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Characterization of TASK-4, a novel member of the pH-sensitive, two-pore domain potassium channel family

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Abstract We report the primary sequence of TASK-4, a novel member of the acid-sensitive subfamily of tandem pore K^+ channels. TASK-4 transcripts are widely expressed in humans, with highest levels in liver, lung, pancreas, placenta, aorta and heart. In *Xenopus* oocytes TASK-4 generated K^+ currents displaying a marked outward rectification which was lost by elevation of extracellular K^+. TASK-4 currents were efficiently blocked by barium (83% inhibition at 2 mM), only weakly inhibited by 1 mM concentrations of quinine, bupivacaine and lidocaine, but not blocked by tetraethylammonium, 4-aminopyridine and Cs^+. TASK-4 was sensitive to extracellular pH, but in contrast to other TASK channels, pH sensitivity was shifted to more alkaline pH. Thus, TASK-4 in concert with other TASK channels might regulate cellular membrane potential over a wide range of extracellular pH.

Key words: Cloning; TASK; Two-pore domain K^+ channel; Anesthetic

1. Introduction

Two-pore domain K^+ channels are an emerging superfamily of background (leak) K^+ channels, which are expected to strongly contribute to the resting membrane potential in various tissues. Two principal types of 2-P domain structures are known, having either eight or four transmembrane segments (2P/8TM, or 2P/4TM) [1,2]. In particular, the 2P/4TM type of two-pore domain K^+ channels seems to be very common since only the genome of the nematode *Caenorhabditis elegans* contains over 50 genes, which may encode K^+ channels with this pattern [3–6]. TWIK-1 is the founding member of the mammalian class of 2P/4TM K^+ channels [7], which in addition to TWIK includes three other families named TASK, TREK and TRAAK. The members of these gene families usually share low sequence similarity but they encode K^+ channels that are regulated by a variety of physiological stimuli and are characterized by a unique pharmacology. TWIK-1 and TWIK-2 express weak inwardly rectifying currents that are regulated by protein kinase C and are inhibited by intracellular pH [7,8]. TREK-1 and TREK-2, similar to TRAAK channels, are activated by mechanical stretch and free fatty acids [9,10]. TASK-1, TASK-2 and TASK-3 generate outward K^+ currents that are inhibited by a decrease in external pH near the physiological range. Outward rectification of TASK currents is lost at high external K^+ concentration, identifying TASK channels as open rectifiers, meaning that their currents obey the Goldman–Hodgkin–Katz equation for an open channel.

While 2-P K^+ channels in general are relatively insensitive to classical K^+ channel blockers, some of them can be activated by inhalational general anesthetics and the neuroprotective drug riluzole [11].

Here we report the primary sequence, tissue distribution, functional expression and pharmacological properties of TASK-4, a new member of the TASK family. In general, sequence similarity of TASK-4 to other 2P/4TM K^+ channels is low. Within the transmembrane spanning region (M1–M4) TASK-4 shares 38% similarity to TASK-2. Interestingly, while TASK-4 is widely distributed in human tissues, it is restricted to the atrium and the atrioventricular node in the heart.

2. Materials and methods

2.1. Molecular cloning and expression of TASK-4

A BLAST search of the Incyte LifeSeq database, using the sequences of mammalian 2-P domain K^+ channels, led to the identification of an EST sequence with significant similarity to the human TRAAK channel [11]. RACE was used to isolate the missing 5′-end from adult human kidney cDNA library (Edge Biosystems). A 0.7 kb fragment was amplified by nested PCR using vector (pEAK8) primers 5′-CTTGGCACTTGATGTAATTCTCCTTGG-3′ and 5′-TGCCCTTTTTGAGTTTGGATCTTGG-3′ and the gene-specific primers 5′-TGTCTCCAGCTTTGGGACTTG-3′ and 5′-TCATTCCAATCACGTAGTCGCC-3′. Oligonucleotide primers derived from the 5′- and 3′-ends were used to amplify the full-length cDNA from human adrenal cDNA, which was subcloned into the *Xenopus* expression vector pSGEM [12]. The cDNA was fully sequenced on both strands using an automated DNA sequencer (ABI 310). The sequence is identical to a recently published sequence (GenBank accession number AL136087) derived from a human genomic DNA clone RP1-137F1 from chromosome 6p21.1–21.2. For *Xenopus* oocyte expression, capped cRNA was synthesized using the T7 mMessage mMachine kit (Ambion).

2.2. Analysis of TASK-4 mRNA distribution

Human multiple tissue cDNAs (Clontech) were used as templates

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Abbreviations: EST, expressed sequence tag; P, pore; PCR, polymerase chain reaction; pK_i, pH of 50% inhibition; RACE, rapid amplification of cDNA ends; RT, reverse transcriptase; S.E.M., standard error of the mean; TASK, TWIK-related acid-sensitive K^+ channel; TM, transmembrane; TREK, TWIK-related K^+ channel; TWIK, tandem of P domains in a weak inward rectifying K^+ channel; V_{rest}, resting membrane potential; TEA, tetraethylammonium

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for PCR amplification (*Taq* DNA polymerase, Gibco BRL), using TASK-4 primers (5′-CCTTCATCACCCTCAGCACC-3′ and 5′-ACACCCGAAAGT-CACATCCC-3′) and GAPDH (Clontech) primers. For 'touch down PCR' the conditions were five cycles of 30 s at 92°C, 30 s at 63°C, and 45 s at 72°C followed by 35 cycles of 30 s at 92°C, 30 s at 60°C, and 45 s at 72°C and a final extension time of 7 min. PCR products were separated by electrophoresis and detected by ethidium bromide staining. The identity of the PCR products was confirmed by automated sequencing (ABI 310).

2.3. Electrophysiology

Xenopus laevis oocytes were obtained from tricaine anesthetized animals. Ovaries were collagenase treated (1 mg/1 ml, Worthington, type II) in OR2 solution (NaCl 82.5 mM, KCl 2 mM, $MgCl_2$ 1 mM, HEPES 5 mM, pH 7.4) for 120 min and subsequently stored in recording solution ND 96 (NaCl 96 mM, KCl 2 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1 mM, HEPES 5 mM, pH 7.4) with additional Na-pyruvate (275 mg/l), theophylline (90 mg/l) and gentamicin (50 mg/l) at 18°C. Oocytes were individually injected with 10 ng cRNA encoding hTASK-4. Standard two-electrode voltage-clamp recordings were performed at room temperature (21–22°C) with a Turbo Tec 10CD (NPI) amplifier, an ITC-16 interface combined with Pulse software (Heka) and Origin version 5.0 (Microcal Software) for data acquisition on Pentium II PC. Macroscopic currents were recorded 2–4 days after injection. The pH of the perfusion solutions was adjusted daily. The pipette solution contained 3 M KCl. GenBank accession numbers for sequences used for sequence alignment: hTASK-1 (AF006823), hTASK-2 (AF084830), hTASK-3 (AF212829), hTASK-4 (AF339912), hTWIK-1 (U33632), hTWIK-2 (AF117708), hTREK-1 (AF129399), rTREK-2 (AF196965), hTRAAK (NM016611), mKCNK6 (AF110521), hKCNK7 (AF110522), mKCNK8 (NM010609).

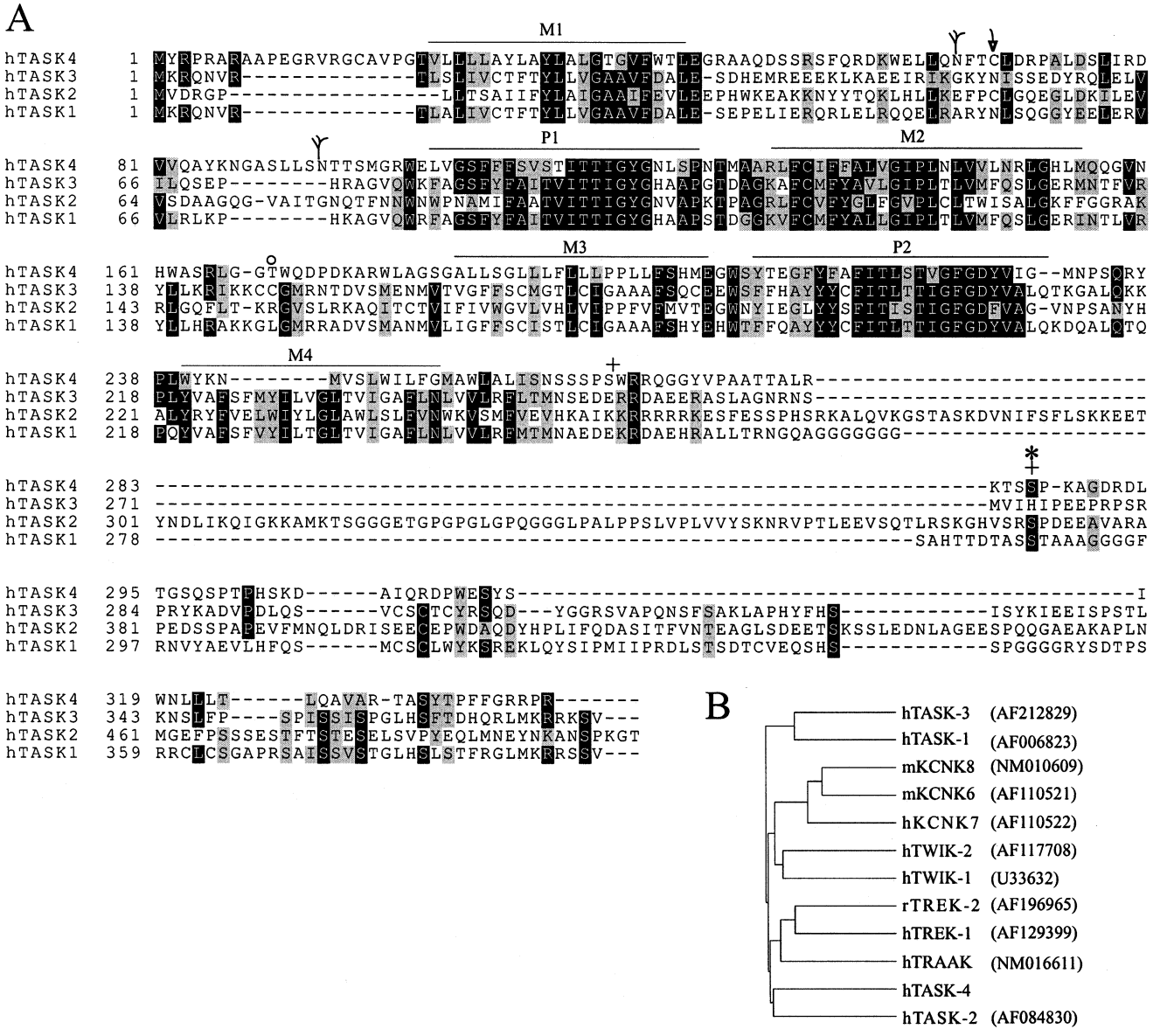

Fig. 1. Protein sequence of TASK-4 and comparison with other TASK proteins. A: Alignment of amino acid sequence of TASK-4 (accession number AF339912) with other TASK channels. The four transmembrane segments, M1–M4, and the two pore-forming regions P1 and P2 are indicated. Identical and conserved residues are in black and gray, respectively. Potential cytoplasmatic sites for protein kinase A (*), protein kinase C (+) and casein kinase II (°) are shown. Consensus sites for *N*-linked glycosylation (branch sign) on the extracellular M1P1 loop are indicated. The conserved cysteine residue (position 68) within the M1P1 loop is marked with an arrow. B: Phylogenetic tree of the 12 currently cloned mammalian KCNK K^+ channels.

3. Results

3.1. Molecular cloning and primary structure of TASK-4

Search of the Incyte LifeSeq database revealed a human EST clone with significant homology to the TRAAK potassium channel. The sequence information was used to isolate the missing 5′-coding sequence from a human kidney cDNA library (Edge Biosystems). A full-length cDNA clone was amplified from adrenal cDNA. The initiator methionine of the cDNA was assigned to the first ATG in frame and was not preceded by a stop codon in the same frame. Although the context of the ATG does not represent the optimal consensus site for translation initiation [13], sequence alignment with the later published human genomic DNA clone (GenBank accession number AL136087) revealed a stop codon in the same frame 250 bp upstream of the ATG. The full-length TASK-4 cDNA encoded a protein of 343 amino acids (Fig. 1) with a predicted molecular mass of ≈38 kDa. Hydropathy analysis supported a topological model with four transmembrane domains and two intervening P domains. Within the two-pore domain K^+ channels, TASK-4 shares the highest homology with TASK-2 (38% identity within the M1–M4 transmembrane domains). An extended extracellular M1P1 interdomain that is characteristic of this channel family is also found. The human TASK-4 protein sequence contains two consensus sites for *N*-linked glycosylation (residues 65 and 94) within this M1P1 interdomain and a cysteine (residue 68), which is conserved in TWIK-1, TREK-1, TRAAK and TASK-2. In TWIK-1, this cysteine residue is involved in homodimer formation [14]. The TASK-4 protein contains consensus sites for phosphorylation by casein kinase II (residue 169), protein kinase A (residue 286) and protein kinase C (residues 266 and 286). Similar to TASK-2 and in contrast to TASK-1 and TASK-3, TASK-4 does not possess a histidine immediately downstream of the 'GYG' pore motif, which mediates proton block of TASK-3 [15].

3.2. Tissue distribution of TASK-4

The expression of TASK-4 in human tissues was examined by RT-PCR. PCR of cDNA from multiple tissues showed that TASK-4 is widely distributed (data not shown). In contrast to TASK-2 [16] it is also present in the brain. Similar to most 2P/4TM K^+ channels [3], TASK-4 is absent in human skeletal muscle. A lower size PCR product was observed in skeletal muscle and identified as myosin light chain 3, also referred to as both the slow skeletal muscle isoform and the ventricular isoform [17]. Consistent with this, it was also amplified from ventricular cDNA. Strongest TASK-4 signals were observed in liver, lung, placenta, pancreas, small intestine and aorta. TASK-4 was also detected in the human heart, colon, ovary, peripheral blood leukocytes, prostate, spleen, testis and thymus. In the heart it is preferentially expressed in the atrial regions. Signals indicate mRNA expression in left and right atrium, the auricles and in the atrioventricular node. A faint signal was detected in the interventricular septum, whereas TASK-4 could not be detected in the ventricles, the apex or in fetal heart.

3.3. Biophysical properties of TASK-4

48 h after injection of TASK-4 cRNA in *Xenopus* oocytes, a rise in external pH caused quasi-instantaneous and non-inactivating currents (Fig. 2) that were not present in uninjected oocytes. TASK-4 currents were highly sensitive to changes in extracellular pH (Fig. 2), a hallmark of the TASK family. The current–voltage (*I–V*) relationships were outwardly rectifying and almost no inward currents were recorded in the ND 96 external medium containing 2 mM K^+. Like other members of the TASK family and other background K^+ channels, TASK-4 is open at all membrane potentials once block by external protons is terminated, as a result of an increase in pH. Thus its activity is a function of how far the membrane potential is set from the reversal potential. As shown in Fig. 3A,B, increase of pH_o from 7.5 to 8.5 or 7.5 to 9.0 caused magnifications of the outward currents by factors of 6.3 ± 0.2 ($n = 22$) and 17.4 ± 2.9 ($n = 9$), respectively. However, decrease of pH_o to 6.0 reduced the currents only by a factor of 0.7 ± 0.1 ($n = 7$). The rise in current amplitude showed no saturation within the pH range 6.0 to 10.5 (Fig. 3A), therefore it was not possible to estimate a pK_i (pH of 50% inhibition). Unlike the current amplitude, the hyperpolarization reached a steady state and the rise of pH_o shifted the resting membrane potential (V_{rest}) close to the K^+ equilibrium potential (E_K) (Fig. 3C). These data indicate that TASK-4 is mainly blocked at a pH range of 6.0–7.5. Consistently, unlike TWIK-1, TREK-1, TRAAK, TASK-1 or other 2-P domain K^+ channels [7,11,18,19], the membrane potential of oocytes expressing TASK-4 is only slightly hyperpolarized (-39.3 ± 1.1 mV, $n = 58$) as compared with control oocytes (-35.7 ± 1.4 mV, $n = 13$) at pH 7.5. Therefore TASK-4 possesses a pH dependency shifted towards more alkaline pH in comparison with TASK-1, TASK-2 and TASK-3.

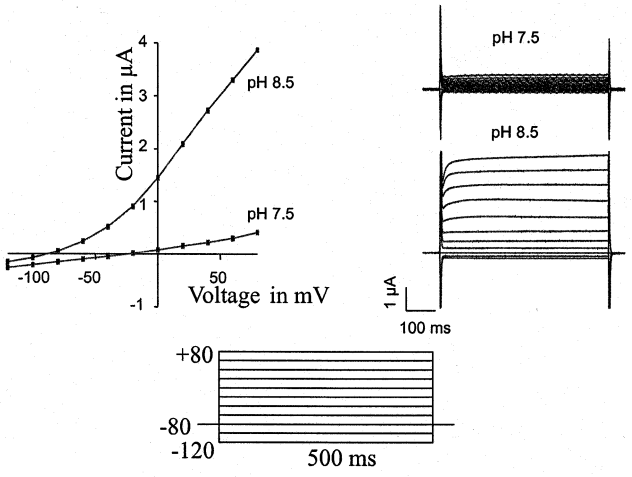

Fig. 2. Functional expression of TASK-4 in *Xenopus* oocytes. Representative current traces and *I–V* relationships from a TASK-4-expressing oocyte subjected to a pulse protocol stepping from holding potential (−80 mV) to test pulses from −120 to +80 mV, in steps of 20 mV, and then back to holding potential. Recordings were performed at pH_o 7.5 and 8.5.

Increase of external K^+ concentration ($[K^+]_o$) revealed inward currents and the oocytes depolarized (Fig. 4A). When cells were perfused with K^+-rich external solution (96 mM), the TASK-4 currents presented an almost linear *I–V* relationship (data not shown). The relationship between the reversal potential and $[K^+]_o$ was close to the predicted Nernst value of 58.4 mV/decade at 21°C. The experimental data were fitted by linear regression with a slope of 51.1 ± 1.0 mV/decade, as expected for selective K^+ channels (Fig. 4C).

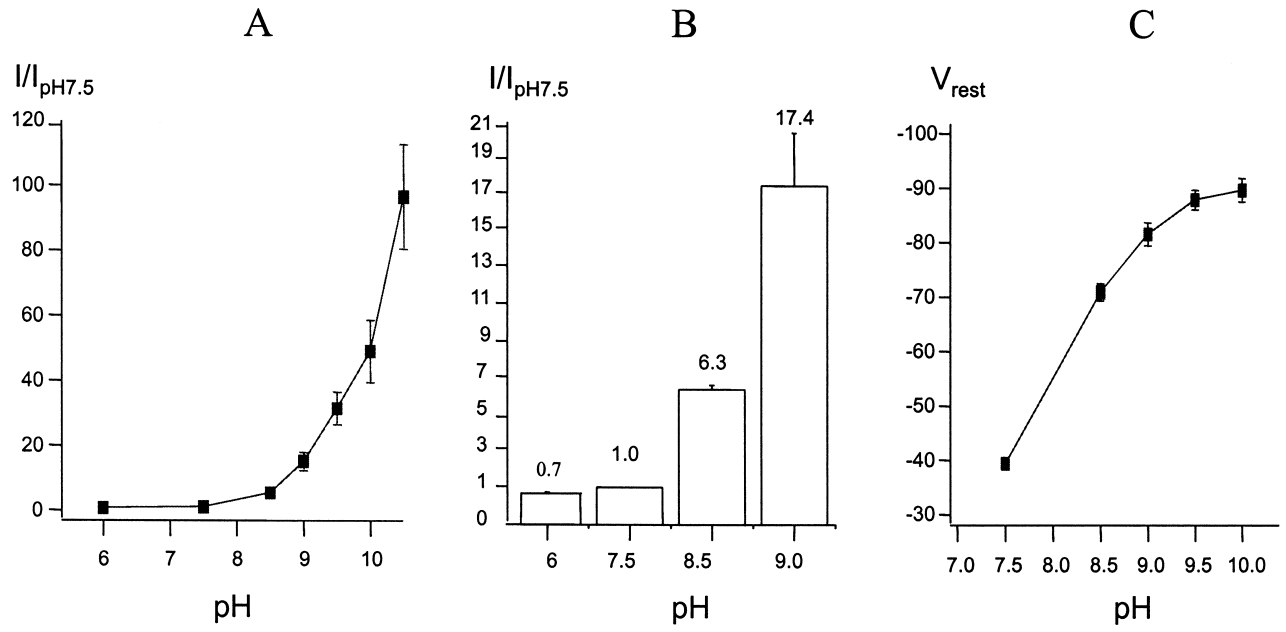

Fig. 3. Sensitivity of TASK-4 currents and V_{rest} to external pH. A: Current amplitude from a TASK-4-injected oocyte subjected to a pulse protocol stepping from −80 to +40 mV. Recordings were done in a pH range from 6.0 to 10.5. Current amplitudes were normalized to the value at pH 7.5; data are means ± S.E.M., $n = 9$–22. B: Enlarged illustration of the pH range 6.5–9.0. C: Dependence of resting membrane potential of TASK-4-injected oocytes of external pH; data are means ± S.E.M., $n = 10$–58.

3.4. Pharmacological properties of TASK-4

The effects of various pharmacological agents were studied on TASK-4. Experiments were carried out at pH 8.5 to activate TASK-4 and inhibition values were determined at +40 mV. The 'classical' K^+ channel blockers tetraethylammonium (TEA, 3 mM), 4-aminopyridine (4-AP, 2 mM) and Cs^+ (2 mM) were inactive on the recorded currents. TASK-4 was only slightly sensitive to quinine (1 mM), which induced a 17.8 ± 4.3% inhibition ($n = 4$). The local anesthetics bupivacaine (1 mM) and lidocaine (1 mM) were weakly effective on TASK-4 current amplitudes. They only showed a transient block with a maximum inhibition of 25.7 ± 5.6% ($n = 8$) and 13.1 ± 1.1% ($n = 8$), which was observed after 2 min of drug application. A strong inhibitory effect was observed with Ba^{2+} (2 mM), showing an inhibition of 82.8 ± 1.6% ($n = 4$). Like other TASK channels, TASK-4 is not sensitive to external application of arachidonic acid (100 μM).

TASK-4 has potential phosphorylation sites for protein ki-

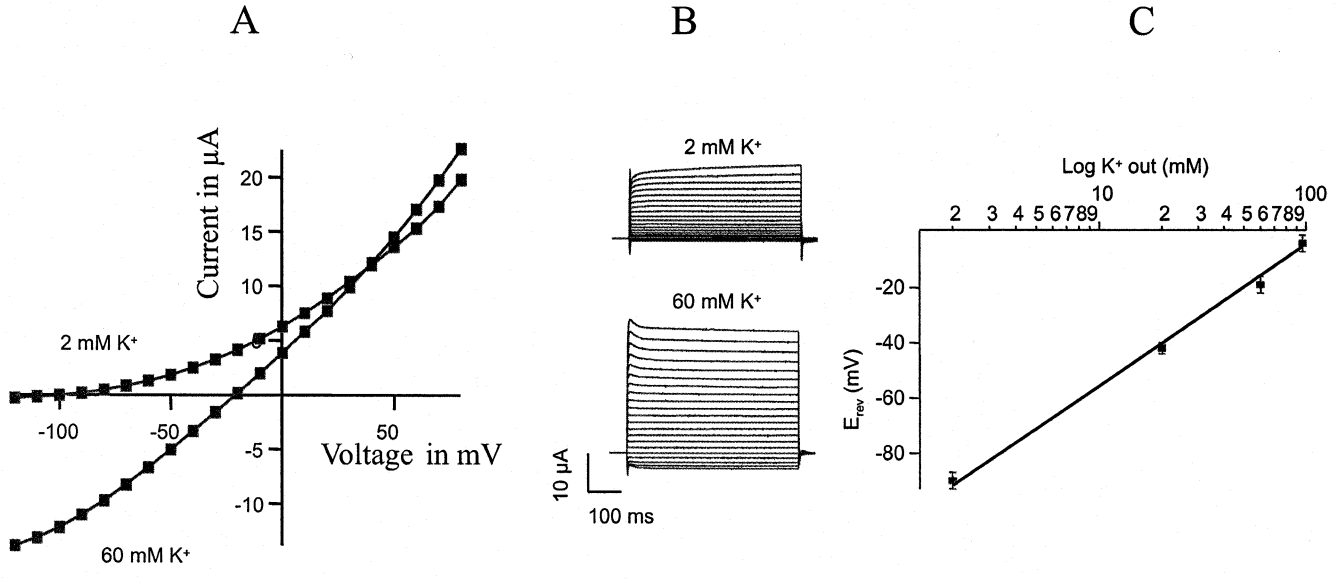

Fig. 4. $[K^+]_o$ dependence of the reversal potential and of TASK-4 currents at pH_o 8.5. A: Representative *I–V* relationships from a TASK-4-injected oocyte subjected to changes in external K^+ concentration. B: Single recordings of TASK-4 with 2 and 60 mM external K^+ concentration. C: Relationship between the reversal potential and the external K^+ concentration; data (mean ± S.E.M., $n = 4$–7) are shown by linear regression (slope 51 mV/decade).

nase A and protein kinase C. However, TASK-4 currents were insensitive to the activation of adenylate cyclase by extracellular application of a mixture containing 1 mM 3-isobutyl-1-methylxanthine (IBMX) and 10 μM forskolin (data not shown). Also theophylline incubation of *Xenopus* oocytes, which should increase intracellular cAMP concentration and activate protein kinase A, did not alter the current amplitude and pH sensitivity of the TASK-4 currents. Activation of protein kinase C by perfusion with phorbol myristine acetate (PMA, 100 nM) failed to alter TASK-4 currents ($n = 5$). Therefore, TASK-4 does not appear to be regulated by protein kinases A and C.

4. Discussion

4.1. TASK-4 is a new member of the TASK family

TASK-4 is belonging to the rapidly growing class of two-pore domain K^+ channels. The TASK family currently includes four members. TASK-4 shares many common features with other family members, including wide tissue distribution, open rectification, fast kinetics displaying quasi-instantaneous currents, pharmacology and sensitivity to external pH. Despite the highest homology to TASK-2, it can be distinguished from it by the shifted pH dependency, the faster activation kinetics and the different sensitivity to various agents.

4.2. pH sensitivity of TASK-4

Although external pH dependency was similar, the point of inhibition by external acidification is shifted to less acidic values. The open probability of TASK-4 channels is low at pH 7.5, so the current amplitude can only be decreased by 30% through acidification. Furthermore, at this pH the TASK-4 current amplitude is not sufficient to drive the resting membrane potential near to the K^+ equilibrium potential. Due to the low activity at pH 7.5, the increase in current amplitude by alkalization is more pronounced than for the other TASK channels. The pH–current relationship is very steep and does not become saturated within the pH range at which recordings are reliable in *Xenopus* oocytes. Therefore we could not estimate a pK_i, which must obviously be shifted towards a more alkaline value. The molecular basis for this altered pH dependency is not yet known. TASK-4 does not possess a histidine in the P1 loop which is conserved in TASK-1 and TASK-3 and which was proven to cause pH-dependent block in TASK-3 [15]. However, this histidine also is not present in TASK-2, which displays a very similar pH sensitivity to TASK-1 and TASK-3. This suggests that other residues may also contribute to the pH sensitivity of TASK channels.

4.3. Pharmacology

Pharmacological properties were determined at pH 8.5 in order to record macroscopic currents of more than 1.5 μA at +40 mV. This minimized inaccuracies due to small current amplitudes. Alkaline pH is a potential source of error because of the pH sensitivity of basic compounds like quinine and the local anesthetics lidocaine and bupivacaine. However, the stronger inhibition of TASK-4 by Ba^{2+}, compared with that of TASK-2, is in contrast to the expected decrease in inhibition potency due to the loss of insoluble Ba^{2+} hydroxide. Therefore the differences in the pharmacology, as compared to TASK-2, are unlikely to result from changed pH alone.

4.4. Physiological relevance of TASK-4

Judging from the pH dependency of TASK-4 observed in *Xenopus* oocytes, it is questionable whether it affects resting membrane potential or other cellular functions at the physiological pH in most tissues. On the other hand, the strong increase observed in current amplitude by a little change of pH in the alkaline direction might have a large impact on the resting membrane potential especially in tissues displaying high input resistances. TASK-4 might be of great relevance in tissues, like those of the bile or pancreas, where physiological pH can range from 7.5 to 8.5. Under these conditions TASK-4 can produce large outward currents, which are able to strongly affect the resting membrane potential. In addition pathophysiological situations are often accompanied by pH shifts, which can be preceded by alkaline pH changes [19].

It is possible that native TASK-4 channels display a different pH dependency to that in the heterologous expression system and therefore, similar to other K^+ leak channels, may be constitutively open under physiological conditions. Although we could not demonstrate effects of PKA and PKC on TASK-4, other effectors remain to be investigated, like G protein-coupled receptor-mediated signalling, as has previously been shown for other 2P/4TM K^+ channels [20–22].

In the heart, background K^+ currents with similar properties to TASK currents are thought to modulate the cardiac action potential [23,24]. In the human heart TASK-4 displays a distinct expression pattern compared to the other TASK family members. While TASK-1 is possibly expressed in atrium and ventricle [3,16,19,25], TASK-2 is not expressed in the human heart [3,16]. Rat TASK-3 seems to be predominant in the ventricles [15], whereas TASK-4 expression is restricted to the atrial regions and the atrioventricular node. Thus native cardiac leak K^+ currents might be generated by different members of the TASK family.

Heterogeneity in the molecular composition of TASK-like background K^+ currents may be further increased by formation of heterodimers. This interesting possibility is suggested by the overall overlapping expression patterns of the different TASK channels. This will be one hypothesis among others to be tested in future studies aimed at understanding TASK function.

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