TASK, a human background K⁺ channel to sense external pH variations near physiological pH

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TASK is a new member of the recently recognized TWIK K⁺ channel family. This 395 amino acid polypeptide has four transmembrane segments and two P domains. In adult human, TASK transcripts are found in pancreas<placenta<brain<lung, prostate<heart, kidney<uterus, small intestine and colon. Electrophysiological properties of TASK were determined after expression in *Xenopus* oocytes and COS cells. TASK currents are K⁺-selective, instantaneous and non-inactivating. They show an outward rectification when external [K⁺] is low ([K⁺]_{out} = 2 mM) which is not observed for high [K⁺]_{out} (98 mM). The rectification can be approximated by the Goldman–Hodgkin–Katz current equation that predicts a curvature of the current–voltage plot in asymmetric K⁺ conditions. This strongly suggests that TASK lacks intrinsic voltage sensitivity. The absence of activation and inactivation kinetics as well as voltage independence are characteristic of conductances referred to as leak or background conductances. For this reason, TASK is designated as a background K⁺ channel. TASK is very sensitive to variations of extracellular pH in a narrow physiological range; as much as 90% of the maximum current is recorded at pH 7.7 and only 10% at pH 6.7. This property is probably essential for its physiological function, and suggests that small pH variations may serve a communication role in the nervous system.

Keywords: *in situ* hybridization/leak/membrane potential/potassium channels/rectification

Introduction

Potassium channels are ubiquitous membrane proteins that form the largest family of ion channels in terms of both function and structure. By determining and modulating the membrane potential, they play a major role in neuronal integration, muscular excitability and hormone secretion (Rudy, 1988; Hille, 1992). More than 40 genes encoding K⁺ channel subunits are now identified in mammals. These subunits fall into two structural classes of pore-forming subunits [*Shaker* and inward rectifier K⁺ channel (IRK)] (Pongs, 1992; Jan and Jan, 1994; Doupnik *et al.*, 1995; Fakler and Ruppersberg, 1996; Köhler *et al.*, 1996)

and four structural classes of auxiliary subunits (Kvβ, Kcaβ, SUR and IsK) (Takumi *et al.*, 1988; Knaus *et al.*, 1994; Pongs, 1995; Inagaki *et al.*, 1996). All *Shaker*-type subunits have a conserved hydrophobic core containing six transmembrane segments (TMS). Associations of *Shaker*-type subunits with accessory subunits such as Kvβ, Kcaβ or IsK give rise to voltage-dependent K⁺ channels (Pongs, 1995; Barhanin *et al.*, 1996; Fink *et al.*, 1996a; Sanguinetti *et al.*, 1996) and Ca²⁺-dependent K⁺ channels (MacCobb *et al.*, 1995; MacManus *et al.*, 1995). Subunits of inward rectifier K⁺ channels (IRK) have only two TMS (Doupnik *et al.*, 1995; Lesage *et al.*, 1995; Fakler and Ruppersberg, 1996). Some IRKs give rise to ATP-sensitive K⁺ channels when they are associated with sulfonylurea receptor (SUR) subunits (Inagaki *et al.*, 1996). Despite a very low overall sequence similarity, *Shaker* and IRK pore-forming subunits share a conserved domain called the P domain. This peculiar motif is an essential element of the K⁺-selective filter of the aqueous pore and is considered as the signature of K⁺ channel-forming proteins (Heginbotham *et al.*, 1994).

We recently have described a new family of mammalian K⁺ channel subunits. Despite a low sequence similarity between them (<28% amino acid identity), both cloned members of this family (TWIK-1 and TREK-1) possess the same overall structure, with four TMS and two P domains (Fink *et al.*, 1996b; Lesage *et al.*, 1996a, 1997). The conservation of this structure is not associated with a conservation of functional properties: TWIK-1 gives rise to weakly inward rectifier K⁺ currents (Lesage *et al.*, 1996a) while TREK-1 produces outward rectifier K⁺ currents (Fink *et al.*, 1996b). However, both channels are open at the resting potential and are able to drive the resting membrane potential near the K⁺ equilibrium potential. This common property suggests that these channels control the resting membrane potential in a large set of cell types. Here, we describe the cloning, tissue distribution and expression of a novel human member of this new structural family. To our knowledge, this channel, called TASK for TWIK-related acid-sensitive K⁺ channel, is the first cloned mammalian channel that produces K⁺ currents that possess all the characteristics of background conductances. They are instantaneous with voltage changes, and their current–voltage relationships fit the curves predicted from the constant field theory for simple electrodiffusion through an open K⁺-selective pore, indicating that TASK currents are voltage insensitive. The activity of this background channel is strongly dependent on the external pH in the physiological range, suggesting that this particular channel is a sensor of external pH variations.

Results

Cloning and primary structure of TASK

TWIK-1 and TREK-1 sequences were used to search related sequences in the GenBank database by using

the Blast alignment program. We identified two mouse expressed sequence tags (ESTs, accession numbers W36852 and W36914) that overlap and give a contig fragment of 560 bp whose deduced amino acid sequence presents significant similarity to TWIK-1 and TREK-1. A corresponding DNA fragment was amplified by RT-PCR and used to screen a mouse brain cDNA library. Eight independent clones were isolated. The 1.8 kb cDNA insert of the longer clone bears in its 5' part an open reading frame (ORF) coding for a 405 amino acid polypeptide (Figure 1). This ORF does not begin with an initiating methionine codon, suggesting that the brain cDNA clones were partial. Ten additional positive clones were isolated from a mouse heart cDNA library. Analysis of their 5' sequences showed that none these clones were longer than the clones previously isolated from brain. The 5' sequence has a very high GC content and is probably associated with secondary structures that could have promoted premature stops of RNA reverse transcription during the construction of both mouse cDNA libraries. To overcome this problem, we cloned the complete cDNA in another species. The DNA probe was used to screen a cDNA library from human kidney, a tissue that express both TWIK-1 and TREK-1 channels. Two hybridizing clones were characterized. Both contain an ORF of 1185 nucleotides encoding a 394 amino acid polypeptide (Figure 1). The human protein sequence contains consensus sites for *N*-linked glycosylation (residue 53), and phosphorylation by protein

The expression of TASK in adult human and mouse tissues was examined by Northern blot analysis. Three different transcripts were detected in the human tissues, with estimated sizes of 6.8, 4.2 and 2.6 kb (Figure 3), the shorter one having the same size as the cloned cDNAs. The other two transcripts (4.2 and 6.8 kb) may result from alternative polyadenylation signals in the 3'-non-coding sequence and/or correspond to alternatively spliced or immature forms of the transcript. TASK is expressed in many different tissues but particularly in pancreas and placenta. Lower levels of expression were found in

Fig. 2. Sequence comparison and membrane topology of TWIK-related channels. (A) Alignment of human TWIK-1, mouse TREK-1 and human TASK sequences. Identical and conserved residues are shown in black and gray, respectively. Dashes indicate gaps introduced for a better alignment. The relative positions of putative transmembrane segments (M1–M4) and P domains (P1 and P2) of human TASK are also indicated. The M1–M4 domains were deduced from a hydropathy profile computed with a window size of 11 amino acids according to the method of Kyte and Doolittle (1982). (B) Putative membrane topology of TWIK-1, TREK-1 and TASK channels.

brain>lung, prostate>heart, kidney>uterus, small intestine and colon. As shown in Figure 4A, the TASK probe detected a single transcript in the mouse with an estimated size of 4.2 kb. TASK is expressed in heart>lung>brain and kidney. No expression was seen in liver and skeletal muscle. The TASK distribution was studied further in adult mouse brain and heart by *in situ* hybridization. A wide and heterogeneous pattern of expression was obtained in the brain (Figure 4B and C). TASK mRNA was detected throughout the cell layers of

Fig. 3. Northern blot analysis of TASK distribution in adult human tissues. Human multiple tissue Northern blots from Clontech were probed at high stringency with a TASK cDNA probe. Each lane contains 2 µg of poly(A)⁺ RNA. Autoradiograms were exposed for 48 h at -70°C . The blots were re-probed with a β -actin cDNA probe for control. sk. muscle, skeletal muscle; sm. intestine, small intestine; PBL, peripheral blood leukocytes.

the cerebral cortex, in the CA1–CA4 pyramidal cell layer, in the granule cells of the dentate gyrus, in the habenula, in the paraventricular thalamic nuclei, in the amyloid nuclei, in the substantia nigra and in the Purkinje and granular cells of the cerebellum. In the heart, a high level of TASK expression was found in the atria (Figure 4D), while ventricular cells did not express this channel.

Biophysical properties of TASK currents

For functional studies, TASK cRNAs were injected into *Xenopus* oocytes. A non-inactivating current, not present in uninjected oocytes (not shown), was measured by two-electrode voltage-clamp (Figure 5A). The activation kinetics of the TASK current are almost instantaneous (<10 ms). The current–voltage (I – V) relationship is outwardly rectifying and almost no inward currents were recorded in the ND96 external medium containing 2 mM K^+ (Figure 5B). However, inward currents were revealed when the external K^+ concentration ($[\text{K}^+]_{\text{out}}$) was increased gradually to 98 mM K^+ (Figure 5A and B). Figure 5B shows the I – V relationships of the current in K^+ -rich solutions ranging from 2 to 98 mM. The relationship between the reversal potential and $[\text{K}^+]_{\text{out}}$ was close to the predicted Nernst value (52.1 mV/decade, $n = 4$), as expected for a highly selective K^+ channel (Figure 5C, upper panel). On the other hand, external K^+ enhanced the outward currents in a concentration-dependent manner as illustrated in Figure 5C (lower panel). The half-maximum activation by K^+ was observed at a $K_{0.5}$ of 2.06 mM. The theoretical I – V relationships in various $[\text{K}^+]_{\text{out}}$ calculated according to the Goldman–Hodgkin–Katz current equation are shown in Figure 5D. These I – V relationships are very close to the I – V relationships corresponding to recorded TASK currents (Figure 5B). This strongly suggests that TASK currents show no rectification other than that predicted from the constant field assumptions, and that TASK lacks intrinsic voltage sensitivity. The slight deviations between experimental and theoretical points are probably due to small endogenous chloride

Fig. 4. Distribution of TASK mRNA in adult mouse. **(A)** Northern blot analysis. Each lane contains 2 μ g of poly(A)⁺ RNA. Autoradiograms were exposed for 72 h at -70°C . The blots were re-probed with a β -actin cDNA probe for control. **(B–D)** *In situ* hybridization analysis from a coronal section at the level of the forebrain **(B)**, the cerebellum **(C)** and the heart **(D)**. Warmer colors represent higher levels of expression. CA1–CA3, fields CA1–3 of Ammon's horn; Cx, cerebral cortex; DG, dentate gyrus; Gl, granular layer; Hb, habenula; SN, substantia nigra; PLCo, postero lateral cortical amygdaloid nuclei; PVP, paraventricular thalamic nucleus; A, atrium; V, ventricle.

conductance and/or a K⁺ loading of the oocytes. We have shown previously that oocytes expressing TWIK-1 or TREK-1 are more polarized than control oocytes, the resting membrane potential (E_m) reaching a value close to the K⁺ equilibrium potential (E_K). In oocytes expressing TASK, the E_m was -85 ± 0.8 mV ($n = 23$, in standard ND96) instead of -44 ± 2.6 mV ($n = 9$) in non-injected oocytes. This result demonstrates that TASK, like other TWIK or TREK channels, is able to drive E_m close to E_K . The effect of various pharmacological agents on currents elicited by voltage pulses to +50 mV has been studied in TASK-expressing oocytes. Less than 20% of TASK currents were inhibited in the presence of quinine (100 μ M), quinacrine (100 μ M) or quinidine (100 μ M). The 'classical' K⁺ channel blockers tetraethylammonium (TEA, 1 mM) and 4-aminopyridine (4AP, 1 mM) were also inactive. Cs⁺ (100 μ M) induced a voltage-dependent block of $31 \pm 2\%$ ($n = 4$) of the inward current, recorded at -150 mV, in 50 mM external K⁺. In the same conditions, Ba²⁺ (100 μ M) was ineffective, with a variation of $6 \pm 1\%$ ($n = 4$) of the inward current.

The biophysical properties of TASK were then verified in transfected COS cells. Untransfected cells did not express this K⁺ channel activity (not shown). Figure 5E shows whole-cell currents recorded in mammalian COS cells transiently transfected with TASK, in external solutions containing 5 and 155 mM K⁺. The currents were instantaneous and non-inactivating, as in *Xenopus* oocytes. Figure 5F presents the I – V relationships of the current in various external K⁺ concentrations. The currents show

the same Goldman–Hodgkin–Katz type outward rectification as in oocytes.

Regulation of the TASK channel

TASK currents were insensitive to internal Ca²⁺ changes obtained by injection of inositol triphosphate (IP3, 1 mM) or EGTA (100 mM), to the activation of adenyl cyclase by perfusion of IBMX (1 mM) and forskolin (10 μ M), or to the activation of protein kinase C (PKC) by application of phorbol 12-myristate 13-acetate (PMA; 40 nM). TASK currents were insensitive to the internal acidification or alkalization obtained by injection of solutions at pH 2 or 8.7 respectively ($n = 3$). However, their very interesting property is that they are highly sensitive to external pH. The current–potential relationships recorded from a TASK-expressing oocyte at pH 6.5, 7.4 and 8.4 are presented in Figure 6A. For an external pH of 6.5, a drastic block was observed at all potentials, while an activation was recorded at pH 8.4, also at all potentials. The inhibition and activation produced no modification of current kinetics (Figure 6A, inset). The pH dependence of the TASK channel is shown in Figure 6B. For currents recorded at +50 mV, the inhibition by acidic pHs was characterized by an apparent pK of 7.34 ± 0.04 units ($n = 3$) and a Hill coefficient of 1.54 ± 0.08 ($n = 3$). For currents recorded at 0 and -50 mV, the pKs were 7.32 ± 0.02 and 7.30 ± 0.01 respectively ($n = 3$), showing that the blocking effect of external protons is not voltage dependent. The resting membrane potential of TASK-expressing oocytes was -84 ± 1 mV ($n = 6$) at pH 7.4 and shifted to $-47 \pm$

6 mV ($n = 6$) at pH 6.4 (not shown). Finally, Figure 6C and D shows that the strong pH sensitivity of TASK currents was also observed in transfected COS cells. A large inhibition or activation of the current was recorded, at all potentials, when the pH was changed from 7.4 to 6.1 or 8.4 respectively (Figure 6C). The kinetics of the current were unmodified at both pH values (Figure 6C, inset). Figure 6D shows that the pH effects were also non-voltage dependent in COS cells. The external pH dependence of TASK, at +50 mV, indicates a pK value of 7.29 ± 0.03 ($n = 5$) and a Hill coefficient of 1.57 ± 0.07 ($n = 5$). Currents recorded at 0 and -50 mV presented pKs of 7.29 ± 0.04 ($n = 5$) and 7.32 ± 0.05 ($n = 4$) respectively. Ten percent of the maximum current was obtained at pH 6.68 ± 0.08 ($n = 4$) and 90% at pH 7.66 ± 0.05 ($n = 4$). These results confirm that TASK is extremely sensitive to extracellular pH in the physiological range.

Discussion

Here we report the isolation and the characterization of a novel human K^+ channel. This channel has an overall

structural similarity to TWIK-1 and TREK-1 channels that suggests a common ancestral origin. Despite this similar structural organization, the amino acid identity between TASK and the two other related mammalian channels is very low (25–28%). Sequence homologies are no higher between TASK and a recently cloned *Drosophila* channel that also belongs to the structural TWIK channel class (Goldstein et al., 1996). The highest degree of sequence conservation is in the two P domains and the M2 segment. In these regions, the amino acid identity reaches ~50%. Like other TWIK-related channels, TASK contains an extended M1P1 interdomain. This peculiar domain has been shown to be extracellular in the case of TWIK-1 and to be important for the self-association of two TWIK-1 subunits. The TWIK-1 homodimers are covalent because of the presence of an interchain disulfide bridge between cysteines 69 located in the M1P1 interdomain (Lesage et al., 1996b). This particular cysteine residue is conserved in TREK-1 (residue 93) but not in TASK, strongly suggesting that TASK probably does not form covalent dimers as observed for TWIK1 (Lesage et al., 1996b) and TREK-1 (unpublished data).

The biophysical and regulation properties of TASK are unique. TWIK-1 has a mild inward rectification that involves an internal block by Mg^{2+} (Lesage et al., 1996a). TREK-1 expresses an outward rectification which seems to result from a voltage sensitivity intrinsic to the channel protein (Fink et al., 1996b). In the case of TASK, the outward rectification observed at physiological external

Fig. 5. Biophysical properties of TASK in *Xenopus* oocytes and COS cells. (A) TASK currents recorded from a *Xenopus* oocyte injected with TASK cRNA and elicited by voltage pulses from -150 to +50 mV in 40 mV steps, 500 ms in duration, from a holding potential of -80 mV in low (2 mM K^+) or high K^+ solutions (98 mM K^+). The zero current level is indicated by an arrow. (B) Current-voltage relationships. Mean currents were measured over the last 50 ms at the end of voltage pulses from -150 to +50 mV in 10 mV steps as in (A). Modified ND96 solutions containing 2 mM K^+ and 96 mM TMA were used, TMA was then substituted by K^+ to obtain solutions ranging from 2 to 98 mM K^+ . TASK currents are not sensitive to external TMA, no changes were observed upon substitution of NaCl by TMA (data not shown). (C) Upper panel: reversal potentials of TASK currents as a function of external K^+ concentration (mean $\pm$ SEM, $n = 3$). Lower panel: slope conductance measured between +10 and +50 mV on current-voltage relationships as in (B), plotted as a function of the external K^+ concentration (mean $\pm$ SEM, $n = 3$). The mean values were fitted with a hyperbolic function. (D) Theoretical current-voltage relationship under the same conditions as in (B), calculated according to the following modified Goldman-Hodgkin-Katz (GHK) current relationship:

$$I_{K^+} = \bar{P}_{K^+} \cdot \left(\frac{[K^+]_{out}}{K_{0.5} + [K^+]_{out}} \right) \cdot \left(\frac{V_m F^2}{RT} \right) \cdot \frac{[K^+]_{in} - [K^+]_{out} \cdot e^{-V_m F/RT}}{1 - e^{-V_m F/RT}}$$

where I_{K^+} is the potassium current, $\bar{P}_{K^+}$ is the apparent permeability for K^+ , $K_{0.5}$ the half maximum activation by K^+ , $[K^+]_{out}$ and $[K^+]_{in}$ are the external and internal K^+ concentrations, V_m the membrane potential, F , R and T have their usual meanings. The classical GHK relationship has been modified with $[K^+]_{out}/K_{0.5} + [K^+]_{out}$ to take into account the sensitivity of the conductance to external K^+ . (E) TASK currents recorded from a transfected COS cell and elicited by voltage pulses from -150 to +50 mV in 40 mV steps, 500 ms in duration, from a holding potential of -80 mV, in low (5 mM K^+) or high K^+ solutions (155 mM K^+). The zero current level is indicated by an arrow. (F) Current-voltage relationships. Mean currents were measured over the last 50 ms at the end of voltage pulses ranging from -150 to +50 mV in 10 mV steps as in (E). Solutions containing 5 mM K^+ and 150 mM TMA were used, TMA was then substituted by K^+ to obtain solutions ranging from 5 to 155 mM K^+ .

Fig. 6. pH regulation of TASK in *Xenopus* oocytes and COS cells. (A) Current–voltage relationships recorded from a TASK-expressing oocyte with a ramp ranging from -150 to $+50$ mV, 500 ms in duration, from a holding potential of -80 mV, in ND96 solution at pH 6.5, 7.4 or 8.4. Inset: currents elicited by voltage pulses to $+50$ mV, 500 ms in duration, under the same conditions as above. The zero current level is indicated by an arrow. (B) pH dependence of TASK activity in a *Xenopus* oocyte recorded at -50 , 0 and $+50$ mV (mean $\pm$ SEM, $n = 3$) as in (A). Data were fitted with a Boltzman relationship. (C) Current–voltage relationship recorded from a TASK-expressing COS cell with a ramp ranging from -150 to $+50$ mV, 500 ms in duration, from a holding potential of -80 mV, in 5 mM K⁺ solution at pH 6.1, 7.4 and 8.4. Inset: currents elicited by voltage pulses to $+50$ mV, 500 ms in duration, under the same conditions as above. The zero current level is indicated by an arrow. (D) pH dependence of TASK activity recorded in a COS cell at -50 , 0 and $+50$ mV (mean $\pm$ SEM, $n = 3$) as in (C). Data were fitted with a Boltzman relationship.

K⁺ concentrations can be approximated to the rectification predicted by the Goldman–Hodgkin–Katz current equation, suggesting that this rectification simply results from the asymmetric concentrations of K⁺ on both sides of the membrane. In other words, this would mean that TASK lacks intrinsic voltage sensitivity and behaves like a K⁺-selective ‘hole’. This behavior is, to our knowledge, unique among cloned mammalian K⁺ channels. Voltage and time independences are classical criteria to describe the so-called leak or background K⁺ channels. Some of these channels have been described in invertebrates, the best characterized of which are the S channels in *Aplysia* sensory neurons (Siegelbaum *et al.*, 1982), and in vertebrates, for example in bullfrog sympathetic ganglia (Koyano *et al.*, 1992), guinea-pig submucosal neurons (Shen *et al.*, 1992), rat carotid bodies (Buckler, 1997), and guinea-pig ventricular myocytes (Backx and Marban, 1993). These channels are open at all membrane potentials and probably play a pivotal role in the control of the resting membrane potential and in the modulation of electrical activity of both neurons and cardiac cells. However, their lack of kinetics, voltage and time sensi-

activities, and their absence of specific pharmacology has delayed their extensive electrophysiological and physiological characterization. Cloning of TASK, the first ‘true’ background mammalian K⁺ channel, should help to characterize further this peculiar functional family of K⁺ channels at the molecular level and identify specific and high affinity pharmacological agents that would block these channels and facilitate analysis of their physiological roles.

TASK behaves as a K⁺-selective ‘hole’, but this does not mean that its activity cannot be modulated. Unlike TWIK-1 and TREK-1 channels, its activity is not changed by activation of protein kinase A or C (Fink *et al.*, 1996b; Lesage *et al.*, 1996a). The probably very important property of TASK is that it is extremely sensitive to extracellular pH in the physiological range, i.e. between 6.5 and 7.8. The Hill coefficient of ~ 1.6 found for the H⁺ concentration dependence of the TASK current is consistent with the idea that the channel is formed by the assembly of two subunits, as previously demonstrated for TWIK1. These two subunits would be in strong cooperative interactions with regard to H⁺.

The modulation by external protons probably has important implications for the physiological function of the TASK channel. Stimulus-elicited pH shifts have been characterized in a wide variety of neural tissues by using extracellular pH-sensitive electrodes (reviewed in Chesler, 1990; Chesler and Kaila, 1992). They can be observed in physiopathological situations such as epileptiform activity and spreading depression in which acid shifts are usually preceded by alkaline transients (Siesjö *et al.*, 1985; Nedergaard *et al.*, 1991). They can be observed of course in ischemia where large acidifications of the extracellular medium have been recorded (Kraig *et al.*, 1983; Mutch and Hansen, 1984). However, they can also be observed in physiological conditions such as electrical stimulation of Schaeffer collateral fibers in the hippocampal slice (Krishtal *et al.*, 1987), or light stimulation of the retina (Borgula *et al.*, 1989; Yamamoto *et al.*, 1992), or parallel fibers in cerebellum (Kraig *et al.*, 1983). All these pH shifts correspond to bursts of H⁺ or OH⁻ creating small pH variations from the external physiological pH value of 7.4 in the alkaline or acidic direction (up to 0.3 pH units) and are rapid, in the 1–30 s range. They might actually be larger in range or shorter in time course in the vicinity of the synaptic cleft. A particularly interesting issue of course is whether these relatively small activity-dependent pH changes have significant modulatory effects. In other words, does H⁺ serve a transmitter role in the nervous system? The discovery of this new TASK channel that can fully open or close within a range of only 0.5 pH unit around the physiological pH (7.4) will certainly strengthen the idea that pH could be a natural modulator of neuronal activity (Chesler and Kaila, 1992).

Materials and methods

Cloning of TASK and RNA analysis

TWIK-1 and TREK-1 were used to search homologs in gene databases by using the tBlastn sequence alignment program (Altschul *et al.*, 1990). Translation of two overlapping EST sequences (GenBank accession Nos W36852 and W36914) in one frame presented significant sequence similarities with TWIK-1 and TREK-1. A 560 bp DNA fragment was amplified by PCR from mouse brain poly(A)⁺ cDNAs and subcloned

into pBluescript (Stratagene) to give pBS-852/914. This fragment was ³²P-labeled and used to screen mouse brain and heart cDNA libraries. Filters were hybridized and washed as previously described (Fink *et al.*, 1996b). Eight positive clones from brain and 10 from heart were obtained. cDNA inserts were characterized by restriction analysis and by partial or complete sequencing on both strands by the dideoxynucleotide chain termination method using an automatic sequencer (Applied Biosystems). All the clones were shown to only contain a partial ORF. The cDNA insert of the longer mouse clone (designated pBS-mTASK) was ³²P-labeled and used to screen a human kidney cDNA library. Two independent hybridizing clones were isolated and sequenced. Both clones (2.5 kb long) were shown to contain the full-length ORF. The longer one was designated pBS-hTASK.

For Northern blot analysis, poly(A)⁺ RNAs were isolated from adult mouse tissues and blotted onto nylon membranes as previously described (Lesage *et al.*, 1992). The blot was probed with the ³²P-labeled insert of pBS-mTASK in 50% formamide, 5× SSPE [0.9 M sodium chloride, 50 mM sodium phosphate (pH 7.4), 5 mM EDTA], 0.1% SDS, 5× Denhardt's solution, 20 mM potassium phosphate (pH 6.5) and 250 µg of denatured salmon sperm DNA at 50°C for 18 h and washed stepwise at 55°C to a final stringency of 0.2× SSC, 0.3% SDS. For hybridization of human multiple tissue Northern blots from Clontech, the procedure was identical except that the probe was derived from pBS-hTASK. The cDNA insert of pBS-hTASK contains different repeat sequences [AluJb, MIR and (CGG)_n] in the untranslated regions (UTR), and a *SmaI*–*Apal* restriction fragment of 1390 bp spanning the coding sequence was chosen as a probe that does not contain these repeats.

In situ hybridization experiments were performed on adult Balb/c mice by using standard procedures (Fink *et al.*, 1996b). An antisense oligonucleotide (48mer, 5'-CACCAGCAGGTAGGTGAAGGTGCAC-ACGATGAGAGCCAACGTGCGCAC-3') complementary to the mouse cDNA sequence of TASK (from nucleotides 7 to 54) was used to detect the expression of TASK transcripts in frozen fixed brain sections (10 µm). The probe was 3'-end-labeled with [α-³³P]dATP. Sections were digested with 5 µg/ml of proteinase K for 15 min at 37°C and acetylated for 10 min in 0.25% acetic anhydride in 0.1 M triethanolamine. Hybridization was carried out overnight at 37°C in 2× SSC, 50% formamide, 10% dextran sulfate, 1× Denhardt's solution, 5% sarcosyl, 500 µg of denatured salmon sperm DNA, 250 mg/ml yeast tRNA, 20 mM dithiothreitol and 20 mM NaPO₄ with 0.2 ng/ml of radiolabeled probe (sp. act. = 8×10⁸ d.p.m./µg). Slides were then washed in 1× SSC before dehydration, drying and apposition to hyperfilm-βmax (Amersham) for 6 days. The specificity of labeling was verified by *in situ* hybridization using cold displacement of radioactive probe with a 500-fold excess of unlabeled oligonucleotide.

Electrophysiological measurements in *Xenopus oocytes*

A 2480 bp *SmaI*–*XhoI* fragment from pBS-hTASK containing 14 bp of 5' UTR, the coding sequence and the entire 3' UTR was subcloned into the pEXO vector (Lingueglia *et al.*, 1993) to give pEXO-TASK. Capped cRNAs were synthesized *in vitro* from the linearized plasmid by using the T7 RNA polymerase (Stratagene). *Xenopus laevis* were purchased from CRBM (Montpellier, France). Preparation and cRNA injection of oocytes have been described elsewhere (Guillemare *et al.*, 1992). Oocytes were used for electrophysiological studies 2–4 days following injection (20 ng/oocyte). In a 0.3 ml perfusion chamber, a single oocyte was impaled with two standard microelectrodes (1–2.5 MΩ resistance) filled with 3 M KCl and maintained under voltage clamp by using a Dagan TEV 200 amplifier, in standard ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 2 mM MgCl₂, 5 mM HEPES, pH 7.4 with NaOH). In some experiments, NaCl was substituted by TMA Cl (tetramethylammonium chloride). Stimulation of the preparation, data acquisition and analysis were performed using pClamp software (Axon instruments, USA). Drugs were applied externally by addition to the superfusate (flow rate: 3 ml/min) or intracellularly injected by using a pressure microinjector (Inject+Matic, Switzerland). All experiments were performed at room temperature (21–22°C).

Patch-clamp recording in transfected COS cells

The 2480 bp *SmaI*–*XhoI* fragment of pBS-TASK was subcloned into the pCi plasmid (Promega) under the control of the cytomegalovirus promoter to give pCi-TASK. COS cells were seeded at a density of 70 000 cells per 35 mm dish 24 h prior transfection. Cells were then transfected by the classical calcium phosphate precipitation method with 2 µg of pCi-TASK and 1 µg of CD8 plasmids. Transfected cells were visualized 48 h after transfection using the anti-CD8 antibody-coated beads method (Jurman *et al.*, 1994). For electrophysiological recordings,

the internal solution contained 150 mM KCl, 3 mM MgCl₂, 5 mM EGTA, and 10 mM HEPES at pH 7.2 with KOH, and the external solution 150 mM NaCl, 5 mM KCl, 3 mM MgCl₂, 10 mM HEPES at pH 7.4 with NaOH.

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