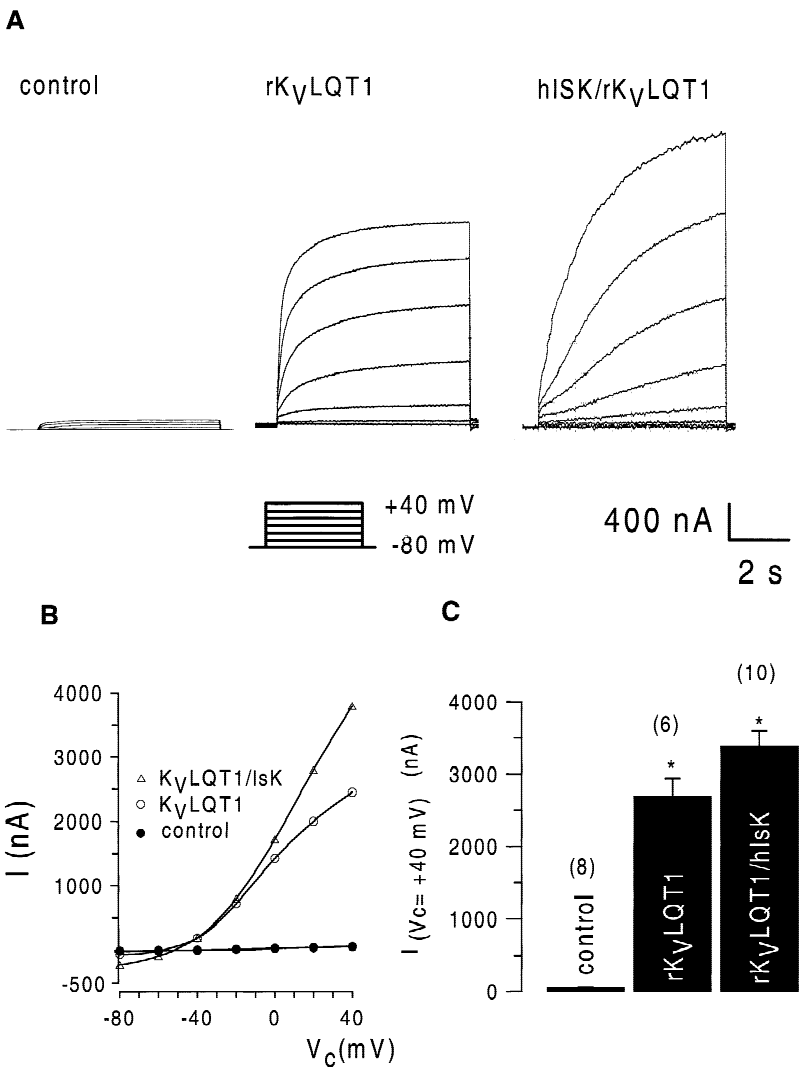

Fig. 2. Expression of rK_VLQT1 in *Xenopus* oocytes. (*A*) Whole cell currents activated by depolarizing voltages pulses applied in 20 mV increments from −80 to +40 mV in control oocytes, or oocytes expressing rK_VLQT1 or coexpressing rK_VLQT1 and hIsK. (*B*) Current-voltage relations obtained for voltage-activated currents shown in *A*. Currents were measured 6 sec after applying voltage pulses. (*C*) Summary of the whole cell currents activated in control oocytes, rK_VLQT1 and hIsK/rK_VLQT1-expressing oocytes after application of voltage pulses from −80 to +40 mV. *Indicate significant difference from control (number of experiments).

cellulose columns. A cDNA library was constructed in lambda ZAP II vector by the custom made library service of Clontech (Palo Alto, CA). The library was screened with a 32P-dCNA probe corresponding to the full length sequence of mouse K_VLQT1 (kindly provided by Bernard Attali). Positive clones were plaque purified and pBluescript plasmids were excised in vivo from the lambda phages using ExAssist helper phage (Stratagene). Two overlapping clones that account for the whole coding sequence of rat K_VLQT1 were identified (submitted to EMBL Nucleotide Sequence Database, accession - number.: AJ133685). cDNA of the cloned rK_VLQT1 was subcloned in an oocyte expressing vector pTLN that contains *Xenopus* β-globin untranslated regions (kindly provided by Prof. Dr. T.J. Jentsch, Zentrum für molekulare Neurobiology, Hamburg, Germany) [16]. Human Isk (hminK) was kindly provided by Dr. S. Waldegger (Zentrum für molekulare Neurobiology, Hamburg, Germany). Total RNA was isolated from isolated rat colonic crypts, reverse transcribed and PCR—amplified using the primers (5′-3′) CGATTTCTGTTGCTATGGAGAC (sense) and CACATCAGATCATAGACACACG (antisense) specific for KCNE3. All cloning and PCR products were confirmed for correct sequence by dideoxynucleotide–termination DNA sequencing (Thermo Sequenase I, Pharmacia) and using a 373A DNA sequencer (Applied Biosystems).

Expression of K_VLQT1 in Epithelial Cells of Rat Colonic Crypts

Preparation of colonic crypts by exposure to Ca^{2+}-free solution has been described in a previous report [8]. Under optical control 50 crypts were divided into crypt-surface and crypt-base. mRNA was isolated from total crypts, crypt-surface and crypt-base separately using Quick-Prep Micro mRNA Purification Kit (Pharmacia Biotech, Sweden) and reverse transcribed (Superscript, Life Technologies, Germany). A 631 bp rK_VLQT1 fragment was amplified using oligonucleotides 5′-CTCCATCTACAGTACGCGTC-3′ (sense) and 5′-ATCTGCGTAGCTGCCAAAC-3′ (antisense) and Taq Polymerase (Life Technology, Germany) according to the manufacturer's introductions. The cycle conditions were: 2 min 95°C, 35 cycles of 95°C for 1 min, 54°C for 30 sec, 72°C for 1,5 min and one cycle of 95°C for 1 min, 54°C for 30 sec, 72°C for 10 min. A rK_VLQT1 encoding fragment (2091 bp) was amplified using oligonucleotides 5′-TGCGCTGCCTTCATCTCTGC-3′ (sense) and 5′-CCTGAACCTCCCTTCTGAGC-3′ (antisense) (2 min 95°C, 35 cycles of 94°C for 1 min, 60°C for 30 sec, 68°C for 2 min and one cycle of 94°C for 1 min, 60°C for 30 sec, 68°C for 10 min). rK_V-LQT1 sequence of both PCR products was confirmed by sequencing.

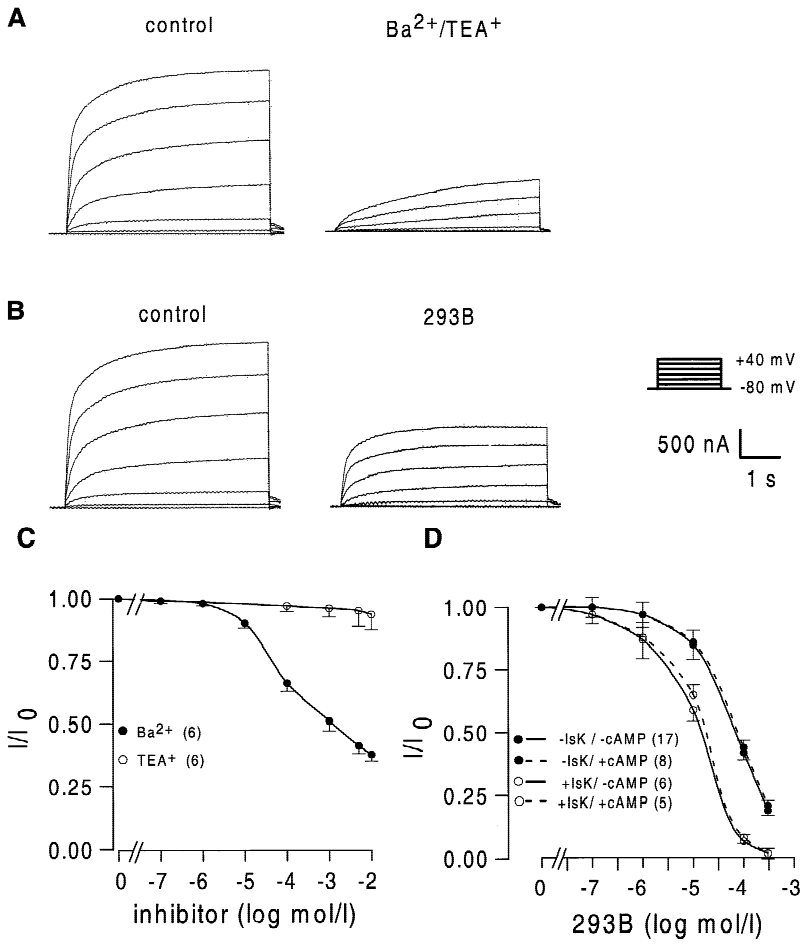

Fig. 3. Whole cell rK_VLQT1 currents activated by depolarizing voltage pulses applied in 20 mV increments from −80 to +40 mV in oocytes expressing rK_VLQT1. Inhibition of rK_VLQT1 by (*A*) Ba^2/TEA^+ (5 mM/10 mM) and (*B*) 293B (100 μM). (*C*) Concentration response curves for the inhibition of rK_VLQT1 by Ba^2 and TEA^+ (number of experiments). (*D*) Concentration response curves for the effects of 293B on both oocytes expressing rK_VLQT1 (−IsK) or coexpressing rK_VLQT1 and IsK (+IsK). Inhibition of rK_VLQT1 and rK_VLQT1/IsK was examined in the presence (+cAMP) or absence (−cAMP) of IBMX and forskolin (1 mM/2 μM). $IC_{50\%}$ values are given in the text (number of experiments).

Preparation of Oocytes, cRNA and Microinjection of cRNA

Isolation and microinjection of oocytes have been described in a previous report [20]. In brief, after isolation from adult *Xenopus laevis* female frogs (H. Kähler, Bedarf für Entwicklungsbiologie, Hamburg, Germany), oocytes were dispersed and defolliculated by a 0.5 hr-treatment with collagenase (type A, Boehringer, Germany). Subsequently, oocytes were rinsed and kept in ND96-buffer (in mM): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, Na-pyruvate 2.5, pH 7.55), supplemented with theophylline (0.5 mM) and gentamycin (5 mg/l) at 18°C. cDNAs were linearized by either *Hpa*I and cRNA was in vitro transcribed using Sp6 polymerases and a 5′ cap (mCAP mRNA capping kit, Stratagene). Oocytes were injected with cRNA of K_VLQT1 (KCNQ1) (10 ng) after dissolving cRNA in about 50 nl double-distilled water (PV830 pneumatic pico pump, WPI, Germany). Oocytes injected with 50 nl double-distilled water served as controls.

Electrophysiological Analysis of *Xenopus* Oocytes

2–4 days after injection oocytes were impaled with two electrodes (Clark instruments) which had resistances of 1 MΩ when filled with 2.7 mol/l KCl. A flowing (2.7 mol/l) KCl electrode served as bath reference in order to minimize junction potentials. Membrane currents were measured by voltage clamping of the oocytes (OOC-1 amplifier, WPI, Germany) in intervals between −80 to +40 mV in steps of 20 mV for 6 sec. Current data were filtered at 400 Hz (OOC-1 amplifier). Between intervals, oocytes were voltage clamped to −80 mV for 5 sec. Data were collected continuously on a computer hard disc at a sample frequency of 1,000 Hz and were analyzed using the programs chart and scope (McLab, AD-Instruments, Macintosh). Typically current values were measured at the time point 6 sec after the voltage step. During the whole experiment the bath was continuously perfused at a rate of 5–10 ml/min. All experiments were conducted at room temperature (22°C).

Ussing Chamber Experiments

Freshly dissected mucosa from rat distal colon was mounted into a modified miniature Ussing chamber [19]. The luminal and basolateral bath were continuously perfused at a rate of 10–20 ml/min (chamber volume 1 ml) with a buffer containing (mM): NaCl 145, KH_2PO_4 0.4, K_2HPO_4 1.6, D-glucose 5, $MgCl_2$ 1, Ca-gluconate 1.3. The pH was adjusted to 7.4. All experiments were carried out at 37°C. Ussing chamber measurements were performed under open-circuit conditions. Transepithelial voltage (V_{te}) was referenced to the serosal side. Transepithelial resistance (R_{te}) and equivalent short circuit current (I_{sc}) were determined according to [19] and by applying Ohm's law.

Whole Cell Patch Clamp Experiments

Whole cell patch-clamp experiments were performed on epithelial cells derived from single isolated crypts of rat colon. Patch pipettes had a resistance of 3–6 MΩ when filled with a solution containing (mM): K-gluconate 95, KCl 30, NaH_2PO_4 1.2, Na_2HPO_4 4.8, glucose 5, $MgCl_2$ 2.38, EGTA 1, Ca-gluconate 0.73, ATP 3. The pH was adjusted to 7.2. The bath was continuously perfused at a rate of 10–20 ml/min with a solution containing (mM): Na-gluconate 115, NaCl 30, KH_2PO_4 0.4, K_2HPO_4 1.6, glucose 5, $MgCl_2$ 1, Ca-gluconate 6. The pH was adjusted to 7.4. All experiments were performed at 37°C.

Materials and Statistical Analysis

All used compounds were of highest available grade of purity. 3-isobutyl-1-methylxanthine (IBMX), forskolin, carbachol and prostaglandin E_2 were all from Sigma (Deisenhofen, Germany). Ba^{2+} and TEA^+ were obtained from Merck (Darmstadt, Germany). 293B was from Hoechst (Frankfurt, Germany). Students *t* test *p* values < 0.05 were accepted to indicate statistical significance (*).

Abbreviations

K_VLQT1, voltage gated K^+ channel; CFTR, cystic fibrosis transmembrane conductance regulator; I_{sc}, equivalent short circuit current; IBMX, 3-isobutyl-1-methylxanthine.

Results and Discussion

rK_VLQT1 Sequence Homology and Functional Properties

K_VLQT1 was cloned from a rat colonic library (rK_VLQT1). A partial sequence has been obtained from rat ventricle (accession No.: U92655) that showed 98% identity. When compared with the amino acid sequences of K_VLQT1 cloned from mouse, human, *Xenopus laevis* and rectal gland of *Squalus acanthias* (dogfish) the sequence identity was 98, 90, 75 and 65%, respectively. Other partial sequences obtained from heart tissue of guinea pig (accession No.: AF049341) and cat (accession No.: AF013961) show 100 and 99% identity. Most sequence differences were found in the cytosolic N-terminal region as well as in C-terminal end (Fig. 1*A*). This suggests the presence of a structurally analogous K_VLQT1 K^+ channel in rat colonic epithelial cells. In fact, RT-PCR analysis of isolated colonic epithelial cells indicates expression of K_VLQT1 in both epithelial cells derived from crypts and surface epithelium (Fig. 1*B*). In addition, a full length cDNA encoding all 669 amino acids was amplified from isolated colonic epithelial cells. Sequencing of both PCR products confirmed the sequence of the cloned rK_VLQT1.

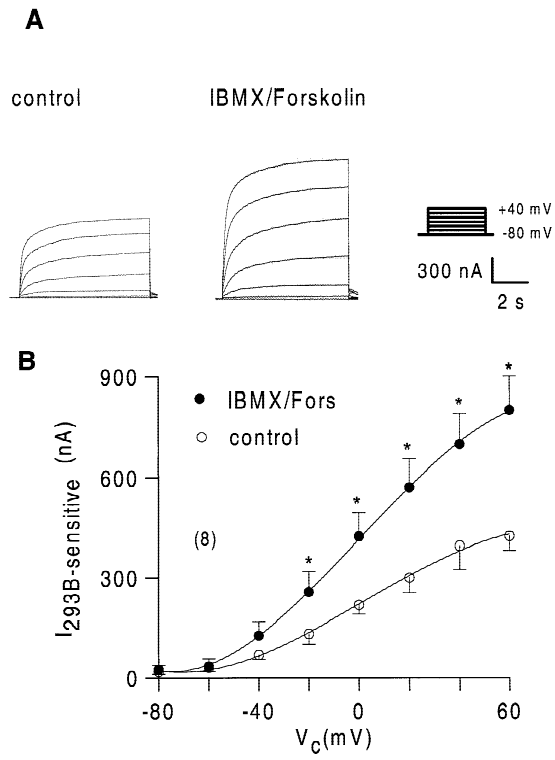

Fig. 4. Activation of 293B-sensitive rK_VLQT1 whole cell currents by increase of intracellular cAMP. (*A*) Whole cell rK_VLQT1 currents activated by depolarizing voltage pulses applied in 20 mV increments from −80 to +40 mV in oocytes expressing rK_VLQT1. Activation by 3-isobutyl-1-methylxanthine (IBMX; 1 mM) and forskolin (Fors; 2 μM). (*B*) Current-voltage relation for rK_VLQT1 whole cell currents activated by voltage pulses in the absence or presence of IBMX and forskolin. *Indicate significant difference from control (number of experiments).

Expression of rK_VLQT1 in *Xenopus* Oocytes

When rK_VLQT1 was expressed in *Xenopus* oocytes the membrane voltage was shifted to hyperpolarized values indicating K^+ selectivity of the cloned channel. Current measurements, as obtained in standard double electrode voltage-clamp experiments, indicate a typically delayed voltage activated K_VLQT1 K^+ current that shows the characteristic outward rectification (Fig. 2). Very little of this K^+ current was observed in water-injected control oocytes, which probably reflects the activity of K_VLQT1 expressed endogenously in *Xenopus* oocytes. The magnitude of the voltage-activated current was enhanced and the time course for channel activation was slowed down when rK_VLQT1 was coexpressed with human IsK (minK) (Fig. 2). This suggests that rK_VLQT1 is able to assemble with IsK to form heteromers as described previously for K_VLQT1 of other species [26].

K^+ currents generated by rK_VLQT1 were inhibited only partially but in a concentration-dependent manner

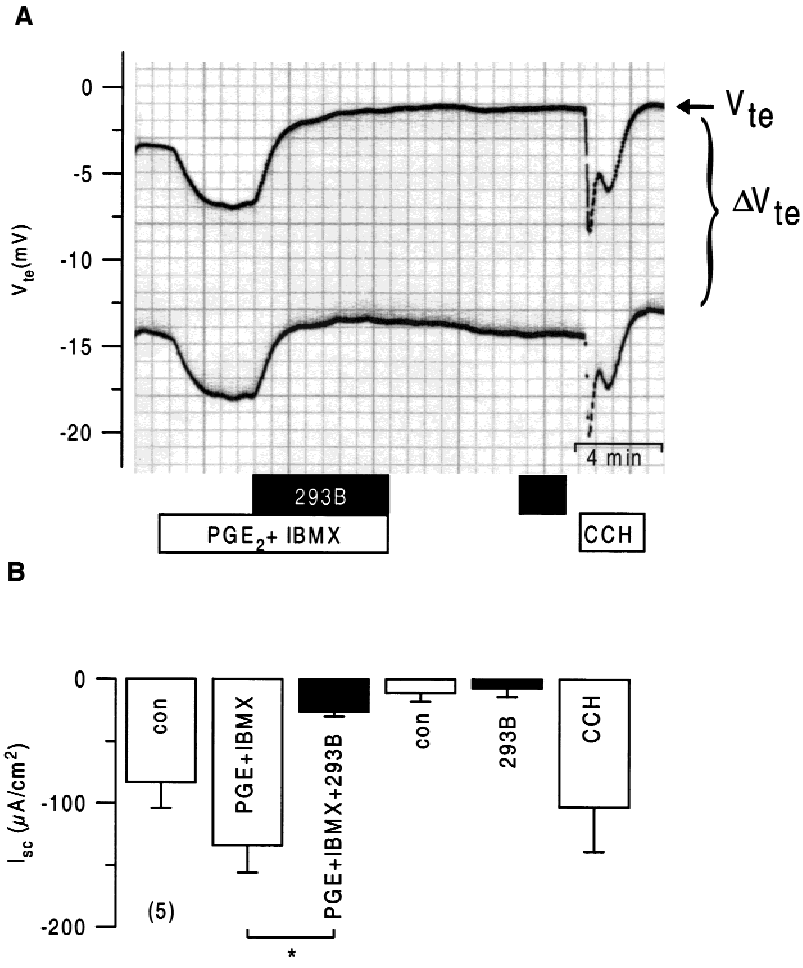

Fig. 5. Effects of stimulatory and inhibitory compounds on transepithelial voltage (V_{te}) of rat colonic epithelium. ΔV_{te} indicates voltage deflections due to current injection. (*A*) Stimulation by prostaglandin (PG) E_2 and 3-isobutyl-1-methylxanthine (IBMX) enhanced lumen negative V_{te}. 293B blocked the effects of PGE2 and IBMX but was without effect in the absence of both secretagogues. 100 μM Carbachol induced a similar albeit more rapid stimulatory response. (*B*) Summary of the equivalent short-circuit currents (I_{sc}). *Indicate statistical significance (number of experiments).

by Ba^{2+} whereas TEA^+ was almost without any effect on rK_VLQT1 up to a concentration of 10 mM (Fig. 3*A*). The $IC_{50\%}$ for the inhibition by Ba^2 was 223 ± 21 μM (n = 6). Incomplete blockage of cAMP-activated whole cell K^+ currents has been observed previously in colonic crypt cells [32[. rK_VLQT1 was also blocked by the chromanol compound 293B [15]. In previous studies, 293B has been demonstrated to be a rather specific blocker for K_VLQT1-type K^+ channels [26]. A relatively low affinity of the channel for the compound 293B was found in this study. Here, fairly high concentrations were required to block the channel and the sensitivity of the channel was not changed by increase of intracellular cAMP by IBMX and forskolin (1 mM/2 μM) (Fig. 3*D*). The $IC_{50\%}$ values for inhibition by 293B were (−cAMP) 85.5 ± 7.6 μM (n = 17) and (+cAMP) 90 ± 9.3 μM (n = 8). However, the sensitivity for 293B was significantly enhanced when the K^+ channel regulator IsK was coexpressed together with rK_VLQT1. Under these conditions, the $IC_{50\%}$ values were (−cAMP) 16 ± 0.8 μM (n = 5) and (+cAMP) 18 ± 0.8 μM (n = 6), respectively. For comparison, clotrimazol, which potently blocks Ca^{2+}-dependent K^+ channels at concentrations below 0.1 μM [6] inhibited only 24 ± 0.05% (n = 7) when applied at a concentration as high as 10 μM.

We further examined regulation of the cloned rK_V-LQT1 by intracellular Ca^{2+} and protein kinase A. Stimulation of the oocytes with 1 μM ionomycin did not show any significant effects on 293B-sensitive currents in rK_V-LQT1 expressing oocytes (1417 ± 165 *vs.* 1478 ± 161 nA, n = 8, measured at Vc = +40 mV). This suggests that rK_VLQT1 is insensitive towards changes of intracellular Ca^{2+}. However, when oocytes were stimulated with 3-isobutyl-1-methylxanthine (1 mM; IBMX) and forskolin (2 μM; Fors), a significant increase in the whole cell current was observed (Fig. 4). This increase was due to activation of a 293B-sensitive K^+ current as demonstrated in Fig. 4*B*. These results indicate that rK_VLQT1, that is already active in nonstimulated *Xenopus* oocytes, can be further activated by stimulation of the cAMP-dependent pathway but not by an increase of intracellular Ca^{2+}.

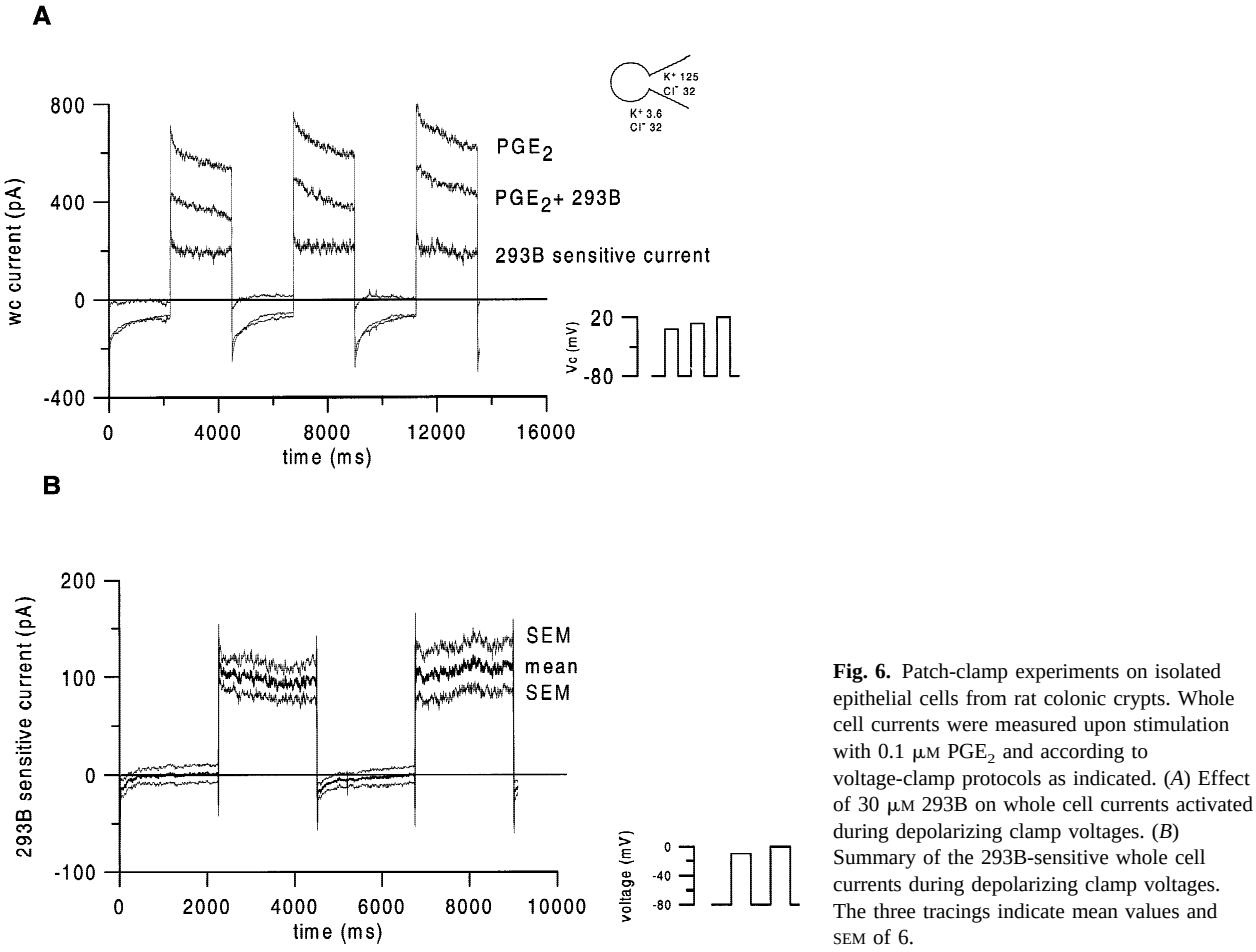

Fig. 6. Patch-clamp experiments on isolated epithelial cells from rat colonic crypts. Whole cell currents were measured upon stimulation with 0.1 μM PGE_2 and according to voltage-clamp protocols as indicated. (*A*) Effect of 30 μM 293B on whole cell currents activated during depolarizing clamp voltages. (*B*) Summary of the 293B-sensitive whole cell currents during depolarizing clamp voltages. The three tracings indicate mean values and SEM of 6.

293B-Sensitive Currents in Rat Colonic Epithelium

Ion transport was examined in rat colonic epithelium by means of a miniature Ussing chamber. Measurements under open-circuit conditions revealed a lumen negative transepithelial voltage (V_{te}) what was further enhanced when the tissue was stimulated with basolaterally applied PGE_2 and IBMX, both enhancing intracellular cAMP. The effects of PGE_2 and IBMX on V_{te} were completely blocked by 10 μM 293B applied from the basolateral side (Fig. 5). However, when applied in the absence of cAMP-dependent secretagogues, 293B was without any effects on V_{te}. Carbachol was applied to show the presence of Ca^{2+}-activated ion transport in this tissue. Equivalent short-circuit currents were calculated and are summarized in Fig. 5*B*. The data indicate a complete inhibition of cAMP-activated ion transport in rat colonic epithelium by 293B. In fact, when examined in whole cell patch-clamp experiments, epithelial cells derived from isolated rat colonic crypts demonstrated 293B-inhibitable whole cell currents. 293B inhibited 1.94 ± 0.28 and 2.52 ± 0.66 nS (n = 13) before and after stimulation with 0.1 μM PGE_2, respectively, and depolarized the cell membrane voltages by 10.2 ± 0.9 and 7.3 ± 0.8 mV. As shown in Fig. 6 whole cell currents activated by depolarizing clamp voltages were instantaneous and did not exhibit any delayed activated component. This became evident when the 293B-sensitive component of the whole cell current was extracted (Fig. 6*B*). We conclude that 293B-sensitive K^+ channels in colonic crypt cells have kinetic properties different from those observed in *Xenopus* oocytes expressing rK_VLQT1. This may suggest expression of additional unidentified modulators of rK_VLQT1 in colonic epithelial cells, similar to IsK, that may change time dependence of the current and sensitivity towards 293. Thus, the results presented here indicate functional expression of K_VLQT1 at basolateral membranes of the rat colonic epithelium as it has been suggested from previous patch-clamp experiments [32]. This K^+ channel serves as the essential pathway for recycling of K^+ for maintaining electrolyte secretion in the colonic epithelium.

There are now numerous studies demonstrating the importance of voltage-activated KVLQT (KCNQ) potas-

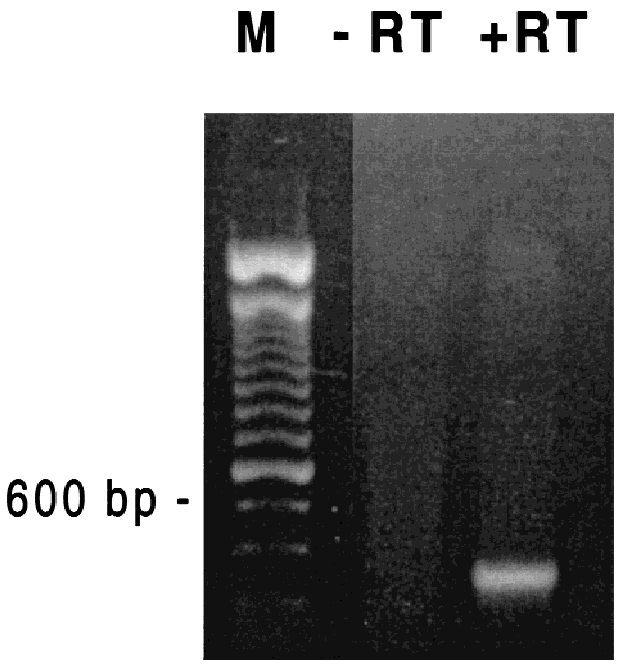

Fig. 7. RT-PCR analysis of RNA obtained from freshly isolated rat colonic crypts. A 330 bp fragment of KCNE3 was obtained after reverse transcription of total RNA (+RT) but not without reverse transcriptase (−RT). Subsequent sequencing of the amplified fragment confirmed amplification of rKCNE3.

sium channels for proper function of various organs such as heart (K_VLQT1), vestibular organ (K_VLQT1), brain (KCNQ2/KCNQ3) and sensory outer hair cells (KCNQ4) [14, 22, 24, 27, 33]. Because of their essential contribution to organ function, several inherited diseases were found to be associated with defects in KCNQ channel function like cardiac arrhythmia, deafness and epilepsy [4, 5, 14]. Moreover, K_VLQT1 was also isolated from *Xenopus laevis* and was shown to be expressed in *Xenopus* oocytes [2, 20]. In addition, K_VLQT1 was cloned from the shark *Squalus acanthias* (dogfish) [30]. sK_VLQT1 is expressed abundantly in the *Squalus* rectal gland, which is a well described secretory gland [11, 12]. There, it probably serves as the basolateral K^+ channel essential for recycling of K^+ ions that are taken up together with NaCl by the $Na^+2Cl^-K^+$-cotransporter [9, 30]. These findings suggest that mammalian secretory epithelia possess a similar type of K^+ channel serving for basolateral recycling of K^+ ions during the process of electrolyte secretion. This assumption is supported by the results of several previous reports: (i) A cAMP-regulated K^+ channel has been described in a patch-clamp study on rat colonic crypt cells [32]. This channel was of very low single channel conductance (<3 pS), only partially inhibited by Ba^2 but reversibly blocked by 293B. (ii) A basolateral K^+ conductance is activated during cAMP-dependent stimulation of electrolyte secretion in the human, murine, rat and rabbit colonic epithelium that is inhibited by the compound 293B [15, 18, 19]. (iii) K_VLQT1 is the target for the inhibitory compound 293B [2, 3, 17]. In the light of these findings, it appears reasonable to suggest expression of K_VLQT1 in basolateral membranes of the rat colonic epithelium. In addition, K_VLQT1 is also present in respiratory epithelial cells [21].

Cloning and functional characterization of rK_V-LQT1 strongly suggest molecular identity with the basolateral cAMP-activated K^+ conductance as detected in patch-clamp and Ussing-chamber experiments. The properties of both the cloned channel and the K^+ current in the intact tissue are overlapping regarding single channel conductance, channel regulation and regarding pharmacological properties. cAMP-dependent activation has not been found in K_VLQT1 cloned from most other species. In fact, the PKA phosphorylation site and the sequence differences in the N-terminus of the protein may account for this. In some respect, however, properties of rK_VLQT1 currents expressed in *Xenopus* oocytes and K^+ currents measured in native epithelial cells show remarkable differences: (i) 293B-sensitive rK_VLQT1 K^+ currents were delayed voltage-activated in *Xenopus* oocytes but largely voltage independent in rat colonic epithelial cells [32]. (ii) rK_VLQT1 currents expressed in *Xenopus* oocytes were largely active under baseline conditions and were enhanced by about 60% by cAMP-dependent stimulation, similar to cAMP dependent activation of hKVLQT in a previous study [23]. Because cAMP levels are rather low in nonstimulated oocytes, we do not assume that rK_VLQT1 activity in the absence of IBMX/forskolin is caused by a high PKA baseline activity. In the native colonic tissue, this K^+ conductance is probably silent under control conditions and relies on activation by increase of intracellular cAMP [31]. (iii) The cloned channel expressed in *Xenopus* oocytes requires fairly high concentrations ($IC_{50\%} \approx 100$ μM) of the inhibitory compound 293B to be clocked as demonstrated in the present study. Much lower concentrations are necessary to block the cAMP-activated K^+ current in the native colonic ($IC_{50\%} \approx 1$ μM) [15, 31]. The affinity towards 293B, the single channel conductance as well as the time dependence for voltage activation of K_VLQT1 channels were shown to be influenced by coexpression with the channel modulator IsK (minK) [13, 26]. An only weak RT-PCR signal for IsK (minK) was detected in epithelial cells from the surface and no signal from the bases of rat colonic crypts (*data not shown*). However, a related protein, KCNE3, was identified recently in human epithelial cells and was demonstrated to interact with KVLQT1 [25]. KCNE3 remarkably changes KVLQT1 properties such as sensitivity towards 293B and voltage dependence and thus renders it similar to those described for K^+ currents detected in the native epithelial tissue [31]. It is therefore very likely that KCNE3 is interacting with KV-

LQT1 also in rat colonic epithelial cells. In fact, RT-PCR analysis of RNA isolated from isolated rat colonic crypts and subsequent sequencing of the PCR product identified expression of a rat homologue of the human KCNE3 (Fig. 7). In summary, knowledge of the molecular identity of the basolateral cAMP-activated K^+ channel in colonic epithelial cells allows for development of both inhibitors or activators of this K^+ conductance [1, 15]. Because proper function of this K^+ channel is essential for maintaining the electrical driving force for electrolyte secretion, new potential pharmacological tools will soon be available for the treatment of secretory diarrhea or cystic fibrosis.

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