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# Genomic and Functional Characteristics of Novel Human Pancreatic 2P Domain $K^+$ Channels

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**We isolated three novel 2P domain $K^+$ channel subunits from human. The first two subunits, TALK-1 and TALK-2, are distantly related to TASK-2. Their genes form a tight cluster of 25 kb on chromosome 6p21.1–p21.2. The corresponding channels produce quasi-instantaneous and non-inactivating currents that are activated at alkaline pHs. These currents are sensitive to $Ba^{2+}$, quinine, quinidine, chloroform, halothane, and isoflurane but are not affected by TEA, 4-AP, $Cs^+$, arachidonic acid, hypertonic solutions, agents activating protein kinases C and A, changes of internal $Ca^{2+}$ concentrations, and by activation of $G_i$ and $G_q$ proteins. TALK-1 is exclusively expressed in the pancreas. TALK-2 is mainly expressed in the pancreas, but is also expressed at a lower level in liver, placenta, heart, and lung. We also cloned a third subunit, named hTHIK-2 which is present in many tissues with high levels again in the pancreas but which could not be functionally expressed.** 

***Key Words:*** **background $K^+$ channel; 2P domain $K^+$ channel; pH; *Xenopus* oocyte; COS cell.**

Potassium channels, by setting and modulating the cellular membrane potential, play a major role in neuronal activity, muscular excitability, and hormone secretion (1–3). More than 70 genes encoding $K^+$ channel subunits have been identified in mammals. Eleven of them belong to the family of 2P domain $K^+$ channels ($K_{2P}$) that have four transmembrane segments and 2P domains in each subunit (4). The $K_{2P}$ channels family can be divided into five structural subfamilies based on the sequence conservation: (i) TWIK-1, TWIK-2 and KCNK7, (ii) TREK-1, TRAAK and TREK-2, (iii) TASK-1, TASK-3, (iv) TASK-2, and (v) THIK-1 and THIK-2. $K_{2P}$ channels are referred as "background channels" because they are opened at all membrane potentials. They display a large variety of pharmacological properties, patterns of regulation and tissue distributions (4). For example TASK-1 is an acid-sensitive background $K^+$ channel and is important for the control of motoneuron and cerebellar granule cell excitability (5, 6), for oxygen sensing in the carotid bodies (7), and for the generation of a high resting membrane potential in adrenal glomerulosa cells (8). The TREK-1, TREK-2, and TRAAK channels are activated by arachidonic acid and membrane stretch (4, 9–13). Here, we report the cloning, tissue distribution, and functional expression of three novel human $K_{2P}$ channels, TALK-1, TALK-2, and hTHIK-2.

## MATERIALS AND METHODS

*Cloning of cDNAs.* Sequences of $K_{2P}$ channels were used to search homologs in public DNA databases by using the Blast program (14). This led to the identification of genomic sequences (Accession Nos. AC009600 and AL136087). In order to characterize the corresponding full-length cDNAs, 5′- and 3′-rapid amplifications of cDNA ends (RACE PCR) were performed on adult human brain (hTHIK2) and pancreas (TALK-1 and TALK-2). For hTHIK2, two antisense (5′-CGATGGTGCTGAAGGTGACG-3′ and 5′-GCACACGCCGAGCAGGATG-3′) and two sense (5′-GGGTGCCACAGTCTTCT-3′ and 5′-ATCGCCTACGG GCTGTTC-3′) specific primers were designed for 5′- and 3′-RACE PCR. For TALK-1, two antisense primers for 5′-RACE (5′-CGAAGTAGAAGCCCTCTG TGTAGCT-3′ and 5′-TGGCCCAGTGGTTTACTCCCTGCTG-3′) and two sense primers for 3′-RACE (5′-GTCCAAGCATACAAAAACGGAGCCA-3′ and 5′-AAC ACGATGGCTGCCCGCCTCTTCT-3′) were derived from genomic sequences. The antisense primers used for 5′-RACE of TALK-2 were 5′-GGTAGTGACAACTG TGCCTGC-3′ and 5′-CGCCTGCCTCTCTAGCAGCTG-3′, and for the 3′-RACE (5′-CCTATGTCTGCTACCTGCTGC-3′ and 5′-GAGAACTACACCTGCCTGGAC-3′). Two successive RACE reactions were carried out by using anchor primers 5′-TAGAATCGAGGTCGACGGTATC-3′ and 5′-GATTTAGGTGACACTAT AGAATCGA-3′. Amplified products were subcloned into pGEMt easy (Promega). The complete coding sequences of the three channels were amplified from human brain (hTHIK-2) or human pancreas cDNAs (TALK-1 and TALK-2) by PCR using a low-error rate DNA polymerase and were subcloned into the pIRES-CD8 vector (pIRES-CD8.hTHIK2, pIRES-CD8.TALK-1 and pIRES-CD8.

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TALK-2). Different pIRES-CD8 clones from independent PCR reactions were sequenced and found to be identical.

*Analysis of channel tissue distributions.* For Northern blot analysis, a Human Multiple Tissue Northern blot from Clontech was probed with the $^{32}$P-labeled insert of pIRES-CD8.TALK-1 in ultrahyb hybridization buffer (Ambion) at 50°C for 18 h, then washed stepwise at 55°C to a final stringency of 0.2× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate) 0.3% SDS. The blot was dehybridized according to the manufacturer's instructions and sequentially reprobed following the same procedure by using hTHIK-2 and TALK-2 probes. Autoradiograms were exposed 24 h at −70°C on BioMax films by using a Transcreen-HE Intensifying Screen (Eastman Kodak Co).

*Electrophysiological measurements in Xenopus oocytes. Xenopus* laevis oocytes were used as previously described (15). They were injected with either 10 ng RNA (TALK-1 and TALK-2) or 1 ng DNA (hTHIK-2). The standard solution (ND96) contained (in mM): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 2 $MgCl_2$, and 5 Hepes (pH 7.4 with NaOH). The high $K^+$ solution (98 mM $K^+$) was obtained by substituting NaCl with KCl. Changes in extracellular solutions were done by rapid perfusion, with a pipette close to the oocyte. Solutions were buffered with HEPES, except in the study of the pH effects where MES (pH < 7), TRIS (7 < pH < 9) and borate (pH > 9) were used. Volatile anesthetics were prepared from saturated solutions as previously shown (16).

*Whole-cell recordings in transfected COS cells.* COS cells were transfected with pIRES-CD8.hTHIK-2, pIRES-CD8.TALK-1 and pIRES-CD8.TALK-2 by using the DEAE-dextran method as previously described (10). The internal solution contained (in mM): 150 KCl, 2 $MgCl_2$, 5 EGTA, and 10 Hepes (pH 7.2 with KOH), and the external solution contained (in mM): 145 NaCl, 5 KCl, 2 $MgSO_4$, 1 $CaCl_2$, and 10 Hepes (pH 7.4 with NaOH). High potassium solutions (150 mM $K^+$) were obtained by substituting NaCl with KCl. Cells were continuously superfused with a microperfusion system (0.1 ml/min). Whole-cell recordings were performed using a RK300 patch-clamp amplifier (Bio-Logic, Grenoble, France), at room temperature. Data were analyzed using pClamp software.

All results are expressed as mean ± SEM with *n* indicating the number of cells tested.

## RESULTS AND DISCUSSION

*Cloning and primary structure of THIK-2, TALK-1 and TALK-2.* Searches in DNA databases, using the Blast alignment program (14) with $K^+$ channel sequences as query, led to the identification of human genomic sequences that encoded three novel 2P domain $K^+$ ($K_{2P}$) channel subunits. Primers were deduced from the potential exonic sequences and used to amplify the corresponding cDNAs from human brain and pancreas by using the RACE-PCR technique. Human THIK-2 cDNA contains an open reading frame (ORF) of 1290 nucleotides that encodes a 430 amino acid (aa) protein (Fig. 1A). During the preparation of this manuscript, the cloning of a 2P domain subunit from rat was reported (17). This clone is 98% identical to human THIK-2. It also presents the same glycosylation sites and the same number of consensus sequences for phosphorylation by protein kinase C, protein kinase A and casein kinase. This suggests that these two subunits are the products of orthologous genes. The two other cDNAs code for novel 2P domain subunits, named TALK-1 and TALK-2. TALK-1 ORF is 930 nucleotides long and codes for a 309 aa polypeptide, whereas TALK-2 ORF is 999 nucleotides long and codes for a 332 aa protein (Fig. 1A). The three proteins, hTHIK-2, TALK-1, and TALK-2, share the same overall structure with the previously described 2P domain subunits. Both TALK-1 and TALK-2 channels have a short cytosolic carboxy terminus of around 60 residues that contains two consensus sites for phosphorylation by protein kinase C (PKC). They have two N-linked glycosylation sites and a cysteine residue in the M1P1 extracellular loop. TALK-1 contains an additional consensus site for phosphorylation by the cAMP-dependent protein kinase (PKA) in its carboxy terminus and a leucine zipper motif in the amino terminal part of the M4 domain (see Fig. 1A). TALK-1 and TALK-2 are 37% identical and 60% homologous. Sequence conservation with the other $K_{2P}$ channels does not exceed 30% of identity. According to the dendrogram shown in Fig. 1C, TALK-1 and TALK-2 are more related to TASK-2 than to any other subunit.

As TASK-2, the TALK-1 and TALK-2 genes are located on chromosome 6p21 (EMBL Accession No. AL136087). Their organization was deduced from the comparison of cDNAs clones and genomic sequences. The 3′-end of the TALK-1 channel ORF is separated by less than 1 kb from the 5′-end of TALK-2 ORF (Fig. 1B). This tight clustering suggests that their transcription could be under the control of common promoter elements and that both proteins might associate to form heteromeric channels, as observed for other types of $K^+$ channels subunits (18, 19). The TALK-1 gene contains six exons and five introns (Fig. 1B). The size of introns ranges from 0.2 to 1.2 kb. The genomic structure of TALK-2 is very similar to TALK-1. This gene contains five exons and four introns. Introns from 0.3 to 6.2 kb are found at the same positions in both channels. However, the intron situated between exons 5 and 6 in TALK-1 is absent in TALK-2. The gene organizations of TALK-1 and TALK-2 are very close to those of TASK-2 and TREK-1 (unpublished results), TREK-2 and TRAAK (10, 20). These observations are in agreement with the dendrogram shown in Fig. 1C that suggests that the TASK-2, TALK-1, TALK-2, TREK-1, TREK-2, and TRAAK channel genes arose from a single common ancestral gene that underwent several duplication events. Finally, an intron site, located in the codon sequence of the first glycine residue belonging to the pore signature sequence G(Y/F/L)G, is present in the novel channel genes like in all mammalian $K_{2P}$ channel genes. The significance of this intron position is not yet established.

*Tissue distribution of hTHIK-2, TALK-1 and TALK-2.* Northern blot analysis using a probe corresponding to TALK-1 indicates that a 3 kb transcript is strongly present in the pancreas (Fig. 2). No signal was found in the other examined tissues. A TALK-2 specific probe revealed a signal at 1.9 kb in pancreas and liver. A fainter

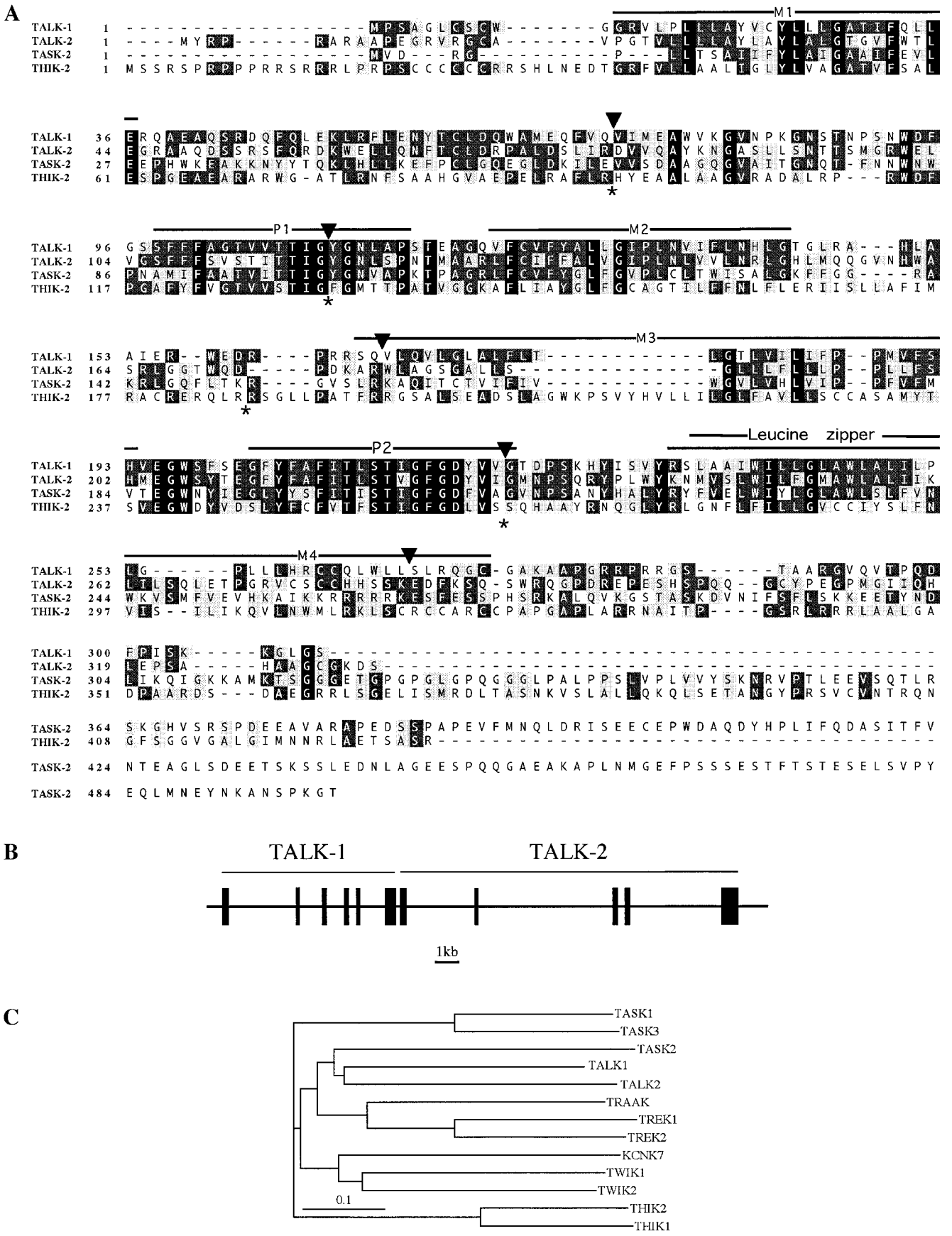


**FIG. 1.** Sequence comparisons and genomic organizations. (A) Alignment of TALK-1, TALK-2, TASK-2, and hTHIK-2 channels. Relative positions of introns are indicated by arrows (TALK-1) or asterisks (TALK-2). M1 to M4 membrane-spanning segments and P1 and P2 pore-domains are indicated. (B) Genomic organization of TALK-1 and TALK-2 genes on chromosome 6. (C) Dendrogram produced by Treeview using a ClustalW alignment of human $K_{2P}$ channels.

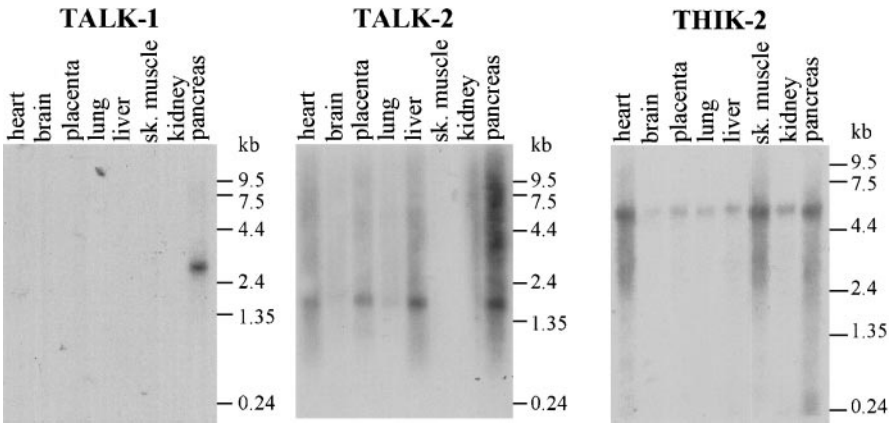


**FIG. 2.** Distribution of TALK-1, TALK-2, and hTHIK-2 in adult human tissues by Northern blot analysis. Each lane containing 2 $\mu$g of poly(A)$^+$ RNA. Sk. muscle, skeletal muscle.

signal was obtained in placenta, heart and lung (Fig. 2). hTHIK-2 has a ubiquitous distribution, a 5 kb transcript being found in all examined tissues and particularly in pancreas, heart and skeletal muscle (Fig. 2). These results were confirmed and extended to other tissues by RT-PCR analysis. hTHIK2 expression was also found in ovary, testis, prostate, colon, peripheral blood leukocytes, small intestine, spleen and thymus (not shown).

*Biophysical properties of TALK-1 and TALK-2 in Xenopus oocytes.* No hTHIK-2 current could be measured in the injected oocytes, as previously observed

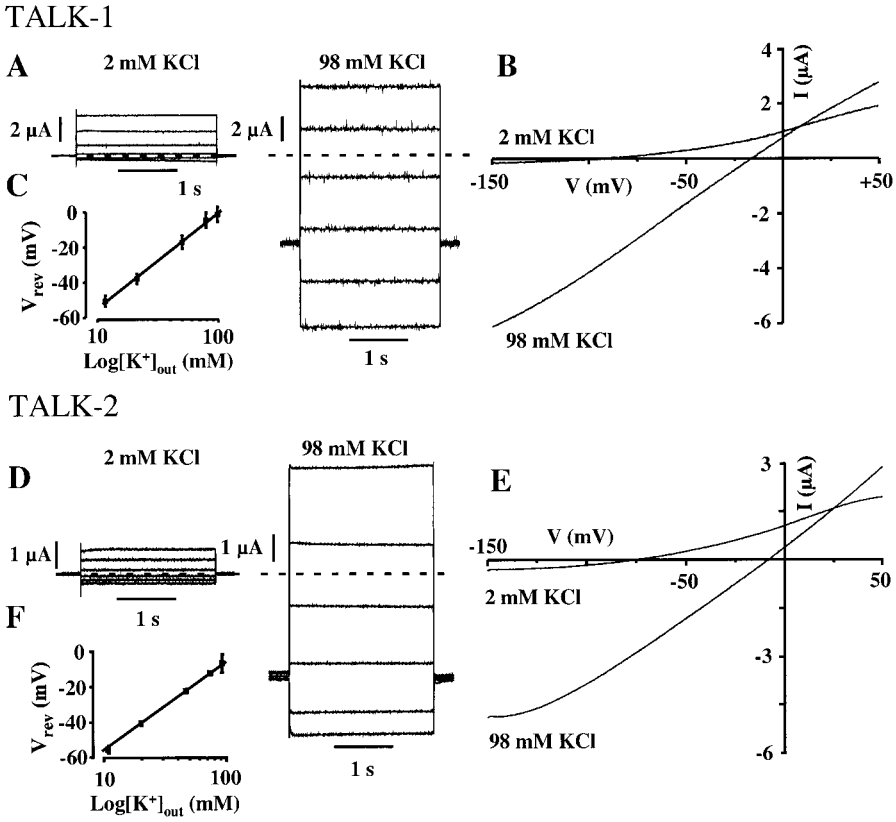


**FIG. 3.** Biophysical properties of TALK-1 and TALK-2 in *Xenopus* oocytes. (A) TALK-1 currents recorded in 2 mM and 98 mM external $K^+$. Currents were elicited by voltage steps from −150 to +50 mV (+40 mV-increments, holding potential of −80 mV). (B) Current–voltage relationships in normal and high $K^+$ using voltage-ramps (from −150 mV to +50 mV, 1 s in duration) from a holding potential of −80 mV. (C) Relationships between reversal potential ($V_{rev}$) and external $K^+$ concentration. The data were fitted by linear regression. (D, E, and F) Same as A, B, and C for TALK-2.

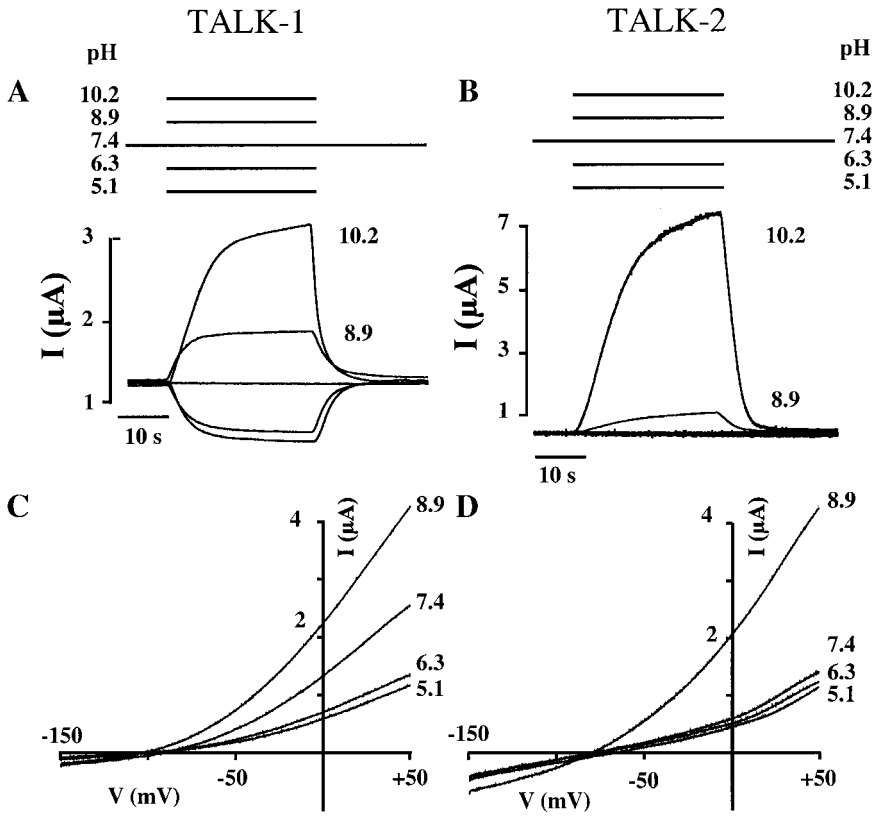


**FIG. 4.** Modulation of TALK-1 and TALK-2 channels by external pH. (A, B) Oocytes expressing TALK-1 (A) and TALK-2 (B) were perfused with external solutions at different pH values: 5.1, 6.3, 7.4 (control), 8.9, and 10.2 (applied during 30 s steps). (C, D) Current-voltage curves (same protocol as in Fig. 3B) of TALK-1 (C) and TALK-2 (D) obtained at different external pH (5.1, 6.3, 7.4, 8.9).

in another study using the rat counterpart (17). Under control conditions, almost instantaneous (<10 ms) and non-inactivating currents were measured in oocytes expressing TALK-1 (1.16 ± 0.18 $\mu$A at +50 mV, $n$ = 21) or TALK-2 (1.14 ± 0.14 $\mu$A at +50 mV, $n$ = 14) (Figs. 3A and 3D). Such currents were not present in non-injected oocytes (0.35 ± 0.08 $\mu$A at +50 mV, $n$ = 12, not shown). The current-voltage (I-V) curves of TALK-1 and TALK-2 were outwardly rectifying in physiological $K^+$ and became linear in symmetrical $K^+$ (Figs. 3B and 3E), with a saturation observed upon hyperpolarisation for the TALK-2 current. In standard ND96, resting membrane potentials of oocytes expressing TALK-1 (−71.9 ± 1.6 mV, $n$ = 65) or TALK-2 (−55.5 ± 2.1 mV, $n$ = 45) were more negative than control oocytes (−26.1 ± 1.6 mV, $n$ = 21). The relationships between the reversal potential and $[K^+]_o$ (54.3 ± 1.4 mV/decade, $n$ = 7, for TALK-1 and 52.2 ± 3.4 mV/decade, $n$ = 4, for TALK-2) were close to the 59 mV/decade predicted by the Nernst equation for $K^+$-selective channels (Figs. 3C and 3F).

*Pharmacological properties of TALK-1 and TALK-2 in Xenopus oocytes.* Partial inhibitions were observed with high concentrations of classical $K^+$ channel blockers like 1 mM TEA (−14.9 ± 4.1%, $n$ = 6, for TALK-1 and −19.9 ± 5.1%, $n$ = 7, for TALK-2) and with 1 mM $Ba^{2+}$ (−51.4 ± 9.9%, $n$ = 7, for TALK-1 and −42.4 ± 3.1%, $n$ = 8, for TALK-2) (not shown). Both currents were also inhibited by 1 mM quinidine (−36.8 ± 1.8%, $n$ = 7, for TALK-1 and −20.5 ± 3.3%, $n$ = 4, for TALK-2). TALK-1 was inhibited by quinine (−45.1 ± 2%, $n$ = 5) while TALK-2 was surprisingly activated by this agent (+99.1 ± 22.2%, $n$ = 5). Neither 4-AP (100 $\mu$M) nor $Cs^+$ (1 mM) were able to affect TALK-1 and TALK-2 currents (less than 10% current inhibition at +50 mV).

Arachidonic acid (20 $\mu$M), which activates some of the cloned 2P domain $K^+$ channels (9–12, 17) failed to modify TALK-1 and TALK-2 currents (less than 10% variation). Some $K_{2P}$ channels are activated by volatile anesthetics (10, 16, 21), others are inhibited (16, 17, 22). TALK-1 and TALK-2 were both inhibited by 800 $\mu$M chloroform (−21.5 ± 2.3%, $n$ = 12 and −44.7 ± 6.5%, $n$ = 5, respectively) and by 800 $\mu$M halothane (−26.8 ± 2.8%, $n$ = 13 and −56.4 ± 7.1%, $n$ = 7, respectively). While TALK-1 was not sensitive to 800 $\mu$M isoflurane (less than 10% variation, $n$ = 5), TALK-2 was activated by this anesthetic (+58.4 ± 16.9%, $n$ = 6).

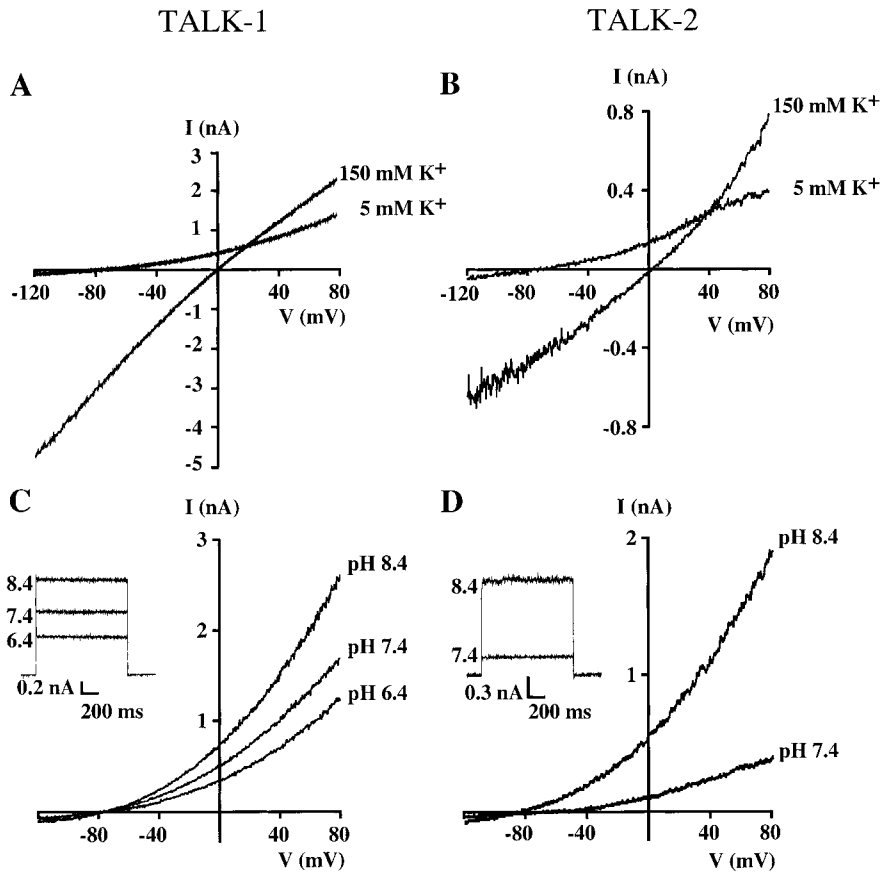


**FIG. 5.** Expression of TALK-1 and TALK-2 channels in COS cells. (A, B) Current–voltage relationships of TALK-1 (A) and TALK-2 (B) obtained in normal $K^+$ (5 mM) and in high $K^+$ (150 mM). Currents were elicited with voltage-ramps ranging from −130 mV to +80 mV (0.8 s in duration, holding potential of −80 mV). (C) Current-voltage curves of TALK-1 recorded in solutions at various external pHs: 7.4 (control), 6.4 (inhibition by 37 ± 1%, $n = 7$, at +50 mV), and 8.4 (activation by 46 ± 1%, $n = 15$, at +50 mV, same protocol as in (A). Inset: Current recorded during 1 s voltage steps to +50 mV from a holding potential of −80 mV. (D and inset) same as in (C) with TALK-2 (activation by 408 ± 32% at pH 8.4, $n = 15$, at +50 mV).

*Regulation of TALK-1 and TALK-2 channel activities.* Modulating activities of PKA (with 300 $\mu$M 8-CPT cAMP or with a mixture of 1 mM IBMX and 10 $\mu$M forskolin) and of PKC (with 400 nM PMA or with 1 $\mu$M chelerythrine) had no significant effect on TALK-1 and TALK-2 currents (less than 10% variation). Injecting 100 mM EGTA/$K^+$ or 100 $\mu$M $CaCl_2$ in oocytes to modify intracellular $Ca^{2+}$ concentrations did not affect TALK-1 nor TALK-2 currents. G-protein-coupled receptors (5-$HT_2$, $\alpha_{2a}$ adrenoceptors) coexpressed with the TALK-1 and TALK-2 channels and activated by the appropriate agonists did not modify these currents (less than 10% variation, $n = 5$). Some $K_{2P}$ channels are sensitive to variations of osmolarity (9), but these channels were not affected by an hyperosmotic shock of 420 mOsm (less than 10% variation, $n = 8$). TALK-1 and TALK-2 are sensitive to variations of the external pH as illustrated in Fig. 4. These effects were rapid and reversible (Figs. 4A and 4B) and voltage-independent (Figs. 4C and 4D). Both TALK-1 and TALK-2 are activated at alkaline pHs (Fig. 4). This is the reason why we named these two channels TALK for **T**wik related **AL**kaline pH activated $\mathbf{K}^+$ channels. Modification of internal pH by using a $NH_4Cl$ solution did not alter the currents (less than 10% current variation, $n = 6$).

*hTHIK-2, TALK-1, and TALK-2 expression in COS cells.* No current was measured in COS cells transfected with hTHIK-2 ($n = 19$). TALK-1 and TALK-2 current-voltage curves were similar to those recorded in oocytes (Figs. 5A and 5B). The reversal potentials measured in physiological $K^+$ (−71 ± 2 mV, $n = 18$, for TALK-1 and −78 ± 2 mV, $n = 16$, for TALK-2) were strongly shifted towards positive values in symmetrical $K^+$ (6 ± 1 mV, $n = 9$, for TALK-1 and −2 ± 2 mV, $n = 9$, for TALK-2) as expected for highly selective $K^+$ channels. The recorded currents at +50 mV in physiological $K^+$ (1004 ± 77 pA, $n = 18$, for TALK-1 and 216 ± 42 pA, $n = 16$ for TALK-2) were significantly larger than

the small endogenous current recorded in mock transfected cells (101 ± 10 pA, $n = 16$). In symmetrical $K^+$, the level of these currents increased (1426 ± 228 pA, $n = 9$, for TALK-1 and 324 ± 76 pA, $n = 9$ for TALK-2) on the contrary to mock transfected cells (126 ± 12 pA, $n = 10$).

Alkalinisation of the external solution increased TALK-1 and TALK-2 without altering the current kinetics or the voltage dependency as illustrated in Figs. 5C and 5D. The endogenous conductance of mock transfected cells was not modified in these conditions (+5 ± 3%, $n = 10$).

*Biological significance of background $K^+$ channels highly specific to the pancreas.* The TALK-1 and TALK-2 channels are open at all membrane potentials and are able to drive the resting potential towards the $K^+$ equilibrium potential. They are not sensitive to the classical $K^+$ channel blockers 4-AP and $Cs^+$, and only mildly sensitive to TEA, $Ba^{2+}$, quinine, and quinidine. These characteristics are typical of those of "background" $K^+$ channels. Activity of background channels can be modulated by various mechanisms as observed for cloned and native channels. These regulations are expected to be important for their physiological functions. For example, in motoneurones and cerebellar granule cells the membrane potential is controlled by a background $K^+$ conductance. The inhibition of this conductance by neurotransmitters leads to a depolarisation and to an increase of cell excitability, TASK-1 is probably at the origin of this background conductance (5, 6). The closure of a similar background TASK-1 conductance by hypoxia or acidosis in type I cells of the carotid body is associated with cell membrane depolarisation and dopamine secretion (7). The unique tissue distribution of TALK-1 and TALK-2, together with their functional properties, suggests a particular function of these background channels in the pancreas which remains to be explored.

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