TASK-3, a New Member of the Tandem Pore K⁺ Channel Family*

Yangmi Kim, Hyoweon Bang‡, and Donghee Kim§

From the Department of Physiology and Biophysics, Finch University of Health Sciences/The Chicago Medical School, North Chicago, Illinois 60064

We have isolated from the rat cerebellum cDNA library a complementary DNA encoding a new member of the tandem pore K⁺ channel family. Its amino acid sequence shares 54% identity with that of TASK-1, but less than 30% with those of TASK-2 and other tandem pore K⁺ channels (TWIK, TREK, TRAAK). Therefore, the new clone was named TASK-3. Reverse transcriptase-polymerase chain reaction analysis showed that TASK-3 mRNA is expressed in many rat tissues including brain, kidney, liver, lung, colon, stomach, spleen, testis, and skeletal muscle, and at very low levels in the heart and small intestine. When expressed in COS-7 cells, TASK-3 exhibited a time-independent, nonactivating K⁺-selective current. Single-channel conductance was 27 pS at −60 mV and 17 pS at 60 mV in symmetrical 140 mM KCl. TASK-3 current was highly sensitive to changes in extracellular pH (pHₑₒₕ), a hallmark of the TASK family of K⁺ channels. Thus, a change in pHₑₒₕ from 7.2 to 6.4 and 6.0 decreased TASK-3 current by 74 and 96%, respectively. Mutation of histidine at position 98 to arginine abolished pHₑₒₕ sensitivity. TASK-3 was blocked by barium (57%, 3 mM), quinidine (37%, 100 μM), and lidocaine (62%, 1 mM). Thus, TASK-3 is a new member of the acid-sensing K⁺ channel subfamily (TASK).

Potassium (K⁺) channels are involved in a variety of cellular functions including regulation of neuronal firing rate, heart rate, muscle contraction, and hormone secretion. Mammalian K⁺ channels can now be grouped into three main structural classes with each subunit possessing two, four, or six transmembrane segments (1–3). A structurally different K⁺ channel having eight transmembrane segments has been cloned from Caenorhabditis elegans (4–6), but a similar channel subunit has not been identified in the mammalian system. Despite the structural diversity, all K⁺ channel subunits share a conserved P domain that is essential for providing K⁺ selectivity (7–9). In Caenorhabditis elegans, ~50 putative K⁺ channels subunits possessing two pore-forming domains and four transmembrane segments (2P/4TM) have been identified by searching the genome sequences of this organism.

* This work was supported by the National Institutes of Health Grant HL55363 and a grant-in-aid from the American Heart Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AF192366 (TASK-3 gene).

‡ Permanent address: Dept. of Physiology, Chung-Ang University, Seoul 156–756, Korea.
§ To whom correspondence should be addressed: Dept. of Physiology & Biophysics, Chicago Medical School, 3333 Green Bay Rd., North Chicago, IL 60064. Tel.: (847)578–3280; Fax: (847)578–3265; E-mail: donghee.kim@finchcms.edu.

1 The abbreviations used are: P; pore; TM, transmembrane; TWIK, tandem of P domains in a weak inward rectifying K⁺ channel; TASK, twik-related acid-sensitive K⁺ channel; bp, base pair(s); kb, kilobase; PCR, polymerase chain reaction; RT, reverse transcriptase; GFP, green fluorescent protein; pS, pico siemens; GTP•S, guanosine 5′-O-(thio)triphosphate.

2 TASK3 has been given the gene name KCNK9 (approved by HUGO).
tively very low levels in the heart, indicating that TASK-3 is unlikely to be the functional partner for TASK-1 in the rat heart. When expressed in COS-7 cells, TASK-3 exhibited an instantaneous and noninactivating $K_1$-selective current with high sensitivity to extracellular pH. The sensitivity to pH was conferred by the histidine residue at position 98 that is located near the selectivity filter of the channel pore. Thus, TASK-3 may help to set the resting membrane potential and contribute to the pH-dependent $K_1$ conductance in different types of cells.

MATERIALS AND METHODS

Cloning of TASK-3—Rat heart and cerebellum cDNA libraries (AZAPII, Stratagene) were screened with the HindIII-KpnI DNA fragment (560 bp) of mouse TASK-1 using a mild stringency wash condition as described previously (23). No positive plaque was obtained from the heart cDNA library. Of 106 plaques screened using the cerebellum cDNA library, one positive colony was found to contain a 1.7-kb insert. DNA sequencing showed that this clone (designated 4f) contained a partial sequence of a new 4TM $K_1$ channel, as judged by the presence of two P domains with amino acids Gly-Tyr-Gly and Gly-Phe-Gly within these regions, respectively. A search of the GenBank database indicated that the new $K_1$ channel clone was 60% similar to TASK-1. To obtain the full sequence, we prepared a 496-bp DNA fragment of 4f by PCR using two specific primers (5'9-TGACTACTATAGGGTTCGGC-3' and 5'9-AAGTAGGTGTTCCTCAGCACG-3') as a probe to rescreen the rat cerebellum cDNA library. After several rounds of screening, the inserts from positive plaques were excised from the phage DNA into pBluescript SK vector. The inserts were analyzed by restriction enzyme digestion.
zymes and by sequencing of both strands using the dideoxynucleotide chain termination method. One clone contained the entire coding sequence of the new \( K^+ \) channel, which we named TASK-3. Single amino acid mutations were performed using QuikChange site-directed mutagenesis kit (Stratagene).

**Tissue Distribution of TASK-3**—Rat multiple tissue Northern blots were purchased from OriGene Technologies, Inc. A TASK-3-specific probe (827 bp) was prepared by PCR using a primer pair (5'-AGCT-TCAGAGAGGATGGGCCTCTAT-3' and 5'-AAGTAGGTGTTCCTCAGCACG-3') and included the 3'-end of the coding sequence that has low homology with TASK-1. Prehybridization (4 h, 42 °C) and hybridization (overnight, 42 °C) were carried out using \( ^{32} \)P-labeled DNA probe. Blots were washed in solution containing 0.1% SDS and 2\( \times \)SSC for 20 min at room temperature and then in solution containing 0.1% SDS and 0.2% SSC for an additional 10–20 min at 50 °C. Blots were exposed to x-ray film for 24–72 h before developing. For RT-PCR experiments, total RNA was isolated from 14 rat tissues (cerebrum, cerebellum, aorta, atrium, ventricle, kidney, liver, lung, colon, stomach, spleen, testis, skeletal muscle, and small intestine) using RNA STAT-60 (TEL-TEST). Total RNA (2 \( \mu \)g) was reverse transcribed to generate first strand cDNA with a Superscript Pre-amplification System (Life Technologies, Inc.). PCR was carried out with TASK-3-specific primers that yield the 496-bp TASK-3 fragment (see above). As control, glyceraldehyde-3-phosphate dehydrogenase was amplified using specific primers (CLONTECH).

**Transfection of TASK-3 into COS-7 Cells**—For transfection into the COS-7 cell, 2.1-kb DNA containing the entire coding region was subcloned into pCDNA3.1 vector (Invitrogen) by ligating into the EcoRV-HindIII sites after cutting TASK-3/pBluscript SK+ with DraI and HindIII. COS-7 cells were seeded at low density (25,000 cells/35-mm dish) for 1 day prior to transfection. COS-7 cells were co-transfected with TASK-3 and green fluorescent protein (GFP) (CLONTECH) in pcDNA3.1 using LipoTaxi (Life Technologies, Inc.) transfection reagent. Green fluorescence from GFP-expressing cells was identified using a Nikon microscope equipped with excitation and barrier filters (470–510 nm) and a mercury lamp light source.

**Electrophysiology**—Gigaseals were formed using Sylgard-coated thin walled borosilicate pipettes (Kimax). Single channel currents were recorded with an Axopatch 200B patch clamp amplifier (Axon Instruments), digitized with a digital data recorder (VR10, Instrutech), and stored on video tape using a video tape recorder. The recorded signal was filtered at 3 kHz using an 8-pole Bessel filter (3 dB, Frequency Devices) and transferred to a computer (Dell) using the Digidata 1200 interface (Axon Instruments) at a sampling rate of 10 kHz. The filter dead time was \( \leq 100 \mu \text{s} \) (3 dB, Frequency Devices) and filtered for \( 50 \mu \text{s} \) will be missed in our analysis. Single channel currents were ana-

**FIG. 2.** Alignment of amino acid sequences of three TASK channels: rat TASK-1 (GenBank™ accession number AF031384), human TASK-2 (GenBank™ accession number AF084830), and rat TASK-3 (GenBank™ accession number AF192366). Identical amino acids are outlined. Dashes indicate gaps in alignment. Four TM segments and two P regions are shown. A proposed phylogenetic tree of mammalian 4TM \( K^+ \) channels is shown. The percent values indicate amino acid identity.
Cloning and Expression of a Novel K⁺ Channel

Cloning of TASK-3—We used a 560-bp DNA fragment that encompasses the pore and C-terminal region of rat TASK-1 and screened rat cardiac and cerebellum cDNA libraries. One positive clone containing a partial sequence (1.7 kb) of a novel two-domain K⁺ channel was obtained from screening the cerebellum cDNA library. After several rounds of screening using the 3’-region of the 1.7-kb DNA fragment as a probe, one positive clone containing the entire open reading frame of 1185 amino acids encoding a 395-amino acid polypeptide with a calculated molecular mass of 44 kDa was finally isolated (Fig. 1A). This clone had only a partial 3’-noncoding region and did not include the poly(A) sequence. Hydrophobicity analysis (25) of the amino acid sequence showed that the new clone belongs to the K⁺ channel family with two pore-forming domains and four transmembrane segments (Fig. 1B). We placed the N terminus in the intracellular side, similar to those of other tandem pore K⁺ channels. Thus, the putative K⁺ channel subunit has a short N terminus, an extended extracellular loop between M1 and P1, and a long C terminus, structural features typical of nearly all 4TM K⁺ channels (Fig. 1C). One N-glycosylation site is present in the extended extracellular loop between M1 and P1, similar to that found in several other K⁺ channels of this class, including TASK-1 and TASK-2. The amino acid sequence of the new clone shows several potential phosphorylation sites. Consensus sites for protein kinase A are found in the intracellular loop between M2 and M3 (Thr-134), at the proximal site in the C terminus (Thr-247), and at the end of the C terminus (Ser-394). Three sites for protein kinase C are found all in the C terminus (Ser-277, Ser-340, Ser-352). Potential phosphorylation sites for tyrosine kinase were not present.

Searching the GenBank™ data base using the BLAST sequence alignment program (26) indicated that the DNA sequence of the new clone is most similar to that of TASK-1, a 4TM K⁺ channel that was cloned earlier (20, 23, 27). A 2P/4TM K⁺ channel clone named TASK-2 has been described recently but has low homology with that of TASK-1 or the new clone (21). We therefore named our new clone TASK-3. A putative homologue of rat TASK-3 was identified in the genome data base of human chromosome 8 (GenBank™ accession number AC007869; locus D8S1741). Combined partial sequences from two locations with an intervening sequence of 84 kb showed 72% identity in the amino acid sequence with rat TASK-3. Within the first 250 amino acids of rat TASK-3 and the human homologue, the identity was 94%. Fig. 2 shows alignment of three TASK sequences, which reveal high homology between TASK-1 and TASK-3, especially within the transmembrane and pore-forming domains. TASK-1 and TASK-3 share 54% identity and 61% similarity in amino acid sequences, whereas TASK-2 is distantly related with 27% amino acid identity with TASK-1 and 26% with TASK-3. Therefore, TASK-1 and TASK-3 probably share close functional similarity, although this has yet to be demonstrated.

RESULTS

Characterization of TASK-3—Basic Electrophysiological Properties of TASK-3—to determine whether TASK-3 is capable of forming a functional ion channel, cDNA was subcloned into a mammalian expression vector (pcDNA3.1) and transfected along with DNA that encodes GFP into COS-7 cells. Whole cell currents were first recorded in solution containing 140 mM KCl, 2 mM MgCl₂, 10 mM HEPES, and 5 mM EGTA (pH 7.2). When studying the effect of pH and protein kinases, macroscopic currents were recorded using a physiological bath solution containing 118 mM NaCl, 1.3 mM K₂HPO₄, 25 mM NaHCO₃, 1.8 mM CaCl₂, 10 mM HEPES, 10 mM glucose, 4.7 mM KCl, and 1 mM MgSO₄. All experiments were performed at 23–25 °C.

To characterize single channel properties of TASK-3, cell-attached patches were first formed. Nearly all COS-7 cells transfected with TASK-3 exhibited robust channel activity that did not decrease with time. Inside-out patches showed similar properties. The probability of a channel being open, Pₒ, was determined from ~1–2 min of current recording. Macroscopic currents from COS-7 cells were recorded using the whole-cell or large outside-out configuration. Current tracings shown in figures were filtered at 1 kHz.

FIG. 3. Reverse-transcriptase PCR analysis of TASK-3 in rat tissues. Approximately 10 µg of total RNA from each tissue was used to prepare first strand cDNAs. TASK-3-specific primers were used to generate an expected PCR product of 496 bp. The amplified PCR products were subcloned into pCR2.1 vector (Invitrogen) and sequenced on one strand to verify the TASK-3 expression. Two controls (last two lanes) included one with the template DNA for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and one that has no cDNA (last lane). The quality of cDNA was checked using glyceraldehyde-3-phosphate dehydrogenase-specific primers (CLONTECH).
solutions contained 140 mM KCl. An amplitude histogram obtained from channel openings at −60 mV shows a single peak (Fig. 4C). The duration histogram obtained from a patch with only one level of channel opening shows that TASK-3 has a mean open time of 1.1 ± 0.1 ms at −60 mV (Fig. 4D; n = 5). The single channel current-voltage relationship shows that TASK-3 is a weak inward rectifier K⁺ channel (Fig. 4E) similar to that observed with TASK-1 (23, 24). The conductances of TASK-3 were 27.1 ± 1.6 pS at −60 mV and 17.0 ± 2.2 pS at +60 mV.

Ion selectivity of TASK-3 was studied by changing the concentration of K⁺ in the bath solution from 10 to 280 mM while maintaining the pipette [K⁺] constant at 140 mM. As shown in Fig. 5A, the reversal potential shifted to the right as [K⁺] in the bath solution was elevated, as expected of an ion channel that is permeable to K⁺ but not to Cl⁻. The plot of the reversal potential as a function of [K⁺] constant at 140 mM, close to the calculated Nernst value of 58 mV (Fig. 5B). These results confirm that TASK-3 is a relatively K⁺-selective ion channel, similar to TASK-1 and other two pore K⁺ channels.

Pharmacological Studies—The effects of various pharmacological agents were examined on the TASK-3 current using outside-out patches from COS-7 cells. TASK-3 was insensitive to low concentrations of Ba²⁺ (<100 μM) and was blocked only at high concentrations (>300 μM). Ba²⁺ at 3 mM applied extracellularly blocked the inward TASK-3 current by 56 ± 9% (n = 4). TASK-3 was insensitive to 1 mM tetraethylammonium, 100 μM zinc chloride, and 170 mM ethanol. Quinidine (100 μM), lidocaine (1 mM), and bupivacaine (100 μM) caused 37 ± 6%, 62 ± 9%, and 56 ± 13%, respectively, inhibition of TASK-3 current (n = 3). Intracellular application of 10 μM Ca²⁺, 30 mM Na⁺, and 4 mM MgATP using inside-out patches had no significant effect on TASK-3 current (n = 3). However, GTPγS applied intracellularly produced a 30 ± 3% (n = 4) decrease in TASK-3 current, suggesting that a GTP-binding protein-dependent pathway regulates TASK-3 activity. Arachidonic acid (10 μM) showed a strong inhibitory activity from the intracellular side of the membrane, reducing TASK-3 current by 59 ± 4% (n = 3). TASK-3 channel activity in cell-attached or inside-out patches was insensitive to suction pressure applied to the patches (0 to −60 mmHg).

TASK-3 possesses potential phosphorylation sites for both protein kinase A and C. Extracellular application of phorbolester acetate (100 nM), an activator of protein kinase C, failed to alter TASK-3 whole-cell current (n = 5). Application of 8-bromo-cyclic AMP (300 μM) and 1-methyl-3-isobutylxanthine (100 μM) together, which should increase cAMP concentration in the cell and activate protein kinase A, also failed to alter TASK-3 current. Forskolin (10 μM), an activator of adenyl cyclase and 1-methyl-3-isobutylxanthine (100 μM) together, also did not affect TASK-3 current. Therefore, TASK-3 does not appear to be regulated via phosphorylation by protein kinases A and C.

Regulation of TASK-3 Current by pH—A hallmark of TASK-1 and TASK-2 K⁺ channels is their sensitivity to pH. To determine whether TASK-3 also possesses similar pH sensitivity, we first examined TASK-3 current using large outside-out patches from COS-7 cells transfected with TASK-3 and GFP. Cell membrane potential was held at −80 mV and then a voltage ramp (−100 to +100 mV; 640 ms duration) was applied.
TASK-3 currents were measured at different pHo values (Fig. 6A). TASK-3 was markedly inhibited by extracellular acidification (pH 6.0–7.2) at all membrane potentials. At pH 6.4 and 6.0, the current decreased to 74 ± 17% and 96 ± 3%, respectively, of that observed at pH 7.2. An increase in pHo above 7.2 caused a small rise in TASK-3 current, showing that TASK-3 is less sensitive to changes in pHo in the alkaline range (7.2–8.4). Averaged currents at different pHo values at −20, +20, and +60 mV were determined from three experiments and plotted in Fig. 6B. The data were fitted to a Hill equation of the form: \[ y = \frac{1/(1 + (k_{1/2}/[H^+]^n))}{k_2} \] where \( k_{1/2} \) is the [H+] at which half maximal inhibition occurs and \( n \) is the Hill coefficient. At −20 mV, apparent \( k_{1/2} \) was 1.8 × 10^{-5} M corresponding to a pK of 6.7, and the Hill coefficient was 2.0 (mean values, \( n = 3 \)). At +20 mV, the pK and Hill coefficient were 6.7 and 1.8, respectively. For currents recorded at +60 mV, the pK and the Hill coefficient were 6.6 and 1.7, respectively.

To study in more detail the effect of changes in pHo and pHi on TASK-3 kinetics, we recorded single channel currents from outside-out and inside-out patches, respectively, at different pHo values. Fig. 6C shows channel openings from an outside-out patch showing the effect of changing the pHo of the bath solution from 7.2 to 6.4, 6.0, 8.0 and then back to 7.2. Amplitude histograms obtained from such tracings at different pHo values are also shown. Current-voltage relationships show that the single channel conductance is not significantly altered between pH 6.0 and 7.2 (Fig. 6D). However, changing pHo from 7.2 to 8.0 caused a significant increase in single channel conductance. Relative channel current was obtained by multiplying \( N_P \), and single channel current (i) at −60 mV and then plotted as a function of pHo. Fig. 6E shows that TASK-3 is particularly sensitive to changes in pHo, ranging from 6.0 and 7.2. These results show that the marked decrease in channel activity observed at low pHo is predominantly because of a decreased frequency of opening. Fig. 6F shows results obtained from inside-out patches in which the pH of the bath solution (pHb) was sequentially changed from 8.0 to 7.2, 6.4, and 6.0. Changing pHb did not significantly affect the single channel conductance. At pH 6.4 and 6.0, TASK-3 current was 81 ± 9% and 77 ± 11%, respectively, of that observed at pH 7.2. These results show that TASK-3 is much more sensitive to pHo than pHb and that the effect of pHo is not mediated via changes in pHb.

**Histidine at Position 98 Confers pHo Sensitivity**—To identify the amino acid residue responsible for the marked sensitivity to pHo, we mutated two histidine residues (His-72 and His-98) that are conserved in TASK-1 and TASK-3 (see Fig. 1). Four mutants TASK-3 (H72D, H72Q, H98D, and H72Q/H98D) were generated and tested for their pHo sensitivity. Outside-out patches from COS-7 cells were used to measure single channel activity at different pHo values. The single channel conductance at pH 7.2 was 26 ± 2 pS for the H98D mutant and 33 ± 2 pS for the H72D mutant (n = 4), indicating that modifications of the long extracellular loop between TM1 and TM2 alter channel conductance. As shown in Fig. 7A, pHo sensitivity of the H72D mutation was similar to that of wild type TASK-3. pHo sensitivity of the H72Q mutant was also similar to that of the wild type TASK3, showing greater than 90% reduction in current by a decrease in pHo from 7.2 to 6.0. However, the H98D or H72Q/H98D mutation abolished the acid-induced decrease in K+ current (Fig. 7B). Therefore, the single histidine residue at position 98 is critical for the acid sensing of TASK-3.

**DISCUSSION**

A New Member of the "TASK" Family of K+ Channels—We successfully isolated a new member of the K+ channel family that possesses two pore-forming domains and four transmembrane segments. We named the new clone TASK-3 as it belonged to the acid-sensing TASK family of K+ channels. TASK-3 is the 9th 4TM K+ channel to be identified in the mammalian system (hence KCN9K), after TWIK-1 (13), TREK-1 (16), TASK-1 (20, 23), TRAAK (17), TASK-2 (21), TWIK-2 (28), KCNK6, and KCNK7 (22). An insect 4TM K+ channel named ORK1 (open rectifier K+ channel 1) has been cloned earlier from D. melanogaster (12). TASK-3 is most closely related to TASK-1 both in nucleotide and amino acid sequences and functional channel behavior. Our study shows that expression of TASK-3 mRNA is very low in the rat heart, indicating that TASK-3 is unlikely to be a functional partner of TASK-1 whose mRNA is highly expressed in the rat heart. Therefore, TASK-3 probably serves as a functionally separate K+ channel to regulate cell K+ conductance in the cells that express it.

**Electrophysiological Behavior of TASK-3**—Single-channel conductances of TASK-1 and TASK-3 expressed in COS-7 cells are 14 and 27 pS, respectively, under identical ionic conditions (symmetrical 140 mM KCl (24)). With the exception of the third transmembrane segment, which has 64% amino acid identity with that of TASK-1, the pore and transmembrane sequences of TASK-3 are nearly identical to those of TASK-1. The kinetic behavior of TASK-3 is similar to that of TASK-1 in that they both open in short bursts with several closings within each burst. TASK-2 shows a much higher single channel conductance (60 pS) under similar ionic conditions and thus can be distinguished from TASK-1 and TASK-3 (21). These differences in single channel properties should help to subsequently identify the native K+ channel with similar characteristics.

Like TASK-1 and other putative background K+ channels, TASK-3 is open at all membrane potentials and its activity is simply a function of how far the membrane potential is set from the reversal potential. As TASK-3 is expressed in the brain, it may be involved in setting the resting membrane potential as...
well as the action potential duration in certain neurons. Different types of background K⁺ channels with lack of intrinsic voltage dependence have been reported in various types of cells (29–33). Therefore, one of the important tasks in the future will be to determine which tandem pore K⁺ channel encodes the various native background K⁺ channels in specific tissues.

Pharmacological Studies—TASK-3 was generally insensitive to K⁺ channel blockers such as tetraethylammonium and Ba²⁺, similar to the low sensitivity exhibited by TASK-1 and TASK-2 (20, 21, 27). TASK-3 is inhibited 40–60% by 100 μM quinidine, 1 mM lidocaine, and 100 μM bupivacaine, similar to TASK-1. However, TASK-3 is little affected by ethanol (170 mM) or zinc (100 μM), which blocks TASK-1 current by 40% (27). Interestingly, TASK-1 has been shown to be activated by inhalation anesthetics such as halothane and isoflurane (34), although the latter has been reported to have no effect in another study (27). We have not tested the effect of these agents on TASK-3 in this study. Inhalation anesthetics have been shown to activate TREK-1 (34), another tandem pore K⁺ channel that is also activated by arachidonic acid and membrane tension. TASK-3 does not possess the PDZ binding motif (T/SXV) that is present in the carboxyl end of TASK-1 (23, 27). Although TASK-3 possesses putative phosphorylation sites for protein kinase A and C, we were unable to detect any effect of activators of these kinases in COS-7 cells. Thus, all three TASK K⁺ channels appear to be generally insensitive to regulation by protein kinase A and C.

pH Sensitivity of TASK Family of K⁺ Channels—The modulation by pH is an interesting and important functional prop-

FIG. 6 pH-dependent changes in TASK-3 current in COS-7 cells. A, macroscopic currents were recorded from large outside-out patches containing many channels. Ramp protocol (−100 mV to +100 mV) was used to generate the current-voltage relations at different extracellular pH. B, relative currents at −20, +20, and +60 mV were determined and plotted as a function of extracellular pH. The points were fitted to a Hill equation and apparent pKₐ (pKₐ) and Hill coefficients were determined (see “Results”). Each point is the mean ± S.D. of three determinations. C, single channel openings from one outside-out patch at different external pH are shown with amplitude histograms. D, current-voltage relationships obtained at different pH are shown. E, bar graph shows relative channel current at different pH. The current at pH 7.2 was taken as 1.0 (NP = 0.22 ± 0.05; n = 4). F, bar graph shows relative channel currents at different pH. The current at pH 7.2 was taken as 1.0. Each bar is the mean ± S.D. of four determinations. The asterisk indicates a significant difference from the value at pH 7.2, as judged by paired t test.

FIG. 7 Histidine at position 98 confers pH sensitivity. Outside-out patches were formed from COS-7 cells transfected with a TASK3 mutant, H72D (A) or H98D (B). The effect of changes in pH was tested as in Fig. 6, and relative channel activities are shown. Each bar is the mean ± S.D. of four determinations. The asterisk indicates a significant difference from the value at pH 7.2.
property of the TASK class of K⁺ channels. Like TASK-1, TASK-3 exhibited greater sensitivity to changes in extracellular than intracellular pH. Our single channel studies using outside-out patches clearly identify the mechanism of the pH₆₇. To TASK-3 as that produced mainly via changes in the number of channel openings in the pH 6.0–7.2 range. Our studies also show that the histidine residue located next to the GYG se-

References

1. Rudy, B. (1988) Neuroscience 25, 729–749
2. Jan, L. Y., and Jan, Y. N. (1994) Nature 371, 119–122
3. Pongs, O. (1992) Physiol. Rev. 72, 569–588
4. Ketchum, K. A., Joiner, W. J., Sellers, A. J., Kaczmarek, L. K., and Goldstein, S. A. N. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13356–13361
5. Lesage, F., Guillemare, E., Fink, M., Duprat, F., Lazdunski, M., Romey, G., and Barhanin, J. (1996) EMBO J. 15, 1004–1011
6. Goldstein, S. A. N., Wang, K.-W., Ilan, N., and Pausch, M. H. (1998) J. Mol. Med. 76, 13–20
7. Poulemy, D. J., Gulakar, I., Vega-Saenz de Miera, E., Holmes, D., Sanganich, M., Rudy, B., Artman, M., and Coetzee, W. A. (1999) FEBS Lett. 456, 191–196
8. Fink, M., Duprat, F., Lesage, F., Reyes, R., Romey, G., Heurteaux, C., and Lazdunski, M. (1998) EMBO J. 15, 6554–6562
9. Fink, M., Lesage, F., Duprat, F., Heurteaux, C., Reyes, R., Fosset, M., and Lazdunski, M. (1998) EMBO J. 17, 3297–3308
10. Patel, A. J., Honore, E., Mainigret, F., Lesage, F., Fink, M., Duprat, F., and Lazdunski, M. (1998) EMBO J. 17, 4283–4290
11. Mainigret, F., Fosset, M., Lesage, F., Lazdunski, M., and Honore, E. (1999) J. Biol. Chem. 274, 1381–1387
12. Duprat, M., Lesage, F., Fink, M., Reyes, R., Heurteaux, C., and Lazdunski, M. (1997) EMBO J. 16, 5464–5471
13. Reyes, R., Duprat, F., Lesage, F., Fink, M., Salinas, M., Farman, N., and Lazdunski, M. (1998) J. Biol. Chem. 273, 30863–30869
14. Salinas, M., Reyes, R., Lesage, F., Fosset, M., Heurteaux, C., Romey, G., and Lazdunski, M. (1999) J. Biol. Chem. 274, 11751–11760
15. Kim, D., Fujita, A., Hario, Y., and Kurachi, Y. (1998) Circ. Res. 82, 513–518
16. Kim, Y., Bang, H. W., and Kim, D. (1999) Am. J. Physiol. 277, H1669–H1678
17. Kyte, J., and Doolittle, R. (1982) J. Mol. Biol. 157, 162–166
18. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
19. Leonardakis, D., Gray, A. T., Winegar, B. D., Kindler, C. H., Harada, M., Taylor, D. M., Chavez, R. A., Forsayeth, J. R., and Yost, C. S. (1998) Neuron. 18, 665–675
20. Chavez, R. A., Gray, A. T., Zhao, B. B., Kindler, C. H., Mazurek, M. J., Mehta, Y., Forsayeth, J. R., and Yost, C. S. (1999) J. Biol. Chem. 274, 7877–7892
21. Baker, M., Bostock, H., Graf, P., and Martius, P. (1987) J. Physiol. (Lond.) 383, 45–67
22. Koh, D. S., Jonas, P., Brau, M. E., and Vogel, W. (1992) J. Membr. Biol. 130, 149–162
23. Shen, K. Z., North, R. A., and Surprenant, A. (1992) J. Physiol. (Lond.) 455, 581–599
24. Koyano, K., Tanaka, K., and Kuba, K. (1992) J. Physiol. (Lond.) 458, 313–324
25. O’Kelly, I., Stephens, R. H., Peers, C., and Kemp, P. F. J. (1999) Am. J. Physiol. 276, L96–L104
26. Patel, A. J., Honore, E., Lesage, F., Fink, M., Romey, G., and Lazdunski, M. (1999) Nature Neurosci. 2, 422–426
27. Chesler, M. (1990) Prog. Neurobiol. (N. Y.) 34, 401–427
28. Krishnalal, O. A., Osipchuk, Y. V., Shelest, T. N., and Smirnoff, S. V. (1987) Brain Res. 386, 352–356
29. Siesjo, B. K., von Hanwehr, R., Ljungberg, G., Hultsch, H., and Julius, D. (1997) J. Biol. Chem. 272, 13063–13069
30. Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., and Julius, D. (1997) Nature 389, 816–824
