TWIK-2, an Inactivating 2P Domain K⁺ Channel*

Received for publication, May 3, 2000, and in revised form, June 22, 2000
Published, JBC Papers in Press, July 7, 2000, DOI 10.1074/jbc.M003755200

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The Journal of Biological Chemistry
Vol. 275, No. 37, Issue of September 15, pp. 28722–28730, 2000
Printed in U.S.A.

We cloned human and rat TWIK-2 and expressed this novel 2P domain K⁺ channel in transiently transfected COS cells. TWIK-2 is highly expressed in the gastrointestinal tract, the vasculature, and the immune system. Rat TWIK-2 currents are about 15 times larger than human TWIK-2 currents, but both exhibit outward rectification in a physiological K⁺ gradient and mild inward rectification in symmetrical K⁺ conditions. TWIK-2 channels are inactivating at depolarized potentials, and the kinetic of inactivation is highly temperature-sensitive. TWIK-2 shows an extremely low conductance, which prevents the visualization of discrete single channel events. The inactivation and rectification are intrinsic properties of TWIK-2 channels. In a physiological K⁺ gradient, TWIK-2 is half inhibited by 0.1 mM Ba²⁺, quinine, and quindine. Finally, cysteine 53 in the MIP1 external loop is required for functional expression of TWIK-2 but is not critical for subunit self-assembly. TWIK-2 is the first reported 2P domain K⁺ channel that inactivates. The base-line, transient, and delayed activities of TWIK-2 suggest that this novel 2P domain K⁺ channel may play an important functional role in cell electrogensis.

The mammalian family of two P domain K⁺ channels has rapidly expanded to include TWIK-1, TWIK-2, TREK-1, TREK-2, TRAAK, TASK-1, TASK-2, TASK-3, and KCNK-7 (1–13). These subunits share the same overall structural motif with four transmembrane segments and 2P domains but only 25–50% identity (14). These channels encode K⁺ selective background conductances that are voltage-independent but display distinct functional characteristics. Sequencing of the genomes of Caenorhabditis elegans and Drosophila have revealed that 50 of 80 and 11 of 30 K⁺ channel genes belong to the 2P domain family, respectively (15, 16).

TREK-1 and TRAAK are mechano-gated K⁺ channels that are opened by polyunsaturated fatty acids including arachidonic acid (AA)¹ (17–19). Additionally, TREK-1 is opened by lysophospholipids, mild intracellular acidosis, and inhalational anesthetics (11, 17, 19, 20). TREK-1 is highly expressed in temperature-sensitive neurons of the preoptic hypothalamus and dorsal root ganglions and moreover is activated by heat (21). Finally, opening of TREK-1 has been proposed to be a key event in the neuroprotective effects of polyunsaturated fatty acids against the deleterious effects of brain ischemia and epilepsy (22).

TASK subunits are background K⁺ channels that are inhibited by mild external acidosis (3, 7, 23). The opening of TASK-1 is stimulated by inhalational anesthetics including halothane and isoflurane (11). TASK-1 has been recently proposed to encode the background K⁺ channel present in motoneurons, cerebellum granular cells, and type I carotid body cells (24–26). In motoneurons and cerebellar neurons, the background TASK-1-like K⁺ current is reversibly inhibited by the activation of G₂-coupled receptors, including the muscarinic receptor (25, 26). In the chemoceptor type I carotid body cells, TASK-1-like K⁺ channels are reversibly inhibited by acidosis and hypoxia (24, 27).

TWIK-1, the founding member of the 2P domain mammalian family, is widely expressed in human tissues and is particularly abundant in brain and heart (8). hTWIK-2 currents expressed in Xenopus oocytes are K⁺-selective, are time-independent, and present a nearly linear I-V relationship that rectifies for depolarizations positive to 0 mV (8). TWIK-1 is blocked by Ba²⁺, quinine, and quindine (8). Recently, hTWIK-2 (also called hTOSS), a TASK-1-related gene, was cloned by two independent groups (1, 9). Although both hTWIK-2 and hTOSS sequences are identical, conflicting results were published concerning functional expression in heterologous systems (1, 9). TWIK-2 expressed in Xenopus oocytes was shown to be a nonactivating, time-independent, weak inward rectifier with biophysical properties identical to TWIK-1 (1). Pharmacologically, hTWIK-2 was reported to be different from TWIK-1 with a lack of sensitivity to quinine, quindine, and Ba²⁺ (1). On the contrary, no significant current was observed in hTOSS cRNA-injected Xenopus laevis oocytes or in hTOSS cDNA-transfected HEK293T cells (9). Coinjection of equimolar concentrations of hTWIK-1 and hTOSS cRNA also failed to generate currents in Xenopus oocytes (9). These negative findings have led these authors and others to propose that hTOSS may be targeted to locations other than the plasma membrane or that it may possess a regulatory function, modulating the properties of other principal channel-forming subunits with tissue-specific implications (2, 6, 9, 10).

In the present report, we cloned both human and rat TWIK-2 channels. We demonstrate that TWIK-2 encodes a low conductance inactivating K⁺ channel that is functionally distinct from that previously described for TWIK-1 (8).

EXPERIMENTAL PROCEDURES

Cloning of hTWIK-2, rTWIK-2, and Splice Variants—Two partial expressed sequence tags were identified in the NCBI data base corre-
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Fig. 1. Sequences of human and rat TWIK-2. Transmembrane segments (M1–M4) as well as pore structures (P1 and P2) are indicated. Identical amino acids are boxed in black, conserved amino acids are boxed in gray, and different amino acids are boxed in white. Residues that have been mutated are indicated with diamonds. TWIK-2 splice variants are indicated by an arrow.

Fig. 2. Pattern of expression of human and rat TWIK-2. A, multiple tissue expression array analysis of normalized TWIK-2 expression in various human tissues: 1, whole brain; 2, cerebral cortex; 3, frontal lobe; 4, parietal lobe; 5, occipital lobe; 6, temporal lobe; 7, paracentral gyrus; 8, pons; 9, cerebellum left; 10, cerebellum right; 11, corpus colossum; 12, amygdala; 13, caudate nucleus; 14, hippocampus; 15, medulla oblongata; 16, putamen; 17, substantia nigra; 18, accumbens nucleus; 19, thalamus; 20, pituitary gland; 21, spinal cord; 22, heart; 23, aorta; 24, atrium left; 25, atrium right; 26, ventricle left; 27, ventricle right; 28, interventricular septum; 29, apex of the heart; 30, esophagus; 31, stomach; 32, duodenum; 33, jejunum; 34, ileum; 35, ileocecum; 36, appendix; 37, colon ascending; 38, colon transverse; 39, colon descending; 40, rectum; 41, kidney; 42, skeletal muscle; 43, spleen; 44, thymus; 45, peripheral blood leukocytes; 46, lymph node; 47, bone marrow; 48, trachea; 49, lung; 50, placenta; 51, bladder; 52, uterus; 53, prostate; 54, testis; 55, ovary; 56, liver; 57, pancreas; 58, adrenal gland; 59, thyroid gland; 60, salivary gland; 61, mammary gland; 62, leukemia HLE60; 63, HeLa S3; 64, leukemia K-562; 65, leukemia MOLT-4; 66, Burkitt’s lymphoma Raji; 67, Burkitt’s lymphoma Daudi; 68, colorectal adenocarcinoma SW480; 69, fetal brain; 70, fetal heart; 71, fetal kidney; 72, fetal liver; 73, fetal spleen; 74, fetal thymus; 75, fetal lung. B, pattern of expression of rTWIK-2 analyzed by reverse transcriptase-PCR and normalized to actin expression. 1, brain; 2, blood; 3, kidney; 4, liver; 5, spleen; 6, heart; 7, left ventricle; 8, right ventricle; 9, septum; 10, atria; 11, endometrium; 12, aorta; 13, esophagus; 14, stomach; 15, colon; 16, jejunum; 17, skeletal muscle; 18, pulmonary artery.

The polymerase chain reaction (PCR) was used as described previously to generate the following point mutations: cysteine 53 to serine in hTWIK-2 and rTWIK-2, leucine 217 to phenylalanine in hTWIK-2, tyrosine 109 to alanine in hTWIK-2 (19). All PCRs were performed using the Advantage-GC cDNA polymerase mix (CLONTECH) according to the manufacturer’s protocol. PCR products were cloned into pCHRES-CDS. The clones obtained in this manner were sequenced in their entirety using an automated sequencer (Applied Biosystems).

Preparation of Affinity-purified Antibodies—Rabbit polyclonal antibodies were raised against GST fusion proteins containing the M1P1 loop (amino acids 29–92) and the C-terminal region of TWIK-2 (amino acids 253–313) as described previously (13, 21, 29). The antibodies were affinity-purified using the GST fusion proteins used for the immunization. Briefly, the crude antisera were preincubated for 4 h at 4 °C with 800 μg of GST protein previously transferred to Hybond C-extra nitrocellulose membranes (Amersham Pharmacia Biotech), followed by a similar treatment with GST fusion protein strips. After four washes in PBS (0.1% Tween 20), the anti-TWIK-2 antibodies were recovered by a 1-min elution of each strip with 0.1 M glycine, 20 mg/ml bovine serum albumin, pH 2.8. After the elution, the purified antibodies were rapidly brought to pH 7.6 with 1 M Tris (pH 8.5).

Protein Preparation and Immunodetection—COS cells were transfected using Fugene 6 (Roche Molecular Biochemicals) and harvested 48 h later. Cells were washed three times with PBS and scraped at 4 °C into PBS supplemented with a mixture of protease inhibitors (Sigma.
**Functional expression of TWIK-2 in transfected COS cells.** Whole cell patch clamp configuration was used to record TWIK-2 currents in transiently transfected cells. A, hTWIK-2 recorded in a physiological K⁺ gradient. A hTWIK-2-expressing cell was voltage-clamped at a holding potential of −80 mV, and steps were applied every 10 s with an increment of 20 mV from −110 mV to 90 mV. The zero current is indicated by a dotted line. B, rTWIK-2 currents recorded in a physiological K⁺ gradient. The holding potential was −80 mV, and voltage steps were applied every 20 s with an increment of 20 mV from −110 mV to 90 mV. The zero current is indicated by a dotted line. C, currents elicited with voltage ramps of 800-ms duration from a holding potential of −80 mV were recorded in physiological (5 mM K⁺, NaCl) and symmetrical K⁺ gradients (155 mM K⁺, KCl). A mock CD8-transfected cell is represented in gray, and a hTWIK-2-transfected cell is represented in black. The inset shows the relationship between the reversal potential of hTWIK-2 and the external K⁺ concentration. D, current density of CD8, hTWIK-2, hTWIK-2 splice variant, rTWIK-2, and rTWIK-2 splice variant in COS-transfected cells. Currents were measured at −130 mV in symmetrical K⁺ (KCl, lower bars) and at 100 mV in physiological K⁺ (NaCl; upper bars). Numbers of experiments are indicated. **, p < 0.01; ***, p < 0.001.

**Electrophysiology—** COS-7 cell culture, transfection, and electrophysiology have been extensively described elsewhere (11, 17–21). Briefly, COS cells were routinely transfected using the DEAE-dextran protocol (Life Technologies, Inc.).

**RESULTS**

A partial human expressed sequence tag (AA604914) was identified, and the full coding sequence was subsequently obtained from human brain using 5'- and 3'-RACE PCR. This gene encodes a 313-amino acid polypeptide 100% identical to the previously reported sequences of hTWIK-2 and hTOSS (1, 9). We subsequently identified a partial rat expressed sequence tag (AI454696) coding for rTWIK-2. 5'- and 3'-RACE PCR were used to clone rTWIK-2 in its entirety from rat heart. rTWIK-2 shares 84% identity with hTWIK-2. The greatest divergence between
A conditioning voltage prepulse of 10 s in duration was applied before a test pulse at 100 mV. The holding potential was −80 mV, and increments of 20 mV were applied every 20 s from −110 to 90 mV. B, rTWIK-2 I-V curves measured at peak current (open circles) and at the end of the 10-s duration prepulse (filled circles). The same cell is shown as in A, C, steady-state inactivation curve of rTWIK-2. Normalized currents measured during the test pulse were represented as function of the preconditioning prepulse voltage. Experimental data (n = 9) were fitted with a Boltzmann relationship, and half-inactivation occurred at −65 mV. The inset represents the time constant of inactivation (single exponential decay) as a function of voltage of the cell illustrated in A. D, effects of a K⁺-rich solution (155 mM KCl) on the inactivation kinetic of rTWIK-2. The holding potential was −80 mV, and the cell was depolarized every 20 s to 100 mV. The same cell is shown as in A.
with a phenylalanine in hTWIK-2 had no significant effect on current density (−13.2 ± 4.4 pA/pF in symmetrical K⁺ at −130 mV and 8.8 ± 1.9 pA/pF in physiological K⁺ at 100 mV, n = 14), on the reversal potential (−84.0 ± 2.9 mV, n = 14) or on current kinetics. On the contrary, substitution of tyrosine 109 with an alanine in the first pore domain killed channel activity (−1.5 ± 0.7 pA/pF in symmetrical K⁺ at −130 mV and 2.7 ± 0.4 pA/pF in physiological K⁺ at 100 mV, n = 13).

The inactivation of rTWIK-2 was studied using a standard double voltage pulse protocol (Fig. 4A). rTWIK-2 currents recorded during the conditioning prepotential showed a gradual inactivation upon depolarization. Inactivation became evident at potentials above −30 mV (Fig. 4B). At room temperature, the time constant of inactivation was 1935 ± 159 ms (at 70 mV, n = 7) and remained constant at all potentials studied (Fig. 4C, inset). The current amplitude elicited during the test pulse at 100 mV was gradually decreased with depolarizing preconditioning voltage pulses (Fig. 4, A and C). A steady-state inactivation curve was constructed and indicates that rTWIK-2 was inactivated over the whole voltage range with half-inactivation at +65 mV (Fig. 4C). Finally, the inactivation rate of rTWIK-2 at depolarized potential was largely impaired when currents were recorded in symmetrical K⁺ conditions (n = 6; Fig. 4D). Similar inactivation properties were observed for hTWIK-2 (Fig. 3A).

The inactivation of rTWIK-2 was highly temperature-sensitive (Fig. 5). At physiological temperature, the outward current inactivated with a time constant of 218 ± 42 ms (at 0 mV, n = 15) (Fig. 5, A, B, and D). Moreover, the steady-state outward current became inwardly rectifying at 37 °C (Fig. 5, B and C). The effect of temperature on both current kinetics and rectification occurred within seconds and was fully reversible on returning to room temperature (Fig. 5A). The inward current recorded during hyperpolarization remained time-independent at physiological temperature (Fig. 5B). Inactivation was only partial, and a steady outward current was still present at 37 °C (Fig. 5, A and D). Recovery from inactivation at 37 °C occurred within 200 ms (n = 3) (Fig. 5D).

Outside-out patches were excised from COS cells expressing rTWIK-2. The pipette solution was Mg²⁺-, and Ca²⁺-free. Under these conditions, we observed currents that displayed the same kinetic and rectification characteristics as the macroscopic currents (Fig. 6, A and C). The example illustrated in Fig. 6A shows a patch with a high level of channel expression. The I-V curve recorded in a physiological K⁺ gradient was outwardly rectifying and became mildly inwardly rectifying in a symmetrical K⁺ gradient. Similar data were obtained with patches expressing low levels of currents as well as in the presence of 3 mM Mg²⁺ in the intracellular medium (not shown). In a patch held at −80 mV and expressing a low level of current, application of an external K⁺-rich solution induced an inward current of about 2 pA (Fig. 6A, inset). Discrete channel gating could not be clearly identified (n = 18), and single channel conductance was lower than 5 pS. I-V curves were constructed using voltage pulses (as previously shown for the macroscopic currents) in both physiological and symmetrical K⁺ gradients (Fig. 6B). In a physiological K⁺ gradient, the current displayed the same rectification and inactivation characteristics as the whole cell current (Fig. 6B, left panel). In a symmetrical K⁺ gradient, the current became inward at negative potentials, and as observed in the macroscopic conditions, the inactivation was markedly slowed down (Fig. 6B, right panel). Although the current became more noisy at extreme negative and positive potentials in symmetrical K⁺ gradient, no single-channel event could be clearly observed. In the excised outside-out patch configuration, increasing temperature from 25 to 35 °C speeded up the inactivation kinetic, and the effect was fully reversible (n = 3) (Fig. 6C).

The currents were not altered by removing external Na⁺ (substituted with either TMA⁺ or NMDG⁺) (n = 8). rTWIK-2, recorded in a physiological K⁺ gradient, was resistant to 5 mM TEA, 3 mM 4-aminopyridine, and 10 mM glibenclamide (n = 6). In a physiological K⁺ gradient, the outward and inward rTWIK-2 currents, recorded at positive and negative potentials, respectively, were reversibly blocked by the addition of Ba²⁺ in the bath (Fig. 7, A and B). rTWIK-2 was half-inhibited with about 100 μM Ba²⁺ (Fig. 7B). Interestingly, Ba²⁺ inhibition was not observed in a symmetrical K⁺ gradient even with high Ba²⁺ concentrations (Fig. 7, A and B). Quinidine (100 μM) inhibited rTWIK-2 currents by 73 ± 10% (n = 7) in physiological but not in symmetrical K⁺ gradient (Fig. 7C). A similar block was found with quinine (n = 3). Both blockers speeded up
the apparent rate of relaxation of the outward rTWIK-2 currents, while no effect was found on the inward currents. Finally, 10 mM Cs⁺ blocked inward (−92 ± 2%, n = 8) but not outward currents recorded in a physiological K⁺ gradient (n = 4) (Fig. 7D). rTWIK-2 currents were reversibly inhibited (−36 ± 3%; n = 16) by acidification of the external medium from pH 7.4 to pH 6.4. The inhalational anesthetics chloroform (300 μM) and halothane (750 μM) inhibited rTWIK-2 currents by 32 ± 2% (n = 5) and 27 ± 2% (n = 6), respectively. We investigated the modulation of rTWIK-2 by second messengers using the following protocols: 400 nM phorbol 12-myristate 13-acetate (+12 ± 4%, n = 5), 500 μM cAMP (+28 ± 3%, n = 9), 500 μM cGMP (+12 ± 3%, n = 14), 100 μM sodium nitroprusside (+76 ± 10%, n = 8), and stimulation of mGluR5, co-expressed with TWIK-2, with 50 μM glutamate (+27 ± 8%, n = 12). A similar pharmacological and regulation profile was found for hTWIK-2 (not shown).

TWIK-2 was characterized immunologically by Western blot analysis with polyclonal TWIK-2 antibodies (Fig. 8). Under reducing conditions, no signal was obtained with either mock-transfected COS cells (pCI-RES-CDS) or TWIK-1 (Fig. 8B). Two complexes were detected with molecular masses of 34 and 37 kDa for both hTWIK-2 and rTWIK-2 (Fig. 8B). The size of 34 kDa is in good agreement with that of 33.7 kDa calculated from the sequence of hTWIK-2. Since TWIK-2 has two potential sites for N-linked glycosylation in the external M1P1 loop (Fig. 8A), the protein lysates were treated with N-glycosidase before immunoblotting to determine whether the upper complex (37 kDa) might correspond to a glycosylated form of TWIK-2. After this treatment, only the complex of 34 kDa was detected (Fig. 8B). The splice variant of rat TWIK-2 expressed a protein that migrated at the predicted molecular mass of 20 kDa (Fig. 8B). An upper band migrating at 38 kDa was detected that could correspond to a dimer form.

The proposed topology for TWIK-2 indicates that a cysteine is present at position 53 in the extracellular M1P1 linker domain (Fig. 8A). hTWIK-1 subunits self-assemble via a disulfide bridge formed by this conserved cysteine at position 69, and this assembly is critical for hTWIK-1 channel activity (29). We substituted cysteine 53 with a serine and examined the effects by Western analysis on transfected COS cells using either nonreducing (absence of 2-mercaptoethanol (−βMe)) or reducing conditions (+βMe). Under nonreducing conditions, major bands were detected corresponding to molecular masses of 64–70 kDa with lysates from the wild-type rTWIK-2 transfected conditions (as observed for hTWIK-2; data not shown), probably corresponding to a TWIK-2 dimer (Fig. 8C). Substitution of cysteine 53 with a serine did not alter this migration profile (Fig. 8C) (also observed for hTWIK-2; not shown). Under reducing conditions, the bands moving at 64–70 kDa disappeared as observed for wild-type rTWIK-2 (Fig. 8, B and C).

![TWIK-2 single channel activity in excised membrane patches](image-url)

**Fig. 6.** rTWIK-2 single channel activity in excised membrane patches. A, membrane currents in outside-out membrane patches excised from rTWIK-2-transfected COS cells. Currents elicited with voltage ramps of 800 ms in duration from a holding potential of −80 mV were recorded in a physiological K⁺ gradient (NaCl; 5 mM K⁺) and in a symmetrical K⁺ gradient (KCl; 155 mM K⁺). The internal medium was Mg²⁺-free. The inset shows rTWIK-2 current in an outside-out patch measured at −80 mV in the presence of a physiological (NaCl) and a symmetrical K⁺ gradient (KCl). The apparent rate of relaxation of the outward rTWIK-2 currents, while no effect was found on the inward currents. Finally, 10 mM Cs⁺ blocked inward (−92 ± 2%, n = 8) but not outward currents recorded in a physiological K⁺ gradient (n = 4) (Fig. 7D). rTWIK-2 currents were reversibly inhibited (−36 ± 3%; n = 16) by acidification of the external medium from pH 7.4 to pH 6.4. The inhalational anesthetics chloroform (300 μM) and halothane (750 μM) inhibited rTWIK-2 currents by 32 ± 2% (n = 5) and 27 ± 2% (n = 6), respectively. We investigated the modulation of rTWIK-2 by second messengers using the following protocols: 400 nM phorbol 12-myristate 13-acetate (+12 ± 4%, n = 5), 500 μM cAMP (+28 ± 3%, n = 9), 500 μM cGMP (+12 ± 3%, n = 14), 100 μM sodium nitroprusside (+76 ± 10%, n = 8), and stimulation of mGluR5, co-expressed with TWIK-2, with 50 μM glutamate (+27 ± 8%, n = 12). A similar pharmacological and regulation profile was found for hTWIK-2 (not shown).

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Substitution of cysteine 53 with a serine in the external loop M1P1 of hTWIK-2 and rTWIK-2 significantly reduced current amplitudes recorded at both negative and positive potentials in the two different K⁺ conditions (Fig. 8D).

DISCUSSION

We cloned human and rat TWIK-2 and successfully expressed both channels in transiently transfected COS cells. TWIK-2 is abundantly expressed in visceral and vascular smooth muscle cells. Additionally, the substantial expression of hTWIK-2 in spleen, peripheral blood leukocytes, thymus, lymph node, and bone marrow suggests that TWIK-2 is also a component of the K⁺ channel repertoire of the immune system. TWIK-2 may thus constitute an interesting pharmacological target for the control of immunoreactivity as well as smooth muscle tone.

The currents generated by hTWIK-2 in COS cells were substantially smaller (about 15-fold) than rTWIK-2-mediated currents. The weak currents recorded with hTWIK-2 may thus explain the unsuccessful functional expression of hTOSS in cRNA-injected Xenopus oocyte (9). The most variable region between rTWIK-2 and hTWIK-2 is located in the carboxyl terminus. Interestingly, we have previously demonstrated that the carboxyl-terminal regions of TREK-1 and TASK-1 are critical for channel activity (11, 18–21). Besides the difference in current density, the general biophysical and pharmacological characteristics of the currents are identical between human and rat TWIK-2 channels and moreover are unique among the previously cloned 2P domain K⁺ channels.

So far, TWIK-2 is the only member of the 2P domain K⁺ channel family to display a time-dependent inactivation at depolarized potentials. Increasing temperature from 22 to 37 °C dramatically sped up the rate of inactivation by about 10-fold. Increased inactivation at physiological temperature resulted in a decrease in the steady-state current amplitude and produced a marked inward rectification. TWIK-2 is thus a heat-inactivated K⁺ channel, contrasting with TREK-1, a heat-activated K⁺ channel in hypothalamic and dorsal root ganglion thermosensitive neurons (21). At physiological temperature and even at very depolarized potentials, TWIK-2 was only partially inactivating, and a steady current was still present. Interestingly, the inactivation of TWIK-2 was impaired in K⁺-rich conditions. The inactivation of Shaker-type K⁺ channels involves the amino-terminal domain (fast N type inactivation) as well as the carboxyl-terminal region (slow C type inactivation). The exact molecular mechanism of TWIK-2 inactivation remains to be determined.

rTWIK-2 was insensitive to TEA and 4-aminopyridine but was reversibly inhibited by external Ba²⁺, quinine, and quinidine with a half-inhibition at about 0.1 mM. Interestingly, the inhibition by these blockers was absent in K⁺-rich conditions whatever the direction of the current. It has previously been reported that hTWIK-2 was a noninactivating weak inward rectifier K⁺ channel (1). Moreover, hTWIK-2 was found to be
resistant to Ba\(^{2+}\), quinine, and quinidine and thus was pharmacologically different from TWIK-1 (1). However, these experiments were performed in high K\(^+\) conditions at voltage values below +40 mV, thus masking the time-dependent inactivation and altering channel pharmacology (1). This observation demonstrates that analysis of these channels should be performed under physiological K\(^+\) conditions. Moreover, these results suggest that occupancy of the pore by K\(^+\) might alter the conformation of the channel as observed for the inactivation and thus influence the pharmacology. Interestingly, the pharmacology of Shaker-type K\(^+\) channels, in addition to the inactivation (see above), is also cation-dependent (31).

As previously observed for TWIK-1, KCNK7, and TREK-1 (13, 21, 29), TWIK-2 self-assembles as a dimer under nonreducing conditions. However, substitution of the conserved cysteine with a serine at position 53 in the M1P1 external loop of TWIK-2 does not impair self-assembly. In the absence of cysteine 53, TWIK-2 current density was strongly reduced, thus demonstrating, as previously observed for TWIK-1 (29), the topology of TWIK-2.

A TWIK-2 splice variant encoding a truncated channel was identified in both human and rat tissues. The TWIK-2 splice variant does not express a current. Using reverse transcriptase-PCR analysis, we identified the colon, the atrium, and the atrioventricular node as differentially expressing the TWIK-2 splice variant. Channel events determined.

The high expression of rTWIK-2 allowed us to record this channel in the outside-out patch configuration. Channel events were not visualized under our recording conditions, indicating a very small single channel conductance. Current inactivation as well as rectification were observed in excised patches in the absence of internal Mg\(^{2+}\). These results suggest that both inactivation and rectification are Mg\(^{2+}\)-independent mechanisms and may be intrinsic properties of the TWIK-2 channels.

The characterization of TWIK-2 clearly differentiates this channel from the other closest 2P domain family member, TWIK-1 (8). First, the patterns of expression of the two channels are completely different, as illustrated by the strong expression of TWIK-1 in the human brain, while TWIK-2 is almost absent. Moreover, mouse TWIK-2 is only expressed in the liver (9), while mouse TWIK-1 is strongly expressed in multiple tissues including brain and heart (8). Second, the functional properties of both channels are radically different. TWIK-1 is time-independent, while TWIK-2 is inactivating at depolarized potentials (8). TWIK-1 shows a prominent Mg\(^{2+}\)-dependent inward rectification, while the mild inward rectification of TWIK-2 is Mg\(^{2+}\)-independent (8). The single channel conductance of TWIK-1 is large (34 pS), whereas TWIK-2 single channel conductance is below 5 pS (8). The cysteine in the M1P1 loop is critical for TWIK-1 dimerization (29), whereas it is not for TWIK-2 self-assembly. The common functional features between TWIK-1 and TWIK-2 are the background time-independent activity at negative potentials; the sensitivity to Ba\(^{2+}\), quinine, and quinidine in physiological K\(^+\); the insensitivity to TEA and 4-aminopyridine; and the importance of the conserved cysteine at position 53 for channel activity (8, 29).

TWIK-2 has unique biophysical, pharmacological, and regulation properties among the 2P domain K\(^+\) channels. For instance, TREK-1 and TASK-1 currents are stimulated by inhalational anesthetics (11, 19), unlike TWIK-2, which is blocked.
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TASK-1 is highly sensitive to external acidosis and is fully blocked at pH 6.4 (3), while TWIK-2 is only depressed by 36%. TREK-1 and TRAAK are strongly stimulated by polyunsaturated fatty acids including arachidonic acid (5, 17, 19, 20), while TWIK-2 is only stimulated by 76%. TREK-1, TREK-2, and TRAAK are mechano-gated K⁺ channels (2, 17–19), while TWIK-2 is constitutively active at atmospheric pressure. TREK-1 is blocked by the cAMP/protein kinase A pathway as well as the protein kinase C pathway (19, 20), while TWIK-2 is only slightly potentiated. TREK-1 is opened by heat (21), while TWIK-2 is inactivated. TREK-like channels are characterized by intermediate conductances (14–27 pS) (6, 7, 19), while TWIK-2 is characterized by a high conductance (100 pS) (2, 17, 19), and TASK-like channels are characterized by intermediate conductances (14–27 pS) (6, 7, 10, 19), while TWIK-2 is characterized by a conductance lower than 5 pS. Taken together, these functional properties clearly differentiate TWIK-2 from the other 2P domain K⁺ channels.

The base-line activity of TWIK-2 suggests that these channels may contribute to the setting of cellular resting membrane potential. The inactivation of TWIK-2, which is fast at physiological temperature, transforms this background K⁺ channel into a A-type K⁺ channel at depolarized potentials. TWIK-2 behaves like Kv1.4, Kv4.2, and Kv4.3 at depolarized potentials and thus might contribute to the early repolarization of the action potential. The lack of complete inactivation of TWIK-2 even at very depolarized potentials also implies that the steady outward TWIK-2 current will probably contribute to the late phase of repolarization of the action potential. TWIK-2 should then be seen as a K⁺ channel contributing to the setting of the resting membrane potential and influencing both early and late phases of action potential repolarization.

Acknowledgments—We thank Valerie Lopez for secretarial assistance, Martine Jodar for technical assistance, and Franck Aguila for help with artwork. We are grateful to Florian Lesage and Jacques Barhanin for stimulating discussions. We thank Roberto Reyes for help in generating hTWIK-2 antibodies.

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J. Biol. Chem. 2000, 275:28722-28730.
doi: 10.1074/jbc.M003755200 originally published online July 7, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M003755200

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