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Regulation and Properties of KCNQ1 (K_VLQT1) and Impact of the Cystic Fibrosis Transmembrane Conductance Regulator

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Abstract. The K^+ channel KCNQ1 (K_VLQT1) is a voltage-gated K^+ channel, coexpressed with regulatory subunits such as KCNE1 (IsK, mink) or KCNE3, depending on the tissue examined. Here, we investigate regulation and properties of human and rat KCNQ1 and the impact of regulators such as KCNE1 and KCNE3. Because the cystic fibrosis transmembrane conductance regulator (CFTR) has also been suggested to regulate KCNQ1 channels we studied the effects of CFTR on KCNQ1 in *Xenopus oocytes*. Expression of both human and rat KCNQ1 induced time dependent K^+ currents that were sensitive to Ba^{2+} and 293B. Coexpression with KCNE1 delayed voltage activation, while coexpression with KCNE3 accelerated current activation. KCNQ1 currents were activated by an increase in intracellular cAMP, independent of coexpression with KCNE1 or KCNE3. cAMP dependent activation was abolished in N-terminal truncated hKCNQ1 but was still detectable after deletion of a single PKA phosphorylation motif. In the presence but not in the absence of KCNE1 or KCNE3, K^+ currents were activated by the Ca^{2+} ionophore ionomycin. Coexpression of CFTR with either human or rat KCNQ1 had no impact on regulation of KCNQ1 K^+ currents by cAMP but slightly shifted the concentration response curve for 293B. Thus, KCNQ1 expressed in *Xenopus oocytes* is regulated by cAMP and Ca^{2+} but is not affected by CFTR.

Key words: K_VLQT1 — KCNQ1 — KCNE1 — IsK — minK — KCNE3 — rat colon — K^+ channel — electrolyte secretion — *Xenopus oocytes* — 293B — cAMP

Introduction

KCNQ1 (K_VLQT1) proteins are expressed ubiquitously in both nonepithelial and epithelial cells where they function as α-subunits of voltage dependent K^+ channels [2, 26]. K_VLQT1 was cloned initially from heart cells where it forms a functional complex together with IsK (minK, KNCE1) [1, 21, 30]. The channel is located in both colon and airways epithelial cells [9, 14, 17, 28]. It is activated by increase in intracellular cAMP and serves as a K^+ recycling pathway in basolateral membranes of epithelial cells. K_VLQT1 is therefore essential for maintaining secretion and absorption of electrolytes [4]. Parallel activation of basolateral K^+ channels and luminal CFTR Cl^- channels is essential for epithelial Cl^- secretion. K_VLQT1 is associated with the small regulatory β-subunits KCNE [28]. In heart cells, KCNQ1 does form a complex with KCNE1 (IsK, minK) and generates slowly depolarization-activated K^+ channels (Iks) [1, 21]. In colonic crypt cells, KCNQ1 interacts with KCNE3, which leads to constitutively open K^+ currents [23]. These instantaneous currents have been detected in patch-clamp experiments on isolated colonic crypt cells [9, 23]. This suggests that KCNQ1 interacts with different KCNE β-subunits in a tissue-specific manner. Although it has been demonstrated that KCNE β-subunits largely influence the voltage dependence of KCNQ1 K^+ channels, the impact of KCNE on the regulatory properties of KCNQ1 currents has not yet been examined in detail. We therefore coexpressed both KCNE1 and KCNE3 together with KCNQ1 in *Xenopus* oocytes and studied the impact of these β-subunits on regulation of KCNQ1 by increase in the common intracellular second messengers cAMP and Ca^{2+}. To that end, KCNE3 was cloned from rat colonic crypt cells and was coexpressed with rKCNQ1, which has been cloned recently in our laboratory, while human KCNQ1 was coexpressed together with human KCNE1 [9].

It has been postulated that the cystic fibrosis transmembrane conductance regulator (CFTR) activates KCNQ1 K^+ currents [16]. CFTR is a cAMP-regulated Cl^- channel which has been shown to influence

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both function and properties of other membrane conductances such as epithelial Na^+ channels, ROMK potassium channels, outwardly rectifying Cl^- channels (ICOR, ORCC) and aquaporin 3 [8, 24]. Experiments in *Xenopus* oocytes demonstrated that CFTR is also able to activate KCNQ1 K^+ channels expressed endogenously in *Xenopus* oocytes. Mutant CFTR such as ΔF508-CFTR is unable to activate xKCNQ1 [3, 16]. Similar results have been obtained in studies with the epithelial cell line CFPAC. In these cells, the authors demonstrated cAMP-dependent activation of a K^+ conductance in parallel with stimulation of a Cl^- conductance in CFTR-expressing CFPAC cells. Such an activation of KCNQ1 K^+ channels was not observed in the parental CFPAC cell line which does express mutant CFTR only. From these results the authors concluded that wild-type CFTR controls the activation of cAMP-dependent K^+ channels in epithelial cells [13]. These results are in contrast to those obtained in another study demonstrating the existence of cAMP-activated K^+ conductance in CFPAC cells without expression of functional CFTR [29]. Moreover, no evidence has been found for defective basolateral cAMP-activated K^+ conductance in human intestinal or airway epithelial cells derived from patients with cystic fibrosis [15, 18]. Taken together, these studies do not give a conclusive answer to the question whether or not CFTR controls the activity of KCNQ1 K^+ currents. We therefore coexpressed CFTR together with KCNQ1 in both absence or presence of the regulatory β subunits KCNE1 and KCNE3 and examined a possible regulatory impact of CFTR on KCNQ1 in *Xenopus* oocytes. The data suggest that apart from a slight increase in the sensitivity for the K^+ channel blocker 293B, CFTR does not otherwise interfere with the properties of KCNQ1 K^+ channels.[1]

Methods and Materials

Cloning of rKCNE3

Messenger RNA was isolated from single isolated rat colon crypts [10] and was reverse transcribed using oligo d(T) primer and reverse transcriptase (Superscript, GIBCO). Second-strand cDNA was synthesized from the first-strand cDNA using RNase H, *E. coli* DNA polymerase I and *E. coli* DNA ligase. Degenerated oligonucleotide primers were designed corresponding to human and mouse KCNE3 (all 5′-3′): Sense primer: CAATMYCTGTTGCCATGGAGAC; antisense primer: CACATCAGATCATAGACACACG. Abbreviations of degenerated primer sites: M = A and C, Y = C and T. PCR amplification was performed using 0.5 μmol/l of sense and antisense primer (1 min at 94°C, 30 sec at 50°C, 3 min at 72°C, 45 cycles followed by 1 min at 94°C, 30 sec at 50°C, 10 min at 72°C, 1 cycle). PCR products (341 bp) were subcloned into pBluescript SK(−) and sequenced using a fluorescence DNA sequencer (373A, Applied Biosystem). A *Nco*I/*Xho*I fragment of rKCNE3 was subcloned into oocyte expression vector pTLN, that used the *Xenopus* β-globin untranslated regions to boost expression in oocytes (kindly provided by Dr. T.J. Jentsch, Hamburg, Germany [12]). Full-length cDNA of rKCNE3 was cloned using the Marathon cDNA Amplification Kit (Clontech, Palo Alto, USA). A library of adapter-ligated, double stranded cDNA was synthesized from poly-A^+ RNA isolated from colon crypts (QuickPrep, Pharmacia Biotech, Sweden). RACE-PCR was carried out with the gene-specific sense primer 5′- ACACAACCCTTCACAGTCAC-3′ and antisense primer 5′- GGTCACTACGTTTGTCCAC-3′. A 3′-RACE fragment (672 bp) and a 5′-RACE fragment (515 bp) were subcloned in pBluescript SK(−) and sequenced (EMBO, Accession number AJ271742).

cRNAs of hKCNQ1, hKCNE1 (IsK, minK), rKCNQ1 and CFTR

cDNAs encoding human KCNQ1 isoforms with a truncated N-terminus [21] or full length N-terminus [30] and human KCNE1 (minK, IsK) were kindly provided by Dr. M.C. Sanguinetti (Howard Hughes Medical Institute, Salt Lake City) and Dr. A.E. Busch (Aventis, Bad-Soden, Germany). The rat KCNQ1 has been cloned previously by our group [9]. A rK_VLQT1 mutant was generated carrying a single point-mutation in a protein kinase A phosphorylation motif at the N-terminal end of the protein. This S27A mutation was generated by applying standard PCR methods (Quickchange, Stratagene). Using similar methods, an N-terminal-truncated rKCNQ1 was synthesized, which lacks the first 27 amino acids and thus does not contain the PKA phosphorylation motif RRGS (M27-rKCNQ1). cDNAs encoding hKCNQ1, hKCNE1, rKCNQ1, rKCNE3 and CFTR were linearized at the 3′ end using *Mlu*I, *Not*I, *Xho*I and *Hpa*I and was in vitro transcribed using SP6 polymerase and a 5′cap (mMessage mMachine, Ambion).

Expression in *Xenopus* ooyctes

Isolation and microinjection of oocytes have been described in a previous report [6]. In brief, after isolation from adult *Xenopus laevis* female frogs (Xenopus Express, South Africa), 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. Oocytes were injected with cRNA (1–10 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. 2–4 days after injection oocytes were impaled with two electrodes (Clark Instruments) which had resistances of <1 MΩ when filled with 2.7 M KCl. A flowing (2.7 mM) KCl electrode served as bath reference (serial resistance 0.7 kΩ) 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 1000 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

[1] *Abbreviations:* KCNQ1 (K_VLQT1), voltage gated K^+ channel; CFTR, cystic fibrosis transmembrane conductance regulator; IBMX, 3-isobutyl-1-methylxanthine; Fors, Forskolin; TEA^+, tetraethylammonium. rKNCE3 (EMBO Accession number AJ 271742)

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                1                                                    50
rat KCNE3       METSNGTETW YKSLHAVLKA LNTTLHSHLL CRPGPGPGS GTGPDNQTEDH
human KCNE3     METTNGTETW YESLHAVLKA LNATLHSNLL CRPGPGLG. ...PDNQTEER
mouse KCNE3     METSNGTETW YMSLHAVLKA LNTTLHSHLL CRPGPGPG. ...PDNQTEDR

                51                                                  100
rat KCNE3       RASLPGRNDN SYMYILFVMF LFAVTVGSLI LGYTRSRKVD KRSDPYHVYI
human KCNE3     RASLPGRDDN SYMYILFVMF LFAVTVGSLI LGYTRSRKVD KRSDPYHVYI
mouse KCNE3     RASLPGRNDN SYMYILFVMF LFAVTVGSLI LGYTRSRKVD KRSDPYHVYI

                101
rat KCNE3       KNRVSMI        aa 107
human KCNE3     KNRVSMI        aa 103
mouse KCNE3     KNRVSMI        aa 103
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Fig. 1. Sequence alignment of rat, human and mouse KCNE3. Differences in amino acids are printed in bold. The putative transmembrane region is underlined. The amino-acid identity of rat KCNE3 to that of human and mouse is 88.8% and 94.4%, respectively. The amino-acid homology of rat KCNE3 to human and mouse KCNE3 is 95.3%. Accession numbers for rat, human and mouse KCNE3 are AJ271742, AF076531 and AF076532. The bar indicates the sequence of the putative transmembrane domain.

whole experiment the bath was continuously perfused at a rate of 5–10 ml/min. All experiments were conducted at room temperature (22°C).

Materials and Statistical Analysis

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

Results

Cloning of KCNE3 from Rat Colonic Epithelial Cells

RNA was prepared from isolated colonic crypts and RT-PCR was performed as described in methods. A full-length sequence of rKCNE3 was obtained by RACE-PCR. As shown in Fig. 1, rKCNE3 shares a high degree of homology with KCNE3 isolated from human and mouse. It is, however considerably different from KCNE1, which is only about 30% identical to KCNE3. rKCNE3 is coexpressed together with rKCNQ1 in colonic epithelial cells [9]. When coexpressed together with rKCNQ1 in *Xenopus* oocytes, an almost instantaneously activated K^+ current can be detected, similar to the K^+ channel formed by human KCNQ1 and KCNE3 [23] (Fig. 2).

Inhibition of KCNQ1

We examined the inhibition of KCNQ1 by Ba^{2+}, TEA^+ and the chromanol compound 293B. 293B has been demonstrated to block specifically KCNQ1-type K^+ currents. Fig. 2 shows K^+ currents induced by expression of either human or rat KCNQ1 and it demonstrates the impact of coexpression of both hKCNE1 and rKCNE3. Coexpression of hKCNQ1 with hKCNE1 induced a significant delay in voltage activation of the hKCNQ1 as described in previous reports [1, 21, 30]. Coexpression of rKCNE3 together with rKCNQ1 had the opposite effect and induced rapidly activated, almost instantaneous K^+ currents. All KCNQ1 currents were inhibited only at high concentrations of Ba^{2+}. As shown in Fig. 2*A*, inhibition of K^+ currents was only partial, even at 5 mM Ba^{2+}. Concentration response curves were obtained for the different K^+ currents and concentrations for half maximal inhibition ($IC_{50\%}$) were calculated. $IC_{50\%}$ values were 3.0 mM (hKCNQ1, dotted line), 100 mM (hKCNQ1/hKCNE1, continuous line), 4 mM (rKCNQ1, continuous line) and 400 μM (rKCNQ1/rKCNE3, dotted line). Thus, coexpression of the β-subunits KCNE1 or KCNE3 change the sensitivity towards the nonspecific K^+ channel blocker Ba^{2+}. In contrast, there was no detectable

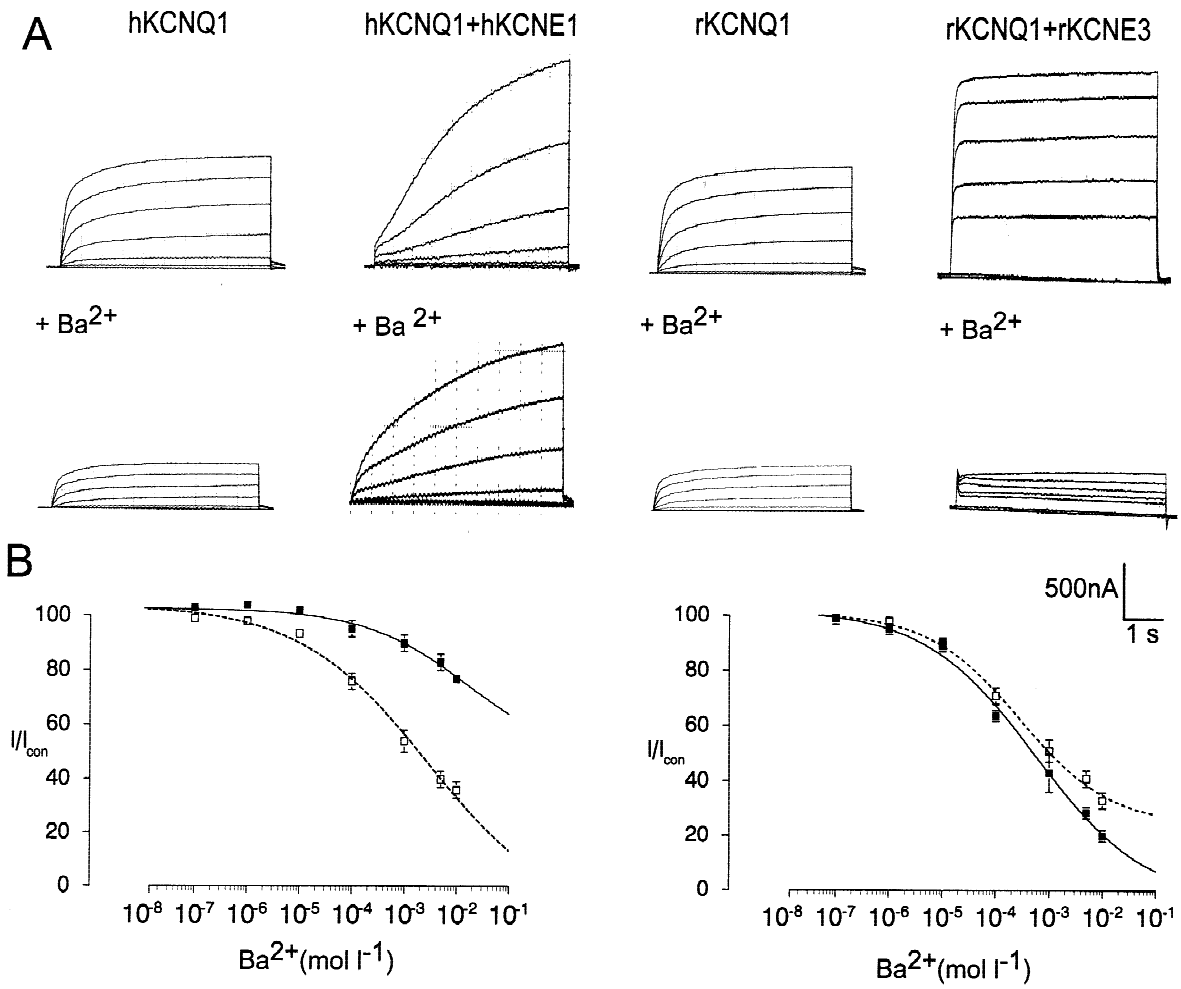

Fig. 2. (*A*) First row: Whole-cell currents activated by depolarizing voltage pulses applied in 20 mV increments from −80 mV to +40 mV in oocytes expressing hKCNQ1 or rKCNQ1 in either absence or presence of hKCNE1 or rKCNE3. Second row: Inhibition by Ba^{2+} (5 mM). (*B*) Concentration/response curves for the effects of Ba^{2+} on the various KCNQ1 currents described above. Left diagram: Dotted line = hKCNQ1 ($n = 7$); Continuous line = hKCNQ1 + hKCNE1 ($n = 9$). Right diagram: Continuous line = rKCNQ1 ($n = 9$); Dotted line = rKCNQ1 + rKCNE3 ($n = 8$). $IC_{50\%}$ values are given in the text.

inhibition of hKCNQ1 or rKCNQ1 K^+ currents by TEA^+ (0.1 μM–100 mM; $n = 4$ each), independent of the presence of either hKCNE1 or rKCNE3 or increase in intracellular cAMP (*data not shown*).

293B has been demonstrated to specifically block KCNQ1 channels [27]. Coexpression of the β-subunits hKCNE1 and rKCNE3 affected the sensitivity of KCNQ1 K^+ currents for 293B. As shown in Fig. 3, expression of hKCNE1 enhanced the sensitivity and shifted the $IC_{50\%}$ (all in μM) for 293B: 70 (hKCNQ1; filled circles, dotted line; $n = 20$) vs. 5.9 (hKCNQ1/hKCNE1; filled circles, continuous line; $n = 14$). A similar shift in the $IC_{50\%}$ value was observed for rKCNE3: 93 (rKCNQ1; open circles, dotted line; $n = 19$) *vs.* 3.5 (rKCNQ1/rKCNE3; open circles, solid line; $n = 21$). Moreover, $IC_{50\%}$ for 293B-dependent inhibition of an N-terminal-truncated hKCNQ1 [1, 21] was also shifted from 68 μM (−hKCNE1, $n = 7$) to 5.1 μM (+hKCNE1, $n = 20$), similar to full-length hKCNQ1 [30]. In addition, the $IC_{50\%}$ for inhibition of N-terminal-truncated hKCNQ1/hKCNE1 by 293B was not significantly altered by stimulation of the oocytes with IBMX and forskolin ($IC_{50\%} = 67$ (−IBMX/Fors) *vs.* 6 (+IBMX/Fors); $n = 7$). Similarly, for both rKCNQ1 and rKCNQ1/KCNE3 expressing oocytes, the $IC_{50\%}$ values for inhibition by 293B were not changed after IBMX/Fors and were 95 μM (n = 6; rKCNQ1) and 3 μM (n = 11; rKCNQ1/rKCNE3). Taken together, under all experimental conditions examined here, coexpression of both KCNE1 or KCNE3 with KCNQ1 does enhance the sensitivity of kCNQ1 for 293B by about one order of magnitude.

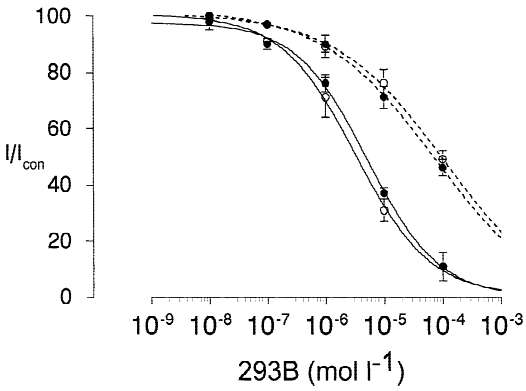

Fig. 3. Concentration/response curves for the effects of the inhibitory chromanol 293B on K^+ currents (*I*) detected in oocytes expressing hKCNQ1 or rKCNQ1 in either absence or presence of hKCNE1 or rKCNE3, respectively. Dotted lines: rKCNQ1 (open circles, $n = 8$); hKCNQ1 (filled circles, $n = 9$). Continuous line: rKCNQ1 + rKCNE3 (open circles, $n = 7$); hKCNQ1 + hKCNE1 (filled circles, $n = 8$). $IC_{50\%}$ values are given in the text. Currents *I* were normalized to control currents I_{con} recorded in the absence of blockers.

Regulation of KCNQ1 by cAMP and Ca^{2+}

We examined whether an increase in intracellular cAMP or Ca^{2+} regulates the activity of KCNQ1. Whole-cell KCNQ1 K^+ currents were augmented when oocytes were stimulated with IBMX (1 mM) and forskolin (2 μM). The summary of this set of experiments is shown in Fig. 4*A*. The data show activation of KCNQ1 K^+ currents, which was largely independent of coexpression with KCNE1 or KCNE3 (Fig. 4*B*). Thus, protein kinase-dependent regulation of KCNQ1 does not seem to be modulated by β-subunits. However, hKCNQ1 with a truncated N-terminus was not activated by increase of intracellular cAMP (Fig. 4*C*). Stimulation with higher concentrations of forskolin (10 μM) or membrane permeable dibutyryl-cAMP (500 μM) were unable to augment these K^+ currents (*data not shown*). hKCNQ1 and rKCNQ1 are largely identical in their N-terminal sequence and both contain the PKA phosphorylation motif RRGS (amino acids 24–27) in their cytosolic N-terminal tail. We examined the contribution of this PKA motif to the cAMP-dependent activation of the channel and therefore generated rKCNQ1 mutants which either lack the first 27 amino acids (M27-rKCNQ1) or which carry a single point mutation (S27A-rKCNQ1). However, both mutants were still activated by IBMX and forskolin, indicating that the putative PKA-phosphorylation site is not essential for cAMP-dependent stimulation (Fig. 4*C*).

We further examined whether increase of intracellular Ca^{2+} affects KCNQ1 activity. In these experiments all but 5 mM Cl^- was removed from the extracellular bath solution and was replaced by the impermeable anion gluconate. Thus, contamination by endogenous Ca^{2+}-activated Cl^- currents was avoided. Under these conditions stimulation of water-injected oocytes with 1 μM of the Ca^{2+} ionophore ionomycin ($n = 12$) did not show any current activation (*data not shown*). Similarly, K^+ currents generated by hKCNQ1 or rKCNQ1 were not changed by exposure to the Ca^{2+}-enhancing agonist ionomycin (1 μM; Fig. 5*A*). This suggests that KCNQ1 is not sensitive towards increase in intracellular Ca^{2+}. However, when hKCNQ1 and rKCNQ1 were coexpressed together with hKCNE1 or rKCNE3, K^+ currents were augmented slightly but significantly by 1 μM ionomycin (Fig. 5*B*). Thus, both KCNE1 and KCNE3 enable

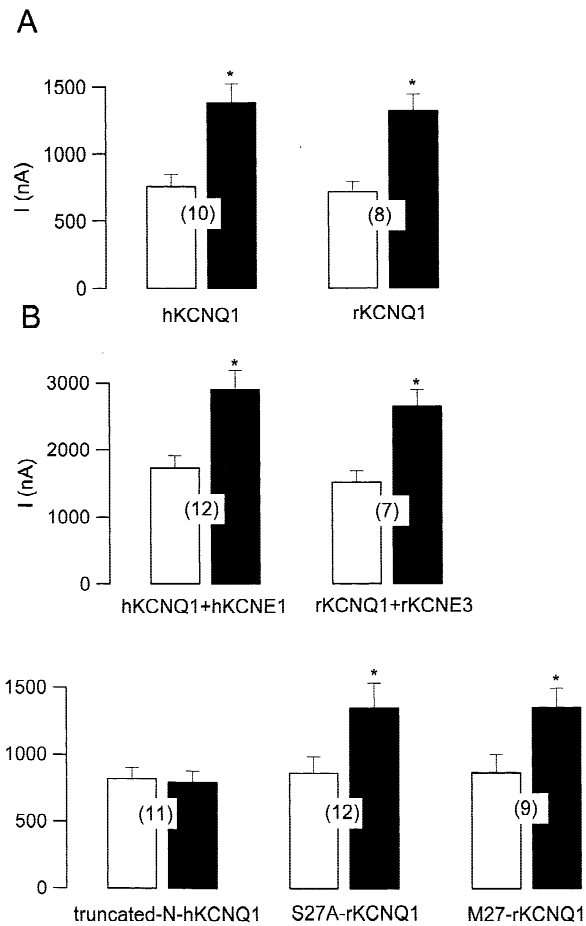

Fig. 4. Summary of the effects of increase of intracellular cAMP by IBMX and forskolin. Activation of whole-cell K^+ currents by stimulation with IBMX and forskolin (black bars, 1 mM/2 μM). Currents were measured at the end of (6 sec) depolarizing voltages pulses (from −80 mV to +40 mV) in oocytes expressing hKCNQ1 or rKCNQ1 (*A*) or hKCNQ1 + hKCNE1 and rKCNQ1 + rKCNE3 (*B*). Effects of IBMX and forskolin on truncated hKCNQ1 and rKCNQ1 without the PKA phosphorylation motif RGGS in the N-terminal tail (S27A-rKCNQ1, M27-rKCNQ1) (*C*). * indicates significant difference ($P < 0.05$) from control (white bars). The number of experiments is given in parentheses.

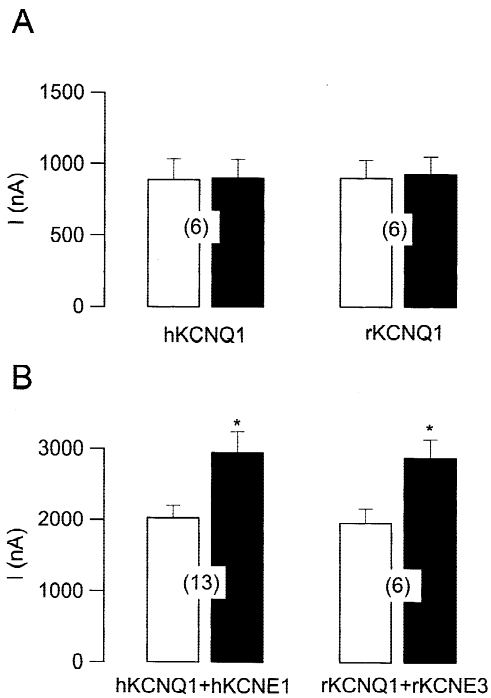

Fig. 5. Summary of the effects of increase of intracellular Ca^{2+} by ionomycin. Stimulation of *Xenopus* oocytes expressing hKCNQ1 or rKCNQ1 (*A*) or hKCNQ1 + hKCNE1 or rKCNQ1 + rKCNE3 (*B*), with ionomycin (black bars, 1 μM). Currents were measured at the end (6 sec) of depolarizing voltages pulses (from −80 mV to +40 mV). Asterisk indicates significant ($P < 0.05$) difference from control (white bars). In parentheses, the number of experiments.

Ca^{2+}-dependent regulation of KCNQ1 K^+ currents which are otherwise Ca^{2+} insensitive.

Impact of CFTR on cAMP-dependent Regulation of KCNQ1

Because previous studies suggested an impact of CFTR on KCNQ1 currents expressed endogenously in *Xenopus* oocytes, we examined the impact of CFTR on the regulation of both human and rat KCNQ1 in the absence or presence of hKCNE1 and rKCNE3. When KCNQ1 was coexpressed with KCNE1 or KCNE3 and CFTR, a whole-cell Cl^- current was activated by IBMX and Forskolin that was largely inhibited by gluconate (Fig. 6). Ba^{2+}-(5 mM) sensitive KCNQ1 K^+ currents were examined before and after stimulation of the oocytes with IBMX and forskolin (1 mM/2 μM). Ba^{2+}-sensitive K^+ currents were activated by increase of cAMP independent of coexpression with hKCNE1 or rKCNE3 (Fig. 7*A,B*). Moreover, coexpression with CFTR did not affect cAMP-dependent regulation of KCNQ1, i.e., activation of Ba^{2+}-sensitive K^+ currents by IBMX and forskolin was similar in the absence or presence of CFTR (Fig. 4*AB*, 8*AB*). Moreover, also in experiments with N-terminal-truncated hKCNQ1 coexpression with CFTR in presence ($n = 6$) or absence ($n = 6$) of hKCNE1 did not affect regulation by IBMX and forskolin. Therefore, CFTR does not seem to influence cAMP-dependent regulation of kCNQ1.

We further examined a possible impact of CFTR on 293B dependent inhibition of KCNQ1. 293B (300 μM)-sensitive KCNQ1 currents were activated by IBMX/Fors to the same degree, independent of coexpression of CFTR. Moreover, coexpression and stimulation of G551D-CFTR had no further impact (*data not shown*). In addition, we examined concentration-dependent inhibition of KCNQ1 by 293B in the presence of CFTR before (open circles) and after (filled circles) stimulation with IBMX and forskolin and in both absence (dotted lines) or presence (continuous lines) of KCNE1 and KCNE3. In absence of IBMX/Fors, $IC_{50\%}$ values (μM) were slightly lower than those obtained without CFTR: hKCNQ1 35 ($n = 12$, Fig. 8*A*); hKCNQ1/hKCNE1 2.5 ($n = 14$, Fig. 8*A*); rKCNQ1 25 ($n = 12$, Fig. 8*B*); rKCNQ1/rKCNE1 5 ($n = 6$, Fig. 8*B*). After stimulation with IBMX/Fors, $IC_{50\%}$ values were shifted slightly further to lower values and were 20 (hKCNQ1; $n = 13$; Fig. 8*A*), 1.0 (hKCNQ1/hKCNE1; $n = 11$; Fig. 8B), 15 (rKCNQ1; $n = 13$; Fig. 8*A*) and 1 (rKCNQ1/rKKCNE3; $n = 11$; Fig. 8*B*). However, whole-cell Cl^- currents activated in CFTR-expressing oocytes remained essentially unaffected by 0.1 and 100 μM 293B (*data not shown*). Taken together, these data indicate that apart from a slight increase in the sensitivity for 293B, CFTR had no further effects on the activity of KCNQ1 K^+ currents.

Discussion

The present paper examines properties and regulation of KCNQ1 K^+ currents and the possible impact of CFTR. Because our particular interest is in the function of KCNQ1 currents in epithelial cells and because most work has been done in rat colonic epithelial cells, we decided to clone the β-subunit KCNE3 from rat colon. rKCNE3 demonstrates a high degree of homology when compared to human KCNE3 [23]. KCNE3 co-assembles with KCNQ1 in colonic epithelial cells where it largely affects voltage-dependence and -sensitivity towards the inhibitor 293B [23]. Similar results were found in the present study with rKCNE3: KCNQ1 currents were almost instantaneous and $IC_{50\%}$ values for inhibition with 293B were lowered due to coexpression of rKCNQ1 and rKCNE3. Moreover, we detected an increase in the affinity for the nonspecific K^+ channel blocker Ba^{2+}. This might be a consequence of the changes in kinetic properties of the KCNQ1/KCNE3 current. The opposite should be expected for coexpression with KCNE1, which slows down voltage dependent activation of the KCNQ1 currents [1, 21, 23]. In fact, the $IC_{50\%}$ value for inhibition by Ba^{2+} was higher for hKCNQ1/hKCNE1 currents.

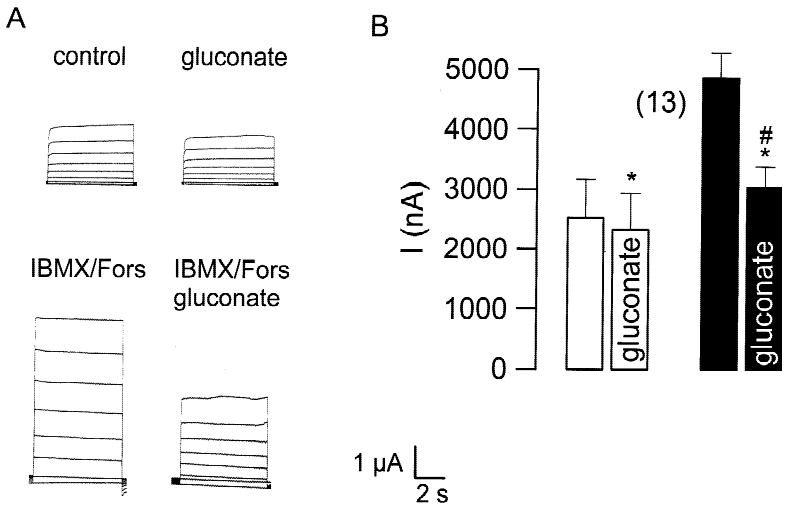

Fig. 6. Activation of Cl^- currents by IBMX and forskolin. (*A*) Whole-cell currents of an oocyte coexpressing CFTR, rKCNQ1 and rKCNE3. Whole-cell currents were activated by depolarizing voltages pulses applied in 20 mV increments from −80 mV to +40 mV. Effects of partial replacement of extracellular Cl^- by gluconate before and after stimulation with IBMX (1 mM) and forskolin (2 µM). (*B*) Summary of the effects of gluconate on oocytes coexpressing CFTR, rKCNQ1 and rKCNE3. The effect of gluconate was examined before (white bars) and after (black bars) stimulation with IBMX/Fors. Asterisk indicates significant effect of Cl^- replacement by gluconate; # indicates significant difference of the effects of gluconate before and after stimulation with IBMX/Fors. Number of experiments in parentheses.

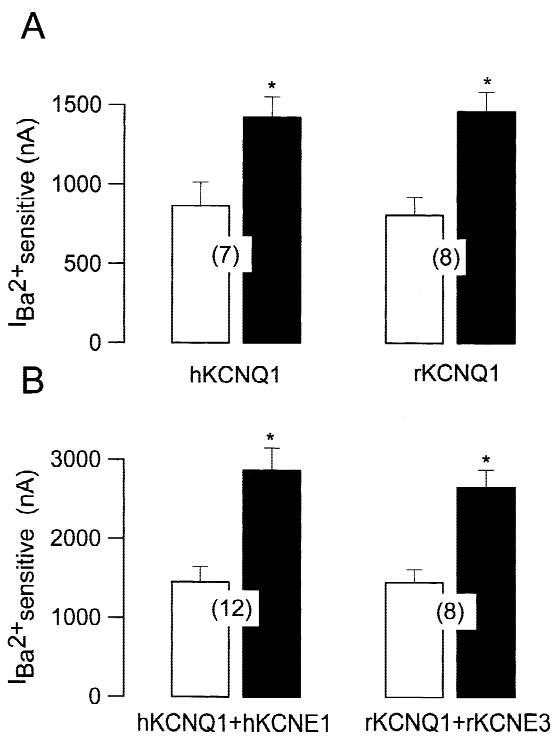

Fig. 7. Summary of the effects of increase of intracellular cAMP by IBMX and forskolin (black bars) on KCNQ1 in the presence of CFTR. Activation of Ba^{2+} sensitive whole-cell K^+ currents by increase of intracellular cAMP due to stimulation with IBMX and forskolin (1 mM/2 µM). Currents were measured at the end of (6 sec) depolarizing voltages pulses (from −80 mV to +40 mV) in oocytes expressing hKCNQ1 or rKCNQ1 in either absence (*A*) or presence (*B*) of hKCNE1 or rKCNE3. Asterisk indicates significant difference from control (white bars). Number of experiments in parentheses.

Previous reports described cAMP-dependent regulation of KCNQ1 [23, 30]. Here we demonstrate that activation by increase of intracellular cAMP of either hKCNQ1 or rKCNQ1 is independent of coexpression with the regulatory β-subunits hKCNE1 and rKCNE3, respectively. This result and the fact that both hKCNQ1 and rKCNQ1 contain the PKA phosphorylation motif RRGS in the N-terminal tail suggest that KCNQ1 is activated by direct phosphorylation at this motif. However, the present data rather suggest that although the N-terminal tail is necessary for PKA-dependent regulation of KCNQ1, the RRGS motif is not required. Different results were obtained for the regulation of KCNQ1 by intracellular Ca^{2+}. Stimulation of KCNQ1 K^+ currents by increase in intracellular Ca^{2+} requires coexpression of either hKCNE1 or rKCNE3. These results do support previous studies demonstrating that slowly-activating Iks currents in mouse heart and *Xenopus* oocytes are activated by A23187 and are reduced by the Ca^{2+} chelator BABTA [26]. Moreover, Ca^{2+}- and protein kinase C-dependent regulation of KCNQ1 were suggested recently [2, 28]. As demonstrated in the present study, the effects of Ca^{2+} are mediated by the regulatory KCNE subunits since KCNQ1 K^+ currents were not activated by ionomycin in the absence of either hKCNE1 or rKCNE3.

In the present paper we asked the question to what degree CFTR is able to control the activity of exogenously expressed KCNQ1 currents. This seemed to be a timely question since previous studies had suggested activation of endogenous K^+ currents by CFTR in *Xenopus* oocytes and cultured mammalian cells [13, 16]. Because CFTR has been demonstrated in numerous previous studies to have a dual function as cAMP-regulated Cl^- channel as well as regulator of other ion channels, it is reasonable to suggest that such a control mechanism also applies to KCNQ1 K^+ channels [8, 11, 24]. Co-regulation of both CFTR and basolateral KCNQ1 K^+ channels would facilitate coordinated electrolyte secretion in epithelial cells [4, 5]. However, in the present study we did not find evidence for activation of KCNQ1

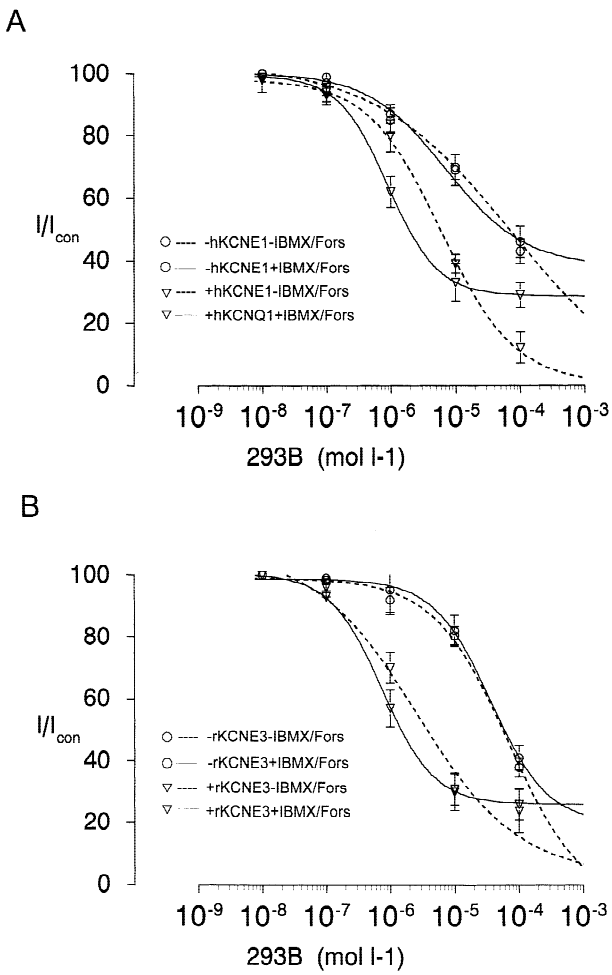

Fig. 8. Concentration/response curves for the effects of the inhibitory chromanol 293B on (*A*) hKCNQ1- and (*B*) rKCNQ1 K^+ currents coexpressed in *Xenopus* oocytes together with CFTR in the absence (dotted lines) or presence of hKCNE1 or rKCNE3 (continuous lines). $IC_{50\%}$ values are given in the text. The effects of 293B were examined in the absence (open circles) or presence (filled circles) of IBMX (1 mM) and forskolin (2 μM).

currents by CFTR. KCNQ1 currents were activated by an increase in intracellular cAMP, independent of coexpression and stimulation of CFTR. Moreover, the presence of the regulatory subunits KCNE1 or KCNE3 did not change these results. This leaves some room for further speculations. It could be that another protein is missing that facilitates an interaction of CFTR with KCNQ1. Another explanation for the previous results could be the slightly higher affinity of KCNQ1 for the inhibitory compound 293B after stimulation of CFTR. Thus, an apparent increase in 293B-sensitive endogenous K^+ currents expressed in *Xenopus* oocytes after activation of CFTR might be due to a higher affinity of this channel for 293B [16]. Such a shift in the affinity of other K^+ channels and inhibitory compounds by coexpression of CFTR has been observed recently [7, 19]. The mechanism of these CFTR/K^+ channel interactions remains obscure. A recent report points out the importance of CFTR's first nucleotide-binding domain in controlling the glibenclamide sensitivity of ROMK K^+ channels [20]. Interestingly, NBF1 has been found to be involved in several regulatory processes, such as control of epithelial Na^+ channels and outwardly rectifying Cl^- channels [22, 25]. In preliminary experiments we found that expression of only NBF1 of CFTR is able to mimic the effects of full-length CFTR on endogenous *Xenopus* KCNQ1 currents (unpublished data from the author's laboratory). Subsequent study will now have to demonstrate how NBF1 interacts with the K^+ channels and other membrane proteins.

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