Modulation of the Two-pore Domain Acid-sensitive K⁺ Channel TASK-2 (KCNK5) by Changes in Cell Volume*

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María Isabel Niemeyer‡, L. Pablo Cid, L. Felipe Barros, and Francisco V. Sepúlveda§
From the Centro de Estudios Científicos, Av. Arturo Prat 514, Casilla 1469, Valdivia, Chile

The molecular identity of K⁺ channels involved in Ehrlich cell volume regulation is unknown. A background K⁺ conductance is activated by cell swelling and is also modulated by extracellular pH. These characteristics are most similar to those of newly emerging TASK (TWIK-related acid-sensitive K⁺ channels)-type of two-pore-domain K⁺ channels, mTASK-2, but not TASK-1 or -3, is present in Ehrlich cells and mouse kidney tissue from where the full coding sequences were obtained. Heterologous expression of mTASK-2 cDNA in HEK-293 cells generated K⁺ currents in the absence intracellular Ca²⁺. Exposure to hypotonicity enhanced mTASK-2 currents and osmotic cell shrinkage led to inhibition. This occurred without altering voltage dependence and with only slight decrease in pKᵣ in hypotonicity but no change in hypertonicity. Replacement with other cations yields a permselectivity sequence for mTASK-2 of K⁺ > Rb⁺ > Cs⁺ > NH₄⁺ > Na⁺ ≈ Li⁺, similar to that for the native conductance (Iₖ,vol). Clofilium, a quaternary ammonium blocker of Iₖ,vol, blocked the mTASK-2-mediated K⁺ current with an IC₅₀ of 25 μM. The presence of mTASK-2 in Ehrlich cells, its functional similarities with Iₖ,vol and its modulation by changes in cell volume suggest that this two-pore domain K⁺ channel participates in the regulatory volume decrease phenomenon.

Potassium channels are multimeric membrane proteins capable of allowing the passage of K⁺ ions across the membrane down their electrochemical potential gradient. Their functions range from the propagation of the action potential and the control of excitability to transepithelial transport and the homeostasis of cell volume. There are many varieties of K⁺ channels distinguishable by their functional properties and pharmacological sensitivities. From the molecular point of view, three major families have been distinguished (1): voltage-gated Kᵥ channels, Kir inward rectifiers and SKCa/KICa Ca²⁺-dependent K⁺ channels. These previously described K⁺ channels have only one pore domain (P) and form tetramers with each monomer contributing one P domain to the selectivity filter.

A novel family of K⁺ channels which, exceptionally, have two P regions in tandem and four putative transmembrane helices (2P-4TM¹) has recently emerged, with 13 mammalian homologues described at the time of this writing. In heterologous systems they give rise to K⁺-selective conductances open at all voltages and, generally, showing little rectification besides that expected from the Goldman-Hodgkin-Katz (GHK) prediction (2, 3). A diagnostic feature of these channels is their insensitivity (or low sensitivity) to a range of conventional K⁺ channel blockers, including various toxins, Ba²⁺, tetraethylammonium, and 4-aminopyridine.

2P-4TM channels are thought to underlie the leak or background conductances. These conductances maintain the passive properties of the cell. They have also been implicated in the regulation of excitability by neurotransmitters, second messengers, O₂, or volatile anesthetics. The discovery of the 2P-4TM channel family provides molecular counterparts for these relatively ill-understood conductances and allows the study of their modulation (1–3). The best studied is TASK-1 (KCNK3), which is thought to be the background K⁺ conductance closed by neurotransmitters to enhance excitability in the central nervous system (4, 5).

An increase in cell volume is followed in most cells by regulatory volume decrease (RVD) mediated by efflux of K⁺, Cl⁻, organic osmolytes and osmotically obliged water leading to volume recovery. The pathway through which K⁺ exits Ehrlich cells during RVD is not known at the molecular level but has been characterized recently through electrophysiology and flux measurements (6–10). This current (Iₑ,vol) is independent of intracellular Ca²⁺, has a current-voltage relation that obeys the GHK formalism, suggesting the channels involved lack intrinsic voltage dependence, and is selective to K⁺ and Rb⁺, with P_K > P_Rb. Iₑ,vol is rather insensitive to a number of conventional K⁺ channel inhibitors but is efficiently blocked by the quaternary ammonium derivative clofilium. Iₑ,vol in Ehrlich cells is markedly dependent upon extracellular pH, being strongly inhibited at pH 6.4 and enhanced at pH 8.4, compared with the control at pH 7.4.

The properties of Iₑ,vol in Ehrlich cells, particularly the lack of voltage dependence and insensitivity to many conventional blockers, is reminiscent of the characteristics of the 2P-4TM K⁺ channels. In addition, its strong dependence on extracellular pH approaches them to a group within the 2P-4TM family that can be distinguished by their sensitivity to extracellular pH. These have been termed TASK, for TWIK (Tandem of P domains in Weak Inward rectifier K⁺ channels)-related acid-

¹ The abbreviations used are: 2P-4TM, family of K⁺ channels with two P regions in tandem and four putative transmembrane helices; GHK, Goldman-Hodgkin-Katz; RVD, regulatory volume decrease; TWIK, tandem of P domains in weak inward rectifier K⁺ channel; TASK, TWIK-related acid-sensitive K⁺ channel; TALK, TWIK-related alkali-activated K⁺ channel; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s); h, human; EST, expressed sequence tag; CAPS, 3-cyclohexylamino)propanesulfonic acid; MES, 4-morpholineethanesulfonic acid; kb, kilobase(s).
sensitive K⁺ channels, and more recently TALK, for alkali-activated K⁺ channels.² TASK channels, but not TALK-1 and TALK-2 (=TASK-4), which are active only at alkaline pH (11, 12), provide good candidates for \( I_K\)vol. The aim of this work was to investigate whether a member of the TASK group of the 2P-4TM channel family could be responsible for \( K^+\) efflux during RVFD. It is demonstrated here that, of the three murine TASK K⁺ channels known this far, only mTASK-2 transcript is present in Ehrlich cells. mTASK-2, studied by heterologous expression in HEK-293 cells, is shown to share pharmacological blockade and ion selectivity with the native conductance activated by osmotic swelling of Ehrlich cells. Importantly, osmotic cell swelling can enhance the activity of TASK-2 whereas shrinkage decreases its activity. We therefore propose it to be the molecular counterpart of \( I_K\)vol.

**EXPERIMENTAL PROCEDURES**

**RNA and cDNA Preparation**—Total RNA was prepared from adult mouse tissue immediately after euthanasia by cervical dislocation or from Ehrlich cells grown and collected as described before (7). RNA was isolated using the RNasy kit (Qiagen) according to the manufacturer’s instructions, and cDNA was synthesized from 2–3 μg of RNA using SuperScript (Life Technologies, Inc.), oligo(dT), and random primers in the presence of RNase inhibitors (RNasin, Promega). All animal manipulations were approved by the local ethics committee.

**PCR and Northern Blot Analyses**—The PCR amplification products were carried out as described before (13). For mTASK-2 (14) the primers used were: sense 5’-CGTGTGTCGCGCCTCAA-3’ and antisense 5’-ACGGCGGGTTGCTGCTG-3’, corresponding to nucleotides coding for Arg⁴⁴-Ala⁵⁰ and Arg²⁴⁵-Ala²⁵¹ in the M. musculus TBAK-1 (GenBank™ accession number AB008537). The expected product is 624 bp long. Conditions were: denaturation at 94 °C for 2 min, followed by 30 cycles at 94 °C for 15 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. mTASK-2 primers, based on the rat homologue (15), were: sense 5’-ATGGCGAAGGAGAAAAC-3’ and antisense 5’-TCTTGTAGCCCTGTTACG-3’. They correspond to peptide segments Met³⁵-Leu¹⁴ and Thr¹⁵-Ser¹⁰ of rTASK-5 (GenBank™ accession number AF192366). The expected product is 338 bp long. PCR conditions were as for mTASK-2. For hTASK-2 (16) was used to search for murine expressed sequence tags (ESTs) with the NCBI tBLASTn program in the National Library of Medicine data bases. ESTs AW31846 and AW31955 were used to design primers 5’-AGTGATAGTGAACCCGGG-3’ (sense) and 5’-CCAGTGCTCTCTCCACGG-3’, which should correspond to 5’- and 3’-untranslated segments. The expected size of the ampiclon is around 1623 bp. PCR conditions were: initial denaturation at 94 °C for 2 min, followed by 5 cycles at 94 °C for 15 s, annealing at 68 °C for 45 s, extension at 72 °C for 1 min, 25 cycles at 94 °C for 15 s, annealing at 54 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products resolved by agarose gel electrophoresis were excised and extracted for DNA, which was cloned into pGEM-T vector (Promega). Sequencing was performed automatically.

For Northern analysis, total RNA was run in a denaturing agarose gel. After transferring to a nylon membrane, it was probed with digoxigenin-labeled antisense riboprobe (50 ng ml⁻¹) synthesized by in vitro transcription using mTASK-2 cDNA as a template. Hybridization was conducted at 68 °C. Detection was with an anti-digoxigenin-alkaline phosphatase conjugate (Roche Molecular Biochemicals, Mannheim, Germany), which was used for colorimetric visualization.

**Immunoblotting**—Western blot was done with crude membrane fractions (17). Ehrlich cells, grown as described elsewhere (7), were homogenized in a buffer containing 250 mM sucrose and 10 mM triethanolamine. Homogenates were centrifuged at 2000 × g for 10 min at 4 °C. The supernatants were spun down at 100,000 × g for 1 h at 4 °C, and pellets were resuspended. Protein concentration was determined by the Bradford method. SDS-polyacrylamide gel electrophoresis was done using Laemmli buffers on 10% polyacrylamide minigels. Enhanced chemiluminescence was used to reveal antigen-antibody reaction. The anti-TASK-2 antibody (APC-07, Alomone Laboratories, Israel) was used at 1:200 dilution. Preadsorption was done by preincubating the antibody with the antigen peptide for 1 h at a 1:1 μg ratio.

**Transient Transfections and Electrophysiology Studies**—The mTASK-2 plasmid used in the electrophysiology studies was subcloned in the expression vector pCR3.1 (Invitrogen) and transfected into HEK-293 cells as described previously (13). CD8 cotransfection was used to identify effectively transfected cells. The CD8 antigen was revealed with microspheres (Dynabeads) coated with an anti-CD8 antigen. In cation selectivity studies, the bath solution contained 135 mM KC1, 2 mM CaCl₂, 1 mM MgCl₂, 50 mM sucrose, 10 mM Hepes/Tris, pH 7.4. X stands for either RB, Cs, Li, Na, K, or NH₄, as indicated. The pipette solution contained 140 mM KC1, 1 mM MgCl₂, 10 mM EGTA, 1 mM NaATP, 0.1 mM GTP, 10 mM Hepes, pH 7.4. Alternatively, in experiments at low Cl⁻ concentration this anion was replaced by gluconate to give a final Cl⁻ concentration of 10 mM solution either in the pipette or bath. In experiments to measure the pH dependence of the currents HEPES (used for pH 7.0, 7.5, and 8.0) in the bathing medium was replaced with CAPS (pH 10 and 11), Tris (pH 8.5 and 9), or MES (pH 6.0).

Standard whole-cell patch-clamp recordings were performed as described elsewhere (13, 18). All chemicals were from Sigma Chemical Co.
FIG. 2. Effect of cation replacement on K⁺ currents in mTASK-2-transfected HEK-293 cells. Currents were measured in the whole-cell recording mode of the patch-clamp technique, using the voltage protocol in A. The intracellular solution contained 140 mM K⁺. In B, the extracellular medium had 135 mM Na⁺ and 5 mM K⁺. In C, D, and E extracellular Na⁺ was replaced by equimolar amounts of Rb⁺, Cs⁺, and K⁺, respectively. F shows the current-voltage relations for traces in B (circles) and E (triangles). The solid lines are fits to the GHK current equation. G shows current-voltage relations for traces in C (circles) and D (squares) and for a record taken in 135 mM Li⁺ (triangles), which is not illustrated. H, open columns, permselectivity of mTASK-2 (means ± S.D.). Comparison with data for mTASK-1 given as cross-hatched columns (23).
(St. Louis, MO). When necessary, calculated correction for changes in junction potential were made (19).

**Cell Volume Measurements**—Changes in cell water volume were assessed in single cells by measuring changes in concentration of an intracellularly trapped fluorescent dye (20) exactly as described previously (21). HEK-293 transfected with mTASK-2 cDNA were loaded on 25-mm No. 1 round coverslips, loaded with calcine-AM (5 μM, for 5 min) and then superfused with iso-osmotic solution for 30 min before starting the experiment. The experiments were performed using a confocal laser imaging system (LSM5 Pascal, Carl Zeiss, Germany). Excitation light was 488 nm, and emitted light was measured at wavelengths longer than 515 nm. Pictures were obtained at 30-s intervals, and fluorescence of a selected area inside the cell was measured. Under the conditions of the experiment there was no apparent dye photobleaching. The data are presented as $F_t/F_0$, where $F_0$ = fluorescence in iso-osmotic solution, at $t = 0$, and $F_t$ = fluorescence at time $t$. The ratio $F_t/F_0$ is proportional to cell volume. Transfected cells were identified by the presence of microbeads as described above.

**RESULTS**

*Mouse Ehrlich Cells Express TASK-2 mRNA*—The possible presence of TASK transcripts in Ehrlich cells was assayed in RT-PCR experiments. In Fig. 1A specific primers for mTASK-1 were used. When using RNA from mouse heart as a positive control, a product of a size compatible with the 624 nucleotides of the expected amplicon was seen in the electrophoresis run. However, there was no detectable amplification of mTASK-1 using RNA from Ehrlich cells or from mouse liver, a negative control known to lack mTASK-1 mRNA (2, 22, 23). A similar result was obtained for mTASK-3, as seen in Fig. 1B. There was clear amplification of a product of a size compatible with the expected 338 nucleotides amplicon in brain, the site of most abundance of this transcript (15, 24). No detectable amplification was seen in either Ehrlich cells or small intestine, a negative control (15, 24). To search for the presence of mTASK-2 in Ehrlich cells and mouse kidney by RT-PCR, the protein sequence for (human) hTASK-2 (16) was used to search for murine expressed sequence tags (ESTs). Two ESTs (AW31846 and AW31955) were identified that contained putative start and stop codons for an mTASK-2 (mouse). Primers were designed to flank these, and RT-PCR with mouse kidney and Ehrlich cell RNA gave products of around 1600 nucleotides as shown in Fig. 1C, suggesting that mTASK-2 was present in Ehrlich cells. This was confirmed by the Northern blot shown in Fig. 1D that revealed a ~3.3-kb transcript. The amplicons were subcloned and sequenced confirming the presence of an open reading frame that on translation gave a 502-amino acid sequence for (human) hTASK-2 (16) that on translation gave a 502-amino acid sequence containing the two P regions in tandem as well as four putative trans-membrane helices. This predicted polypeptide was 88.8% homologous to hTASK-2. The mTASK-2 sequence information has been deposited in the GenBank under accession number AF319542. These data suggest that Ehrlich cells express TASK-2 but not TASK-1 or 3. The mTASK-2 expression was also checked by Western blot of membranes from Ehrlich cells, as shown in Fig. 1E. The analysis revealed a major band of about 70 kDa (*lane 1*). This, as well as other minor bands, could be abolished by previous incubation of the antibody with the antigenic peptide (*lane 2*). The mass of mTASK-2 simply derived from the predicted amino acid sequence is 55 kDa. Glycosylation, for which a consensus site is present, could account for this discrepancy.

**Functional Characteristics of TASK-2 Resemble Those of I₉ᵥvol**—No K⁺ currents were seen in mock- or untransfected HEK-293 cells (not shown). After transfection with mTASK-2 cDNA, sizeable currents occurred that had voltage and pH dependence characteristics identical to those reported for the human orthologue (16). These currents showed GHK behavior at all pH values tested (not shown). The selectivity of mTASK-2 was analyzed by cation replacement. Fig. 2 shows currents evoked by the pulse protocol in Fig. 2A. The pipette solution was high in K⁺ (140 mS), and the external solution contained 140 (Fig. 2E) or 5 mS K⁺ and 135 mS of either Na⁺, Rb⁺, or Cs⁺ (Fig. 2, B–D). In Na⁺-rich medium, instant rectification and a moderate activation/deactivation were observed. With K⁺-rich medium the currents appeared Ohmic with decreased time dependence. In Cs⁺ the rectification was more marked, and there was little evidence of time dependence. In Rb⁺-rich medium, on the other hand, the instantaneous current was large at all potentials and relaxed slightly to a lower absolute value toward the end of the pulse at negative potentials. The current-voltage relations for these experiments as measured at the end of the pulse are shown in Fig. 2 (F and G). In symmetrical K⁺ solutions, it was linear with a reversal potential at 0 mV. Replacement of all but 5 mS K⁺ by Na⁺ shifted $E_{rev}$ to more negative than 70 mV. The current-voltage relation was described by the GHK formalism albeit with a lower $P_K$ value than for symmetrical K⁺ condition ($4.8 \times 10^{-11}$ and 2.8 $\times 10^{-11}$ cm$^2$ s$^{-1}$ respectively). In low K⁺ solution and at very depolarized voltages, the fit deviated from the experimental points, which are lower than expected. Fig. 2G compares the
Fig. 4. Effect of hypotonicity upon K\(^+\) currents in mTASK-2-transfected HEK-293 cells. A, current recorded by pulsing to 0 mV (E\(_{C_l}\), upper panel in A) from a holding potential of −80 mV. The lower panel in A shows Cl\(^-\) current measured at E\(_{C_l}\). The upper bar shows changes in tonicity of the extracellular solution from 300 to 200 (hypotonicity) mosM. The intracellular solution contained 116 mM K\(^+\) and 12 mM Cl\(^-\), calculated to be diluted 0.83-fold to 96 and 10 mM, respectively, upon cell swelling (6). Extracellular solution had 5 mM K\(^+\) except for periods in symmetrical K\(^+\) during which this was raised to 96 mM K\(^+\) by equimolar replacement of Na\(^+\). Change in tonicity was achieved by removal of D-mannitol without change in ion composition. Detailed solution compositions were as those published previously (7).

B, current-voltage relations at 300 mosM in physiological (circles) or symmetrical (triangles) K\(^+\) gradients. C, same as in B but after the increase of extracellular osmolality. The solid symbols show the effect of increasing extracellular pH to 8.5 (triangle) or decreasing it to 6.0 (circle) measured at 5 mM extracellular K\(^+\).

current-voltage relations measured in Rb\(^-\), Cs\(^-\), and Li\(^-\)-rich solutions. The curve in the Li\(^-\)-replaced solution was indistinguishable from that in Na\(^+\). In Cs\(^+\), although the reversal was as in Na\(^+\), current was depressed in the entire voltage range examined. The reversal in Rb\(^-\)-rich solution was shifted in the depolarizing direction compared with that in Na\(^+\). The permeability ratios for different cations were calculated from the shifts in reversal potential. They are given in Fig. 2H and compared with those reported for mTASK-1 (KCNK3) (23).

Clofilium is an inhibitor of I\(_{K,\text{vol}}\) in Ehrlich cells (7). The effect of this compound on mTASK-2 currents is shown in Fig. 3. In the inset to the graph in Fig. 3A it is seen that 30 \(\mu\)M clofilium inhibited mTASK-2 current evoked by an 80-mV pulse without affecting the kinetics of its development. Current-voltage relations are shown under control conditions (squares) and after superfusing the cells with 20 and 50 \(\mu\)M clofilium. There was a graded inhibition of the current at all potentials explored without affecting the reversal potential. In Fig. 3B a summary of results for clofilium inhibition of mTASK-2-mediated currents is shown. The measurements were taken at 0 mV (E\(_{C_l}\)) and are expressed as percent inhibition caused by the drug. The result obtained in mTASK-2 assays (circles) is compared with that obtained previously for I\(_{K,\text{vol}}\) (7). The solid line represents the best fit to the Hill equation to the mTASK-2 data with an IC\(_{50}\) of 25 \(\mu\)M and n\(_H\) of 2.

Changes in Medium Tonicity Modulate the TASK-2-Mediated Current—The data presented above make TASK-2 a likely candidate to be the molecular counterpart of I\(_{K,\text{vol}}\). The sensitivity of mTASK-2 current to changes in tonicity was, therefore, tested. Swelling untransfected HEK-293 cells, by exposure to hypotonic solution, resulted in the activation of Cl\(^-\) current of a type present in other cells (25, 26), but no K\(^+\) current developed (not shown). In mTASK-2-expressing cells, hypotonic exposure elicited an increase in K\(^+\) current as measured at E\(_{C_l}\) (upper trace in Fig. 4A). As expected, this was accompanied by activation of a small Cl\(^-\) current measured at E\(_{K}\) (lower panel in Fig. 4A). The current-voltage relations measured in isotonicity under physiological (open circles) or symmetrical (triangles) K\(^+\) concentrations (Fig. 4B) showed the expected rectification properties and reversal potentials. The current-voltage relationship measured in physiological K\(^+\) concentration under hypotonicity is shown in Fig. 4C. There was a general increase in current magnitude and a shift in E\(_{rev}\) from E\(_{K}\) to a more depolarized value, consistent with concomitant activation of a Cl\(^-\) conductance. The current at E\(_{C_l}\) retained pH sensitivity as shown by the black triangle (pH 8.5) and circle (pH 6.0). In symmetrical K\(^+\), E\(_{rev}\) changed to 0 mV as expected. As seen in Fig. 4A, the effect of cell swelling was slowly reversible upon return to isotonicity. Notice the slower recovery time for K\(^+\) current, which has been seen before for the native currents (8).

Cells shrunk in hypertonic medium showed a significant reduction in mTASK2-mediated current. Fig. 5A shows an experiment where a cell was superfused with a solution made 100 mosM hypertonic, through the addition of mannitol without change in ion composition. Hypertonic exposure led to a rapid decrease in mTASK-2-mediated current. This decrease was reversible and was not accompanied by alterations in the current at E\(_{K}\). Fig. 5B shows current-voltage plots taken before (circles) and after (downward triangles) shrinking the cell by changing the tonicity of the extracellular fluid from 300 to 400 mosM. The current depressed by this maneuver had the same reversal potential, and the effect was similar in the entire voltage range as shown by experiments with symmetrical K\(^+\) (not shown).

Fig. 6A shows a summary of experiments demonstrating the osmosensitivity of mTASK-2. The average relative changes in mTASK-2 K\(^+\) current at 0 mV (E\(_{C_l}\)) are shown. Statistically
significant increases or decreases in current were seen in hypo- and hypertonicity, respectively. The increase in current in hypotonicity occurred with a small but significant change in pH sensitivity. As shown in Fig. 6B, the pH dependence of $K_\text{a}$ current at 0 mV could be described by a Hill equation with $pK_a$ of 8.30 $\pm$ 0.07 and $n_H$ of 0.81 $\pm$ 0.07 ($n = 13$). In hypotonicity, the respective values were 7.99 $\pm$ 0.07 and 0.81 $\pm$ 0.07 ($n = 8$), with $pK_a$ being significantly reduced with respect to the control ($p = 0.0077$). This small shift in $pK_a$ only accounts for $\sim$60% of the hypotonicity effect. The pH dependence of the current in hypertonicity was not altered significantly, with a $pK_a$ and $n_H$ values of 8.24 $\pm$ 0.13 and 0.87 $\pm$ 0.05 ($n = 4$), respectively.

**RVD Acceleration in mTASK-2-expressing HEK-293 Cells**

The effect of mTASK-2 expression on the ability of cells to undergo regulatory volume decrease was tested. As in previous experiments, co-expression of the CD-8 antigen revealed with an antibody conjugated to microbeads was used to identify transfected cells. Fig. 7A shows a group of such HEK-293 cells loaded with calcein to measure changes in their volume (20, 21). Two of the cells in the group (labeled 3 and 4) are decorated with beads showing that they are expressing the foreign DNA. Two non-expressing cells have been chosen at the opposite end of the cluster (1 and 2) as controls. The fluorescence was monitored in cells 1–4, and Fig. 7B shows a time course of $F/F_0$ (proportional to cell volume). Exposing the cells to a hypertonic medium (200 mosM) led to a rapid cell swelling consistent with osmometric behavior. The amplitude of the response was similar for decorated and non-decorated cells. Cell swelling was followed by a slow decrease in volume in non-expressing cells and a markedly faster shrinking of the decorated cells. The same protocol was repeated in the presence of clofilium. When the drug was present, there was no regulation in cell volume after osmotic-induced swelling in any of the cells.

**DISCUSSION**

RVD is the regulatory volume decrease by which cells avert osmotically induced increases in cell volume. This is a phenomenon of physiological and pathophysiological importance. The role of $K^+$ channels modulated during changes in cell volume is central to RVD, a fact that has long been recognized in great measure thanks to pioneering work on the mouse Ehrlich ascites tumor cells (27). The RVD effectors, $K^+$ and $Cl^-$ channels, have also been proposed as important mediators of what has been termed AVD, apoptotic volume decrease, a prerequisite in programmed cell death (28).

A detailed description of the osmosensitive $K^+$ conductance has been obtained recently in Ehrlich cells through electrophysiology studies (6–8, 10, 29). Its characteristics include independence of intracellular $Ca^{2+}$ and GHK behavior, suggesting lack of intrinsic voltage dependence. It is significantly permeable only to $Rb^+$, besides $K^+$, and is resistant to a number of known $K^+$ channel inhibitors but efficiently blocked by clofilium. The conductance is markedly dependent upon extracellular pH, being strongly inhibited by acidification and enhanced by alkalinization. Further advances into the mechanism of cell volume modulation of conductances could be greatly aided by assigning them to molecular counterparts. The characteristics of $I_{K,\text{vol}}$ are reminiscent of those of members of
the 2P-4TM K⁺ channel family (2, 3). In particular, they resemble those of the acid-sensitive TASK channels, of which three mammalian representatives, TASK-1, -2, and -3, are known to date (15, 16, 22, 24, 30, 31). The recently described TASK-1 and -2 (TASK-4) are active only at alkaline pH (11, 12) and are therefore not considered as likely candidates to mediate I_{K, \text{vol}}.

Various channel types have been suggested to play the role of mediating K⁺ efflux that occurs during the RVD process. These include voltage-gated K⁺ channels as well as Ca²⁺-dependent K⁺ channels (32), but their molecular counterparts had not been defined. It has recently been suggested that IK, a Ca²⁺-dependent K⁺ channel, is the mediator of RVD in mouse erythroid cells (33), human T lymphocytes (34), and human tracheal cells (35). IK currents resemble superficially the Ehrlich cell I_{K, \text{vol}} in that they present only slight inward rectification in symmetrical K⁺ and are outwardly rectified under physiological gradients. In addition to their Ca²⁺ dependence and distinct pharmacology, they differ in having equal permeability for Rb⁺ and K⁺ (36). There are conflicting reports concerning the involvement of intracellular Ca²⁺ in the regulation of K⁺ channels in the RVD process. Two recent studies (9, 37) have examined this issue in great detail in Ehrlich and neuroblastoma cells respectively. In both cell types it was demonstrated that RVD proceeds without increase, or even during a decrease, in [Ca²⁺]_{i}. RVD was not affected by maneuvers preventing Ca²⁺ influx or its intracellular release (9, 37). Cell swelling- and membrane stretch-activated K⁺ channels have been reported in gall bladder epithelium (38). These are voltage- and Ca²⁺-independent and blocked by high concentration of Ba²⁺ but not by tetraethylammonium. They have a greater permeability to K⁺ than to Rb⁺, which is, therefore, similar to that of Ehrlich cell I_{K, \text{vol}}. The K⁺ channel subunit IsK has also been proposed to contribute to regulatory volume adjustments (39–41), but work on a null mutation mouse has produced contradictory evidence (42).

**Fig. 6.** Effect of changes in tonicity upon mTASK-2 current magnitude and pH dependence. A, a summary of data obtained with decreasing tonicity to 200 mosM (hypotonicity) or increasing it to 400 mosM. Values are means ± S.E. of 18 and 8 experiments, respectively. The differences were statistically significant as tested by paired t test: p = 0.0001 and 0.01 for hypotonicity and hypertonicity, respectively. B shows pH dependence curves measured in isotonicity (circles), hypotonicity for (triangles) and hypertonicity (inverted triangles). Results are means ± S.E. of 13, 8, and 4 experiments, respectively. The lines are constructed from average of fitted parameters of the individual experiments (see text).

**Fig. 7.** Effect of mTASK-2 transfection on RVD in HEK-293 cells. A, the image shows a cluster of calcein-loaded cells in which regions of interest have been selected. The red dots are microbeads identifying mTASK-2-expressing cells (cells labeled 3 and 4). The bar represents 20 μm. B, the time course of relative fluorescence (proportional to cell volume) in the four regions of interest indicated in A. Changes in toxicity in the bathing medium are indicated (in milliosmolar).
Here we report the presence of the murine TASK-2 K⁺ channel in Ehrlich cells, demonstrate its functional characteristics consistent with $I_{K \text{vol}}$, in terms of voltage dependence, selectivity, and pharmacological sensitivity, and show that it is modulated by osmotic changes in cell volume. This evidence, coupled to the previously reported pH dependence and GHK behavior of $I_{K \text{vol}}$, suggests strongly that TASK-2 is the K⁺ conductance activated by swelling of Ehrlich and other cells. The possibility that a different channel is responsible for the pH-dependent $I_{K \text{vol}}$ cannot be dismissed. The channel responsible, in that case, would have to exhibit very similar functional characteristics to TASK-2, and its high expression in Ehrlich cells would have to pass unnoticed in the functional assays.

Western and Northern analyses and RT-PCR assays show the presence of TASK-2 in Ehrlich cells. PCR assays, on the other hand, did not detect TASK-1 or -3 transcripts in these cells. The predicted protein coded by the mTASK-2 transcript presents high homology with hTASK-2. When this murine channel is expressed functionally, it has identical characteristics to its human orthologue (16). The pH effects on mTASK-2 expressed in HEK-293 cells occur without change in kinetics of the currents and with no voltage dependence. This is in contrast with the behavior of mTASK-1 (23) for which the pH effects, in addition to being voltage-dependent, are blocked by K⁺-consistent with H⁺ blockade. This is not the case for TASK-2 or for the pH effects upon the K⁺ conductance activated by swelling of Ehrlich cells (10), which are voltage-independent and apparently caused by changes in NP. The pH-modulation of the swelling-sensitive conductance can be therefore better accounted for by the behavior of TASK-2 rather than that observed for TASK-1.

There are no reports on the selectivity of hTASK-2 to monovalent cations. For mTASK-2 the permselectivity sequence found here of K⁺ > Rb⁺ > Cs⁺, NH₄⁺, Na⁺, Li⁺, is consistent with that previously reported for the Ehrlich cell osmosensitive K⁺ conductance (7). This sequence is at variance with that of mTASK-1, the only other TASK channel for which this property has been studied, which shows higher permeability to Rb⁺ than K⁺ (Fig. 2H).

Channels of the 2P-4TM family have a peculiar pharmacology, in that they are resistant to a range of conventional K⁺ channel blockers, a property shared by TASK-2, and for the pH effects upon the K⁺ conductance involved in the RVD process opens the way for mutational analysis of the mechanisms for its modulation by changes in cell volume.

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REFERENCES

1. North, R. A. (2000) Trends Neurosci. 23, 234–235
2. Lessar, F., and Lazdunski, M. (2000) Am. J. Physiol. 279, F703–F708
3. Goldstein, S. A., Bockenhauer, D., O’Kelly, I., and Zilberger, N. (2001) Nat. Rev. Neurosci. 2, 175–184
4. Millar, J. A., Barratt, L., Southan, A. P., Page, K. M., Pyke, R. E., Robertson, K., and Mathie, A. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 3614–3618
5. Talley, E. M., Li, Q., Siria, J. E., and Bayliss, D. A. (2000) Neuron 25, 399–410
6. Riquelme, G., Sepúlveda, F. V., Jorgensen, F., Pedersen, S., and Hoffmann, E. K. (1998) Biochim. Biophys. Acta 1371, 101–106
7. Niemeyer, M. I., Hougaard, C., Hoffmann, E. K., Jørgensen, F., Stutzin, A., and Sepulveda, F. V. (2000) J. Physiol. (London) 524, 757–776
8. Hougaard, C., Niemeyer, M. I., Hoffmann, E. K., and Sepulveda, F. V. (2000) Pflügers Arch. 440, 283–284
9. Jørgensen, N. K., Christensen, S., Harbak, H., Brown, A. M., Lambert, I. H., Hoffmann, E. K., and Simonsen, L. O. (1997) J. Membr. Biol. 157, 281–299
10. Hougaard, C., Jorgensen, F., and Hoffmann, E. K. (2001) Pflügers Arch. 442, 622–633
11. Decher, N., Maier, M., Dittrich, W., Gassenhuber, J., Bruggemann, A., Busch, A. E., and Steinmeyer, K. (2001) FEBS Lett. 492, 84–89
12. Girard, C., Duprat, F., Terrenoire, C., Tinel, N., Fosset, M., Romey, G., Lazdunski, M., and Lesage, F. (2001) Biochim. Biophys. Res. Commun. 282, 249–256
13. Cid, L. P., Niemeyer, J. M., Ramirez, A., and Sepúlveda, F. V. (2000) Am. J. Physiol. 279, C1198–C1210
14. Kim, Y., Bang, H., and Kim, D. (1999) Am. J. Physiol. 277, H1669–H1678
15. Kim, Y., Bang, H., and Kim, D. (2000) J. Biol. Chem. 275, 9340–9347
16. Barad, R., Duprat, F., Leroy, F., Fink, M., Salinas, M., Farman, N., and Lazdunski, M. (1998) J. Biol. Chem. 273, 30863–30869
17. Gallardo, P., Cid, L. P., Vio, C. P., and Sepúlveda, F. V. (2001) Am. J. Physiol. 281, G856–G863
18. Diaz, M., and Sepúlveda, F. V. (1995) Pflügers Arch. 430, 168–180
19. Barry, P. H. (1994) J. Neurosci. Methods 51, 107–116
20. Alvarez-Leefmans, F. J., Altamirano, J., and Crowe, W. E. (1995) Methods Neurosci. 27, 361–391
21. Stutzin, A., Torres, R., Oporto, M., Pacheco, P., Eguiguren, A. L., Cid, L. P., and Sepúlveda, F. V. (1999) Am. J. Physiol. 277, C392–C402
22. Kim, D., Fujita, A., Horio, Y., and Kurachi, Y. (1998) Circ. Res. 82, 513–518
23. Lopes, C. M., Gallagher, P. G., Buc, M. E., Butler, M. H., and Goldstein, S. A. (2000) J. Biol. Chem. 275, 16969–16978
24. Rajan, S., Wischmeyer, E., Daut, J., Karschin, A., and Derst, C. (2000) J. Biol. Chem. 275, 16650–16657
25. Diaz, M., Valverde, M. A., Higgins, C. F., Rucareanu, C., and Sepúlveda, F. V. (1993) Pflügers Arch. 422, 347–353
26. Valverde, M. A., Hardy, S. P., and Sepúlveda, F. V. (1995) FASEB J. 9, 649–655
27. Hoffmann, E. K., and Simonsen, L. O. (1989) Physiol. Rev. 69, 315–382
28. Maeno, E., Ishizaki, Y., Kanaseki, T., Hazama, A., and Okada, Y. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9487–9492
29. Christensen, O., and Hoffmann, E. K. (1992) J. Membr. Biol. 129, 13–36
30. Duprat, F., Lesage, F., Fink, M., Reyes, R., Heurteaux, C., and Lazdunski, M. (1997) EMBO J. 16, 5464–5471
31. Leonoudakis, D., Gray, A. M., Factor, B. D., Kindler, C. H., Harada, M., Taylor, D. M., Chavez, R. A., Forsayeth, J. R., and Yost, C. S. (1998) J. Neurosci. 18, 88–88
32. Lang, F., Ritter, M., Voikl, H., and Hausssinger, D. (1993) Ren. Physiol. Biochem. 16, 48–65
33. Vandorpe, D. H., Shmukler, B. E., Jiang, L., Lim, B., Maylie, J., Adelman, J. P., de Franceschi, L., Cappellini, M. D., Brugnara, C., and Alper, S. L. (1998) J. Biol. Chem. 273, 21542–21553
34. Khanna, R., Chang, M. C., Joiner, W. J., Kaczmarek, L. K., and Schlichter, L. C. (1999) J. Biol. Chem. 274, 14838–14849
35. Vázquez, E., Nobles, M., and Valverde, M. A. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 5329–5334
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36. Jensen, B. S., Strøbak, D., Christophersen, P., Jørgensen, T. D., Hansen, C., Silahataroglou, A., Olesen, S. P., and Ahring, P. K. (1998) Am. J. Physiol. 275, C848–C856
37. Altamirano, J., Brodwick, M. S., and Alvarez-Leefmans, F. J. (1998) J. Gen. Physiol. 112, 145–160
38. Vanoye, C. G., and Reuss, L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6511–6516
39. Lock, H., and Valverde, M. A. (2000) J. Biol. Chem. 275, 34849–34852
40. Wangemann, P., Liu, J., Shen, Z., Shipley, A., and Marcus, D. C. (1995) J. Membr. Biol. 147, 263–273
41. Busch, A. E., Varnum, M., Adelman, J. P., and North, R. A. (1992) Biochem. Biophys. Res. Commun. 184, 804–810
42. Vetter, D. E., Mann, J. R., Wangemann, P., Liu, J., McLaughlin, K. J., Lesage, F., Marcus, D. C., Lazdunski, M., Heinemann, S. F., and Barhanin, J. (1996) Neuron 17, 1251–1264
43. Valverde, M. A., Díaz, M., Sepúlveda, F. V., Gill, D. R., Hyde, S. C., and Higgins, C. F. (1992) Nature 355, 830–833
44. Niemeyer, M. I., Hougaard, C., Hoffmann, E. K., Jørgensen, F., Stutzin, A., and Sepúlveda, F. V. (2000) J. Physiol. (Lond.) 523, 65
45. O’Brien, J. A., Walters, R. J., and Sepúlveda, F. V. (1991) Biochim. Biophys. Acta 1070, 501–504
46. MacLeod, R. J., and Hamilton, J. R. (1991) Am. J. Physiol. 260, G405–G415
47. Montrose-Rafizadeh, C., and Guggino, W. B. (1990) Annu. Rev. Physiol. 52, 761–772
48. Foskett, J. K., Wong, M. M., Sue, A. Q., and Robertson, M. A. (1994) J. Exp. Zool. 268, 104–110