An Extracellular Ion Pathway Plays a Central Role in the Cooperative Gating of a K$_{2P}$ K$^+$ Channel by Extracellular pH*§

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Wendy González§1, Leandro Zúñiga§1,2, L. Pablo Cid§3, Barbara Arévalo§, María Isabel Niemeyer§3, and Francisco V. Sepúlveda§4

From the §Centro de Estudios Científicos (CECs), 5110466 Valdivia and the ¤Centro de Bioinformática y Simulación Molecular, Universidad de Talca, 3465548 Talca, Chile

Background: TASK-3 is gated cooperatively by extracellular pH.

Results: Mutual electrostatic interaction between K$^+$ ions and two pH-sensing histidines occurs in a recently discovered extracellular ion pathway.

Conclusion: Channel opening requires neutralization of both sensing histidines, with neutralization of the second sensor becoming favored by an electrostatic effect K$^+$ ions.

Significance: The work suggests a central role for the extracellular ion pathway in the gating of K$_{2P}$ K$^+$ channels.

Proton-gated TASK-3 K$^+$ channel belongs to the K$_{2P}$ family of proteins that underlie the K$^+$ leak setting the membrane potential in all cells. TASK-3 is under cooperative gating control by extracellular [H$^+$]. Use of recently solved K$_{2P}$ structures allows us to explore the molecular mechanism of TASK-3 cooperative pH gating. Tunnel-like side portals define an extracellular ion pathway to the selectivity filter. We use a combination of molecular modeling and functional assays to show that pH-sensing histidine residues and K$^+$ ions mutually interact electrostatically in the confines of the extracellular ion pathway. K$^+$ ions modulate the pK$_{a}$ of sensing histidine side chains whose charge states in turn determine the open/closed transition of the channel pore. Cooperativity, and therefore steep dependence of TASK-3 K$^+$ channel activity on extracellular pH, is dependent on an effect of the permeant ion on the channel pH$_s$ sensors.

K$^+$ leak or background conductances are responsible for setting the resting membrane potential (1) and play crucial roles in the regulation of neuronal firing, muscle contraction, hormone, neurotransmitter and enzyme secretion, and transepithelial transport (2). K$^+$ channels of a family known as K$_{2P}$ have been found to be the molecular counterparts of these widespread background K$^+$ conductances. Best known families of K$^+$ channels are membrane proteins with six (Kv) or two (Kir) transmembrane-α-helices (TMs)§ and one highly conserved P-domain that forms the K$^+$ selectivity filter in a tetrameric arrangement around the conduction pathway (3). In contrast, K$_{2P}$ K$^+$ channels have four TMs and two P-domains (4, 5) and form dimers with their P-domains arranged in pseudo-fourfold symmetry (6). Although Kv and Kir channels are active at depolarized or hyperpolarized potentials, respectively, and play important roles during large membrane potential excursions typical of excitable cells, ubiquitously expressed K$_{2P}$ channels are essentially voltage-independent and behave as open rectifiers as expected for K$^+$ leakage pathways (2).

Although appearing as passive leaks, K$_{2P}$ channels are finely tuned by a variety of stimuli. Important among these are intracellular [H$^+$] that gate the activity of 10 of the 15 known K$_{2P}$ mammalian proteins (7). Examples are TASK subfamily K$_{2P}$ channels TASK-1 (K$_{2P}$3.1) and TASK-3 (K$_{2P}$9.1), which are exquisitely sensitive to extracellular proton concentration (8–11). TASK-3 is fundamental to various cellular functions including the modulation of sleep (12), aldosterone/renin secretion (13, 14), and O$_2$ sensing (15), and its dysfunction has been linked to the Birk Barel mental retardation dysmorphism syndrome (16). TASK-3 is inactive (closed) at acid extracellular pH (pH$_s$) but activates (opens) as pH$_s$ is increased. The effect of pH$_s$ on gating is cooperative occurring with a Hill number $n_H$ of 2 (10). Although it is known that the pH$_s$ sensor is a histidine residue (His-98) residing near the extracellular mouth of the pore (9, 10), the cooperativity mechanism of the gating process remains unexplored.

Recent insight into the function of K$_{2P}$ channels has come from the x-ray crystallographic resolution of TWIK-related arachidonic acid-stimulated K$^+$ channel (TRAAK) and TWIK-1 structures (17, 18). A distinguishing feature of these structures is an extracellular cap, corresponding to the TM1-P1 extracellular loop, which obstructs direct movement of ions into the pore normal to the plane of the membrane and defines instead two tunnel-like side portals, the extracellular ion pathway (EIP), which gives bilateral access to K$^+$ binding sites in the selectivity filter (SF). We now explore the consequences of such red; AMPSO, N-(1,1-dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid.

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§This article contains supplemental Tables 1–4 and Figs. S1–S6.

1 Both authors contributed equally to this work.

2 Present address: Departamento de Fisiología, Universidad de Concepción, 4070386 Concepción, Chile.

3 To whom correspondence may be addressed: Centro de Estudios Científicos (CECs), Avenida Arturo Prat 514, 5110466 Valdivia, Chile. Tel.: 56-63-234503; Fax: 56-63-234517; E-mail: miniemeyer@cecs.cl.

4 To whom correspondence may be addressed: Centro de Estudios Científicos (CECs), Avenida Arturo Prat 514, 5110466 Valdivia, Chile. Tel.: 56-63-234503; Fax: 56-63-234517; E-mail: fsepulveda@cecs.cl.

5 The abbreviations used are: TM, transmembrane α-helix; EIP, extracellular ion pathway; SF, selectivity filter; MD, molecular dynamics; RR, ruthenium.
an arrangement on the gating of TASK-3 by extracellular protons, in particular on the cooperativity mechanism. Our data strongly suggest that in these dimeric channels, both His-98 pH sensors, which lie close to the wall of the EIP side portals, need to be neutralized for TASK-3 to become active and that neutralization of the sensor in one subunit enhances the propensity of that in the second subunit to become neutral. The permeant ion plays a central role in this change of acidity of the histidine sensor, an effect that is enhanced by the electrostatic effect of a glutamate residue (Glu-70) lining the wall of EIPs communicating the extracellular space and the outer mouth of the SF.

**EXPERIMENTAL PROCEDURES**

**Electrophysiological Assays—Mus musculus TASK-1 (K_2P-3.1, GenBank™ accession number NP_034738) plasmid was obtained from Dr. Donghee Kim (Rosalind Franklin University, Chicago, IL). Cavia porcellus TASK-3 (K_2P-5.1, GenBank accession number AF212827) plasmid was provided by Dr. Jürgen Daut (Marburg University, Germany). DNAs were subcloned into the pCR3.1 vector. Transient transfections were done in HEK-293 cells using CD8 cotransfection to identify effectively transfected cells (19). The CD8 antigen was revealed with microspheres (Dynabeads) coated with an anti-CD8 antibody. Site-directed mutagenesis and chimera constructions were done by PCR (19, 20). Tandem dimer TASK-3 constructs were made after insertion of XbaI restriction sites to facilitate concatenation (21). The validity of the concatenated channel approach rests on the assumption that the linked subunits are both correctly inserted in the membrane and form the dimer. This sometimes requires shortening the linker between subunits, as reported before for the TASK-2 channel (21). There was no need for amino acid deletion for proper function of the concatenated TASK-3 constructs. Indeed the identical behavior of WT-H98N and H98N-WT TASK-3 constructs strongly suggests the correct assembling of concatenated channels.

Cells were transferred to the stage of an inverted microscope for study, where they were continuously superfused with a bathing solution containing (in mM) 67.5 Na_2SO_4, 4 KCl, 1 potassium gluconate, 2 CaCl_2, 1 MgCl_2, 105 sucrose, 10 HEPES/Tris, pH 7.5. The high K^+ solution was obtained by equimolar replacement of Na^+ by K^+. The pipette solution contained (in mM) 8 KCl, 132 potassium gluconate, 1 MgCl_2, 10 EGTA, 1 Na_3ATP, 0.1 GTP, 10 HEPES, pH 7.4. To measure the extracellular pH dependence of the currents, HEPES (used for pH 7.0, 7.5, and 8.0 in the bathing medium) was replaced with AMPSO (pH 8.5 and 9.0), CAPS (pH 9.5, 10, and 11), or MES (pH 6.5, 6.0, 5.5, and 5.0). In all experiments intra- and extracellular chloride was kept at 10 mM.

Standard whole-cell patch clamp recordings were performed as described elsewhere (22, 23). Currents were measured at several potentials as indicated. All chemicals were from Sigma.

The effect of pH on currents was evaluated by plotting current (I) measured at the indicated membrane potential, against extracellular [H^+]_. For graphical representation, average ± S.E. values of I/I_{max} values are used. The data were obtained from individual experiments. When appropriate, fit of a Hill equation to the data was done for each individual experiment. The parameters are defined in Equation 1.

$$I = I_{min} + (I_{max} - I_{min})/(1 + ([H^+] / K_{H^+}^{0.5})) \quad \text{(Eq. 1)}$$

A similar analysis was done for inhibitor effect. Fits were done using the Marquardt-Levenberg algorithm as implemented in the SigmaPlot software.

**Molecular Model for TASK-3—**The TASK-3 homology model was built using Modeller (24). The template used was the TWIK-1 structure (Protein Data Bank (PDB): 3UKM) (18). Modeller created 10 structures, and the homology model with the lower value of root mean squared deviation of the backbone atoms regarding the template (root mean squared deviation = 0.185 Å) was selected. The multiple alignment used to build TASK-3 model is based on that published by Brohawn et al. (17). The homology model of TASK-3 was validated using PROCHECK (25), and the tautomeric state of the neutral His-98 residue was assigned according to Stansfeld et al. (26). The homology model was embedded into a pre-equilibrated phosphatidyl oleoyl phosphatidylcholine bilayer in a periodic boundary condition box (10.2 × 10 × 10.5 nm) with pre-equilibrated TIP3P water molecules (27). Three K^+ ions were associated to the models in positions S0, S2, and S4 of the selectivity filter and two water molecules at sites S1 and S3, respectively (26). Fifty-eight potassium ions were added to the aqueous phase to simulate a concentration of 150 mM K^+.

The initial configuration of the system was first optimized using energy minimization followed by a molecular dynamics (MD) simulation at 300 K for 1 ns. The protonation state of His-98 was assigned to the optimized homology model of TASK-3 using the CHARMM27 (28) force field. Models bearing both neutral (His^0-His^0) and one (His^+-His^0) or both (His^+-His^+) His-98 residues protonated were improved using energy minimization followed by two equilibrated MD simulations at 300 K for 8 ns (supplemental Fig. S1). The MD simulations were done in an isobaric isothermal ensemble using harmonic restraints of 0.5 kcal/mol Å^2 applied to the backbone atoms, but not to the selectivity filter. All MD simulations were performed using the NAMD program (29). The electrostatic interactions were computed with no truncations using the particle mesh Ewald algorithm (30) under periodic boundary conditions.

The mutant TASK-3-E70K model was built in silico using the plug-in mutate of the VMD program on the basis of the optimized homology model of the TASK-3 wild type bearing both neutral His-98 residues. The TASK-3-E70K mutant was inserted into the membrane, minimized, and equilibrated following the same protocol as applied for wild-type TASK-3.

**Electrostatic Potential Calculations—**The electrostatic potential, φ(r), was calculated using the Poisson-Boltzmann equation implemented in the PBEQ module of the program CHARMM following a previously published protocol (31) (see also supplemental Fig. S2).

**RESULTS**

**Molecular Modeling of TASK-3 Structure—**We used TWIK-1 structure (18) to construct homology models for
**K_{2p} Extracellular Ion Pathway and pH_{o} Gating**

![Figure 1](image_url)  

**FIGURE 1.** Modeling the extracellular ion pathway and the effect of the charge state of the pH_{o} sensors of TASK-3 K^{+} channel. A, a molecular model for the TASK-3 pore based on the structure of TWIK-1 is shown with both sensing His-98 residues in the neutral state (His^{0}-His^{0}). Shown is a ribbon representation with K^{+} ions and H_{2}O molecules in the selectivity filter. His-98 pH_{o}-sensing residues and Glu-70 at the base of the extracellular cap are shown in stick representation. CH2 identifies CH2 of the extracellular cap whose C-terminal end points above the selectivity filter. The EIP is drawn as a solid tunnel connecting the extracellular space and the entrance of the selectivity filter. HOLE color code is used: blue, radius >1.15 Å; green, radius 0.6–1.15 Å. B and C show details of the models for the His-98^{−}–His-98^{−} and His-98^{+}–His-98^{−} configurations. All illustrations are taken at the end of 8-ns MD runs. In D, the position of the outermost K^{+} ion during MD runs is plotted for the three states of protonation of the pH_{o}-sensing histidines. The asterisk indicates the moment when the ion escaped to the extracellular space during the MD run.

TASK-3 to gain a better understanding of the gating process. TWIK-1 shares with TASK channels the histidine sensor in P-domain 1. Amino acid sequences for TWIK-1 and TASK-3, on the other hand, are 23% identical and 62% similar in the extracellular cap/P1 region. Secondary structure prediction gives the α-helices likely to constitute the conserved extracellular cap seen in K_{2p} channel structures. Models bearing both neutral (His^{0}-His^{0}) and one (His^{+}-His^{0}) or both (His^{+}-His^{+}) His-98 residues protonated were subjected to MD. Fig. 1, A−C, show representations of the models at the end of 8-ns MD runs, with EIP depicted as a colored cavity. In all cases, the neutral His-98 side chains remain behind the SF at the bottom surface of the side portals near the pore. Protonation is accompanied by a movement of the charged His-98 side chains toward the EIP, which narrows the cavities as indicated by the change in color of EIP walls (Fig. 1, B and C). Both in the singly (His^{+}-His^{0}) and in the doubly (His^{+}-His^{+}) protonated His-98 situations, but not in the His^{0}-His^{0} model, the outermost K^{+} ion bound at the selectivity filter becomes destabilized, implying a nonconductive channel. This is shown in Fig. 1D, which plots the position of the K^{+} ion sited at S0 at the beginning of separate 8-ns MD runs as a function of time. A previous MD study using a TASK-1 homology model proposed that a water molecule located behind the selectivity filter is critical for the stability of the SF, which is disrupted upon His-98 protonation (26). This water molecule is not present in the resolved K_{2p} structures and was not essential for the conformational changes described here. The MD data shown above suggest a requirement for side chain neutralization of both His-98 sensors of TASK-3 for channel activity, with protonation of single sensor leading to channel closure.

**Role of Individual Histidine pH_{o} Sensors of TASK-3**—To test whether neutralization of both pH_{o} sensors is indispensable to lead to the open state of TASK-3, we have performed functional assays using concatenated channels (21). Tandem channels containing either a normal set of pH_{o} sensors, mixed structures containing one able and one neutralized (H98N mutation) pH_{o} sensor, or two neutralized pH_{o} sensors were assayed for pH_{o} sensitivity by patch clamp after expression in HEK-293 cells. Gating by pH_{o} of wild-type TASK-3 channels was cooperative with pK_{a}, 6.0 ± 0.05 and n_{H}, 2.1 ± 0.09 (means ± S.E., n = 9). Extracellular H^{+} gating of the concatenated TASK-3 wild-type channels (WT−WT) did not differ significantly from that of the nonconcatenated equivalent channels (Fig. 2A). Doubly mutated TASK-3-H98N proteins (H98N-H98N) were pH_{o}-insensitive, but the mixed WT−H98N and H98N−WT constructs followed pH_{o} in a noncooperative manner with pK_{a} values of 4.9 and 5.1 (Fig. 2A and supplemental Table S1). Similar results were obtained at physiological 5 mM extracellular K^{+} (Fig. 2B and supplemental Table S1). These results confirm the prediction of a requirement for both sensing histidines to be neutralized for TASK-3 to open. They also suggest that neutralization of a first His-98 sensor affects the pK_{a} of the second, making it more susceptible to H^{+} loss.

**Minimal Kinetic Model for Cooperative pH_{o} Gating of TASK-3**—The results with concatenated TASK-3 channels shown in Fig. 1 suggest that there is a requirement for both sensing histidines (His-98) to be neutralized for TASK-3 to open. The simplest model representing this situation is

\[
\begin{align*}
\text{CH}_{2}^{2+} & \xrightarrow{0.5k_{d1}} \text{CH}^{+} \xrightarrow{H^{+}} \text{C} \xrightarrow{2K_{d2}} \alpha \xrightarrow{\beta} \text{O} \\
\text{REACTION 1}
\end{align*}
\]

where CH_{2}^{2+}, CH^{+}, and C are doubly protonated, singly protonated, and unprotonated closed states and O is an open state. The dissociation constant for the first transition is 0.5K_{d1}, as two charged sensors, in monomers 1 and 2, could be deprotonated. The second transition is governed by 2K_{d2}, as the unprotonated closed state C can be generated from a complex containing a neutralized sensor at monomer 1 or 2. The relative
open probability of the system will be given by

$$\frac{P_o}{P_{o,\text{max}}} = \frac{1}{1 + \frac{[H^+]}{K_{n1}} + \frac{[H^+]^2}{K_{n2}}}$$

(Eq. 2)

where

$$K_{n1} = \frac{\alpha + \beta}{\beta} K_{d2}$$

(Eq. 3)

and

$$K_{n2} = \frac{\alpha + \beta}{\beta} K_{d1} K_{d2}.$$  

(Eq. 4)

Fit of Equation 2 to the data for the WT-WT construct shown in Fig. 1A yielded the $K_{n1}$ and $K_{n2}$ of $5.82 \times 10^{-6} \pm 1.2 \times 10^{-6}$ M and $1.1 \times 10^{-12} \pm 0.3 \times 10^{-12}$ M. From these fitted constants, it is possible to obtain a value for

$$\frac{\alpha + \beta}{\beta} \times \frac{K_{d2}}{K_{d1}}$$

(Eq. 5)

of 14.2. This result implies that neutralization of a first His-98 sensor of TASK-3 affects the $pK_a$ of the second, making it at least 14-fold more susceptible to $H^+$ loss.

**Electrostatic Interactions Determine TASK-3 Gating Cooperativity**—Calculations (supplemental Fig. S3a) along the ion conduction pathway show a strong peak of positive potential imparted upon the SF by the doubly protonated His-98 side chains, the His$^+$. This is much decreased in the His$^+$. On this basis, we would propose that K$^+$ ion occupation of the outermost binding sites of SF would be higher in His$^+$. This provides a rationale for cooperativity due to an increased facility of His-98 deprotonation in His$^+$. When compared with that in His$^+$. Because of an electrostatic influence of K$^+$ ions.

The side portals predicted by modeling TASK-3 are, like those of TWIK-1, funnel-shaped, becoming narrower nearer to the SF opening (Fig. 1A). Amino acids lining the walls of this EIP, including Glu-68, Glu-70, Pro-71, Gly-75, and Glu-209 as well as His-98, should confer a predominantly negative electrostatic potential to the cavity. As the cavity size (supplemental Fig. S4) is of the same order as a scantily hydrated monovalent ion, the electrostatic potential is expected to make a large contribution to local cation concentrations within the EIP. Glutamic acid 70 has been shown to lie near proton-sensing His-98 in TASK-3 and to modulate pH$^-$ sensitivity of concatenated TASK-3 channels. Experiments were done in 140 mM [K$^+$] symmetrical intra/extracellular solutions. pH$^-$ dependence curves for WT-WT and mixed concatenated constructs WT-H98N and H98N-WT obtained with 5 mM extra- and H98N-WT. Results are means ± S.E. Lines are fits of the Hill equation constructed using the average of fitted parameters of the individual experiments (41). Parameters obtained are reported in supplemental Table 1.

**FIGURE 2. Using concatenated TASK-3 channels to reveal the mechanism of cooperativity in pH$^-$ gating.** A and B show the pH$^-$ sensitivity of concatenated constructs of WT and pH$^-$-insensitive TASK-3 channels. A, pH$^-$ dependence curves for the following TASK-3 constructs: WT-WT, H98N-H98N, WT-H98N, and H98N-WT. Experiments were done in 140 mM [K$^+$] symmetrical intra/extracellular solutions. B, pH$^-$ dependence curves for WT-WT and mixed concatenated constructs WT-H98N and H98N-WT obtained with 5 mM extra- and H98N-WT. Results are means ± S.E. Lines are fits of the Hill equation constructed using the average of fitted parameters of the individual experiments (41). Parameters obtained are reported in supplemental Table 1.

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Our results suggest an effect of residue Glu-70 on [H$^+$] and $K^+$ in the vicinity of His-98 pH sensor so that charge reversal in TASK-3-E70K would lead to an apparent acid shift in pK$_{13}$.
and a decrease in cooperativity. The loss of cooperativity in TASK-3-E70K therefore would be due to the increase in positive electrostatic potential (see supplemental Fig. S3b) because of charge reversal leading to a decrease in local K$^+$ concentration. The recovery of TASK-3-E70K cooperativity at higher K$^+$ concentration corroborates this interpretation.

Interestingly, TASK-1 K$^+$ channel, which is another closely related member of the TASK subgroup of K$_{2p}$ channels, has a lysine at the site equivalent to TASK-3 Glu-70, and its pH$_o$ gating is noncooperative. As shown before (11), and confirmed here (supplemental Fig. S5 and supplemental Table S3), pH$_o$ gating of TASK-1 becomes cooperative at high [K$^+$]$_o$. TASK-1 has a lysine residue at the place of TASK-3 Glu-70, but mutating this to glutamic acid, as in TASK-1-K70E, although shifting the pH$_o$ dependence curves did not confer cooperativity at the lower [K$^+$]$_o$. It is interesting to note that side chains on the wall of the putative EIP of TASK-1 make it hostile to cations, with a further positively charged residue, Arg-68 in place of Gln-68 of TASK-3.

Pharmacological Blockade of TASK-3 Is Also Dependent upon EIP Electrostatics—Independent evidence that Glu-70 might be an important determinant of electrostatic potential at the external ion pathway of TASK-3 comes from inhibition experiments by positively charged blockers Zn$^{2+}$ and ruthenium red (RR). Blockade of TASK-3 by Zn$^{2+}$ and RR are K$^+$-dependent and show virtual voltage independence, as expected from an interaction with superficial sites at the selectivity filter. Both Glu-70 and His-98 are proposed to form the blocker binding site. It is also possible that Glu-70 side chain additionally favors the interaction of the blockers by increasing their local concentration electrostatically. Fig. 4A shows that both TASK-3 and its mutant TASK-3-E70K are blocked by Zn$^{2+}$ and that the
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potency of the effect is decreased by increasing K⁺ concentration. Interestingly, as for the H⁺ concentration effect on gating, the effect of Zn²⁺ was also cooperative, suggesting more than one blocker site (Fig. 4A and supplemental Table S4). The shift in the Kₛ (Kₛₐ) of TASK-3-E70K minus TASK-3 for inhibition is compatible with a change in electrostatic potential (Δφ) of 46 mV at the site of action of the blocker. We have used the TWIK-1-based model of TASK-3 (His-98⁰-His-98⁰ configuration) to explore the interaction of Zn²⁺ with the channel. Fig. 4B shows a Zn²⁺ ion positioned at a site in the EIP where the switch from Glu-70 to Lys is predicted to give a Δφ of ~46 mV. After an 8-ns MD run (Fig. 4C), the Zn²⁺ appears coordinated by His-98, which has flipped upwards, Glu-70, and Gln-209 (not shown). This conformational change is accompanied by disruption of the selectivity filter and a switch of the position of the second His-98 from behind the SF toward the EIP as though forming a second Zn²⁺ site and thus providing a rationale for cooperative inhibition. Fig. 4D shows the result of an MD run with two Zn²⁺ ions originally positioned as for Fig. 4B but at opposite ends of the EIP.

In contrast with the Zn²⁺ effect, RR inhibits TASK-3 in a noncooperative manner with a Kₛ of 10 μM (supplemental Fig. S6a). This is explained by the shape of the inhibitor that could plausibly be coordinated by both His-98-Glu-70 pairs in each monomer and thus span across the SF mouth (supplemental Fig. S6b). Use of the TASK-3 minus TASK-3-E70K Δφ value calculated above predicts an RR (valence 6) ratio Kₛₚ TASK-3-E70K/Kₛ of 6 × 10⁴. This prediction matches well the lack of effect of RR up to 1 mM on TASK-3-E70K channels.

DISCUSSION

TASK-3 shares with congener K₂p channels TASK-1 and TWIK-1 a sensitivity to extracellular pH that is transduced through protonation and neutralization of histidine 98 located at P1. Although TWIK-1 pHₐ gating is complex, involving a profound change in selectivity only recently begun to be understood (33), more is known about TASK-1 gating. Gating of K₂p channels by extracellular H⁺ is known to occur at the selectivity filter by a process that has been thought to be, at least superficially, akin to C-type inactivation commonly encountered in other K⁺ channels (reviewed in Ref. 34). K⁺ dependence of TASK-1 pHₐ gating is in accord with this concept (11, 35). Yuill et al. (36) modeled TASK-1 based on the structure of the KcsA channel and first suggested that His-98 sensor was located behind the selectivity filter where its protonation might alter the shape of the SF producing a nonconducting state. The same authors show that site-directed mutagenesis affecting ion selectivity produced concomitant changes in pHₐ dependence, consistent with SF gating. Later MD studies provide the important observation that protonation of His-98 leads to a flipping upwards of the sensing His-98, which, in addition to producing a deformation of the SF moving away K⁺-coordinating backbone carbonyl groups from its lumen, would create "an electro-positive barrier to K⁺ ions at the outer mouth of the channel" (26). This proposal is interesting as it links pHₐ gating with its known K⁺ dependence. It necessitates, however, that the protonated His-98 side chain would somehow avoid the bulk solution bathing the extracellular aspect of the channel to exert this proposed electrostatic effect. Differences in pHₐ sensitivity between TASK-1 and TASK-3 have been attributed to the effect of residues in the large TM1-P1 extracellular loop on the sensing machinery. Indeed Clarke et al. (32) employed TM1-P1 loop exchange TASK-1/TASK-3 chimeras to show its importance in pHₐ dependence and Zn²⁺ blockade, concluding that the loop must lie close to the channel pore. A point not addressed in the literature cited above is that of the higher sensitivity of TASK-3 to external H⁺ that is expressed through the cooperative nature of the gating process.

The recent discovery of the structure of TWIK-1 and TWIK-related arachidonic acid-stimulated K⁺ channel (17, 18), two K₂p K⁺ channels, has revealed the presence of an extracellular helical cap that is likely to be a conserved feature among members of this family of transport proteins. The extracellular cap, made of the TM1-P1 linkers, impedes extracellular access to the SF on the main axis of conduction of the channel. Instead it defines what has been called side portals or the EIP that only allows bilateral access to the mouth of the selectivity filter through rather narrow tunnel-like structures. We show that the presence of these side entrances crucially determines the main gating process of TASK-3, namely the opening and closing of the pore in response to changes in extracellular pH. Our data
indicate that the concentration of K⁺ in the cavity must be a major determinant of pH sensitivity and the cooperativity exhibited by TASK-3 in its gating process. The side chains of pH-sensing histidine residues, one in each monomer of these homodimeric channels, line the side portals and constrain their diameter, bursting into the cavity when protonated. In channel structures lacking the extracellular cap characteristic of K₂p channels, we would expect the protonated His-98 imidazole charge to be screened out by the external solution (31). Instead its electrostatic effect should be heightened in the constrained space of the EIP, particularly after its narrowing by flipping upwards of the His-98 side chain. The negatively charged Glu-70 side chains, already known to be located close to the His-98 sensors (32), also form part of the side portal wall and influence the electrostatic potential in EIP, presumably becoming a major determinant of the EIP K⁺ concentration and affecting the occupancy of the outermost SF K⁺ binding sites.

Independent evidence for the importance of electrostatics of the EIP comes from experiments with TASK-3 blockers Zn²⁺ and RR. The inhibition by these, and other divalent cations, has been shown to depend on the presence of the Glu-70 residue (32, 37–39) and a neutral His-98 (32, 38). Here we show that inhibition of TASK-3 by Zn²⁺, just as pH₂, gating, is cooperative and strongly impeded by increasing extracellular K⁺ concentration. MD experiments suggest that two Zn²⁺ ions might plausibly bind at sites defined by neutral His-98 side chains flipped upwards into the EIP at both sides of the SF entrance. The presence of Glu-70, however, does not appear to be necessary as Zn²⁺ still inhibited TASK-3-E70K channels, albeit with a higher Kᵯ. The results suggest that the main effect of Glu-70 on Zn²⁺ inhibition is in electrostatically affecting its concentration in the EIP. A similar role for Glu-70 was surmised from the effect of divalent cations on TASK-3 single channel conductance (37). Our experiments comparing Zn²⁺ inhibition of TASK-3 and TASK-3-E70K allowed us an estimation of the electrostatic potential due to the charge replacement at a site of action of the divalent cation. Use of this value predicted a lack of effect of RR⁶⁺ on TASK-3-E70K that has been previously reported (39) and is corroborated here. In contrast to Zn²⁺ inhibition, RR blockade was not cooperative, in line with the binding of a single inhibitory molecule. Using the x-ray structure of RR and our model of TASK-3, we show the plausibility of such an interaction with RR spanning between the two Glu-70/His-98 sites. Czirja´k and Enyedi (39), although lacking structural information, had already suspected such an arrangement when proposing that RR acted by tethering the two subunits of TASK-3 through their Glu-70 side chains.

Our results suggest a molecular explanation for the cooperativity and [K⁺] dependence in the gating of TASK-3. We postulate that the vicinity of His-98 charged side chains to the outer part of the SF leads to a decrease in K⁺ occupancy because of an electrostatic effect of the charged imidazole rings. Neutralization of one of the His-98 sensors results in a decrease in electropositive potential accompanied by an increased occupancy of the outermost selectivity filter K⁺ binding sites. Increased K⁺ occupancy should in turn affect the readiness with which the imidazole of the remaining charged His-98 loses its H⁺, thus accounting for the observed increase in acidity of pKₐ observed experimentally for the neutralization of a second pH₀ sensor in TASK-3. Our interpretation provides a good explanation for the loss of cooperativity in TASK-3-E70K and its recovery at higher K⁺ concentration.

The presence of side portals with predominantly negative walls in TASK-3 is crucial in providing a restricted space for the gating process we postulate. The effect of local environment in the modulation of the pKₐ of key residues in both enzymes and ion channels is known to play a role in their catalysis/gating mechanisms (22, 40). The mechanism for cooperativity of channel gating proposed here, a perturbation in environment caused by the transported ion itself leading to a shift in pKₐ of a gating residue, is, to our knowledge, novel, as is the proposed role of the newly discovered extracellular cap structure of K₂p channels in the process. TASK-3 is fundamental to various cellular functions and has been implicated in disease. The structural and functional insights gained here might help in the design of molecules to interact specifically with the gating machinery of the channel and modulate its function.

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