Silent TWIK-1 Potassium Channels Conduct Monovalent Cation Currents

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ABSTRACT TWIK-1 two-pore domain K^+ channels generally produce nonmeasurable or very low levels of K^+ currents in heterologous expression systems under physiologically ionic conditions. Two controversial mechanisms have been proposed to account for this behavior: TWIK-1 K^+ channels are expressed in the cell surface but silenced by sumoylation at a lysine residue (TWIK-1 K274); constitutive and rapid internalization of TWIK-1 causes TWIK-1 channel silencing. Here we report that TWIK-1 K^+ channels heterologously expressed in Chinese hamster ovary cells, which are silent in physiological K^+ gradients, are able to conduct large monovalent cation currents when extracellular ionic conditions change. These results support the hypothesis that TWIK-1 K^+ channels are expressed in the cell surface but silent, and suggest that the TWIK-1 gating behavior rather than the lack of cell surface expression of TWIK-1 results in nondetectable TWIK-1 K^+ currents in heterologous expression systems.

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Mammalian two-pore domain K^+ channels, which are encoded by 15 *KCNK* genes, mediate leak K^+ conductance. They are regulated by physical and chemical stimuli and play important roles in a variety of physiological and pathophysiological functions (1).

TWIK-1, the first cloned mammalian two-pore domain K^+ channel, is highly expressed in the brain, kidney, and heart (2–4). Mice deficient for TWIK-1 exhibit impaired regulation of phosphate and water transports in kidney (5). Our previous studies indicate that TWIK-1 K^+ channels contribute to a large passive K^+ conductance in rat hippocampal astrocytes and account for an inward leak Na^+ current in human cardiac myocytes under hypokalemic conditions (6,7).

When heterologously expressed in mammalian cells or *Xenopus* oocytes, TWIK-1 K^+ channels generate unusually low levels of K^+ currents (2,8). Two mechanisms have been used to explain this feature of TWIK-1 K^+ channels:

The first hypothesis is that TWIK-1 K^+ channels are expressed in the cell surface but silent. Rajan et al. (8) have shown that TWIK-1 K^+ channels with a GFP-tag in the N-terminal reach the plasma membrane of *Xenopus* oocytes. However, the TWIK-1 K^+ channels in the cell surface have a very low open probability because they are silenced by sumoylation, a posttranslational modification of lysine residues by conjugation of a small ubiquitin modifier protein. Desumoylation in TWIK-1 K274 residue leads to the opening of TWIK-1 K^+ channels and such a regulation can occur reversely in inside-out patches of membrane excised from transfected Chinese hamster ovary (CHO) cells (8,9), suggesting that small ubiquitin modifier protein regulates gating behavior of TWIK-1 K^+ channels (10).

Recently, an alternative hypothesis has been proposed: constitutive and rapid retrieval or internalization of TWIK-1 from the cell surface causes the absence or lack of TWIK-1 K^+ channels in the plasma membrane so that no detectable TWIK-1 K^+ current can be measured. Mutation of a diisoleucine repeat located in the cytoplasmic C-terminus (TWIK-1•I293A•I294A) stabilizes TWIK-1 in the plasma membrane and results in robust TWIK-1 K^+ currents (11), suggesting that the diisoleucine-based motif controls the surface expression or retrieval of TWIK-1 K^+ channels.

Here we reexamined the hypothesis that TWIK-1 K^+ channels are expressed in the cell surface, even though in the absence of measurable TWIK-1 K^+ currents, by employing a different strategy. If this hypothesis is reasonable, it is possible to measure ionic currents through TWIK-1 channels when these silent TWIK-1 K^+ channels, which produce nondetectable K^+ currents in transfected CHO cells in physiological K^+ gradients, become functional in response to physical or chemical stimuli. Otherwise, the lack of cell surface expression of TWIK-1 should not produce any measurable TWIK-1 current under the same stimuli. Therefore, we tested whether silent TWIK-1 K^+ channels are able to functionally conduct monovalent cation currents. In this study, we demonstrated that silent TWIK-1 K^+ channels conduct large Na^+, K^+, Rb^+, and NH_4^+ currents in transfected CHO cells when extracellular ionic conditions change.

METHODS

CHO cells with at least 80% confluence were transfected by Lipofectamine 2000 (Invitrogen, Carlsbad, CA) with 3 μg of TWIK-1 plasmids and 1 μg of pEGFP plasmids and studied 24 h later. GFP expression was used to identify effectively transfected CHO cells. Whole-cell patch-clamp recordings

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were performed and data were analyzed, as described previously (7). Briefly, whole-cell currents of TWIK-1 K^+ channels in transfected CHO cells were recorded with a standard 2.2 s voltage ramp from −140 mV to +80 mV each 15 s. In Figs. 1 and 2, whole-cell currents at +80 mV in Na^+-based bath solutions with 5 mM $[K^+]_o$ are <250 pA. The pipette solution contained 140 mM KCl, 1 mM $MgCl_2$, 10 mM EGTA, 1 mM K_2-ATP, and 5 mM HEPES (pH7.4). The Na^+-based bath solution contained 135 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH7.4). The total concentration of Na^+ and K^+ in bath solutions is 140 mM so bath solutions with various $[K^+]_o$ were obtained by exchanges of equimolar K^+ and Na^+. The Rb^+ or $NH4^+$-based bath solutions were obtained by replacing extracellular Na^+ by equimolar monovalent cations.

RESULTS AND DISCUSSION

Silent TWIK-1 K^+ channels conduct large inward Na^+ and outward K^+ currents

We have previously shown that in the transfected CHO cells, by which large TWIK-1 K^+ currents are measured, TWIK-1 K^+ channels change ion selectivity and conduct inward leak Na^+ currents in lowered extracellular K^+ concentrations ($[K^+]_o$) (7). Thus, we investigated whether silent TWIK-1 K^+ channels heterologously expressed in CHO cells exhibit the same characteristic in lowered $[K^+]_o$, become permeable to extracellular Na^+, and conduct inward Na^+ currents. Consistent with previous reports (7,8,11), TWIK-1 K^+ channels do not produce measurable currents in most of the transfected CHO cells in Na^+-based bath solutions with physiological $[K^+]_o$. Whole-cell currents recorded at −140 mV and +80 mV are −95 ± 6 pA and 188 ± 19 pA ($n = 4$), respectively (*black line*, Fig. 1 *B*), within the range of background noises. However, removing 5 mM K^+ in the bath solution converted silent TWIK-1 K^+ channels into nonselective cation channels that conducted large inward Na^+ and outward K^+ currents with a reversal potential of −15.6 ± 1.5 mV ($n = 4$) (*red line*, Fig. 1 *B*) and a Na^+ to K^+ relative permeability of ~0.53. It took ~10 min for TWIK-1 channels to complete the selectivity switch process. Moreover, whole-cell currents recorded in −140 mV (−843 ± 191 pA) and +80 mV (637 ± 119 pA) were significantly increased by 8.8-fold and 3.3-fold, respectively. These results are consistent with previous observations in those transfected CHO cells that large TWIK-1 K^+ currents are detected (7). In contrast, these results were not seen in CHO cells transfected with GFP alone ($n = 20$; Fig. 1 *A*). Such an amount of inward Na^+ and outward K^+ currents in 0 mM $[K^+]_o$ indicates that TWIK-1 K^+ channels are expressed on the surface of transfected CHO cells. This finding supports the hypothesis that TWIK-1 K^+ channels reach the cell surface but are not able to conduct measurable K^+ currents in physiological K^+ gradients.

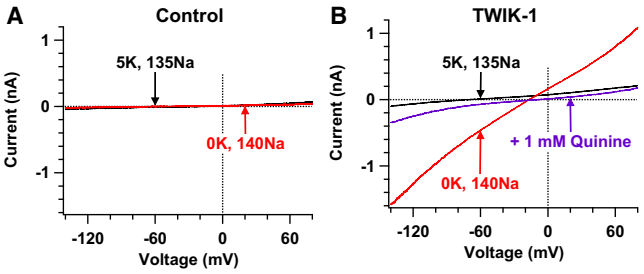

FIGURE 1 Silent TWIK-1 K^+ channels conduct Na^+ and K^+ currents in lowered $[K^+]_o$. Whole-cell currents are shown in CHO cells transfected with GFP plasmids alone (*A*) or with both TWIK-1 and GFP plasmids (*B*) before (*black lines*) and after (*red lines*) removing 5 mM extracellular K^+. At the end of the experiments, quinine blockade confirmed TWIK-1 currents (*purple line*) in panel *B*.

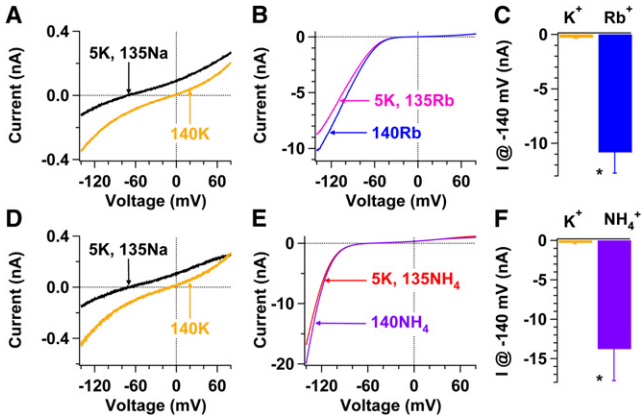

FIGURE 2 Silent TWIK-1 K^+ channels conduct Rb^+ or NH_4^+ currents. (*A* and *B*) Whole-cell currents of TWIK-1 channels were sequentially recorded from the same transfected CHO cell in bath solutions containing 5 mM K^+ and 135 mM Na^+ (*black line*), 140 mM K^+ (*orange line*), 5 mM K^+ and 135 mM Rb^+ (*pink line*), and 140 mM Rb^+ (*blue line*). (*C*) Summary of currents measured at −140 mV in panels *A* and *B* in bath solutions containing 140 mM K^+ (*orange bar*) or 140 mM Rb^+ (*blue bar*) (−311 ± 53 pA vs. −10,843 ± 1850 pA, $n = 4$). (*D* and *E*) Experiments similar to those in panels *A* and *B* were performed except that NH_4^+ replaced Rb^+. (*F*) Summary of currents measured at −140 mV in panels *D* and *E* in bath solutions containing 140 mM K^+ (*orange bar*) or 140 mM NH_4^+ (*purple bar*) (−323 ± 88 pA vs. −13,761 ± 4046 pA, $n = 4$). (*$P < 0.001$).

A potential challenge in interpreting enhanced TWIK-1 currents (*red line*, Fig. 1 *B*), which were induced by removing 5 mM extracellular K^+, is that externalization or insertion of TWIK-1 K^+ channels into the plasma membrane could also result in increases of TWIK-1 currents. However, two lines of evidence argue against this possibility: First, externalization of TWIK-1 K^+ channels should only increase the number of TWIK-1 K^+ channels in the cell surface and the amplitude of TWIK-1 K^+ currents; it should not result in dynamic changes in ion selectivity of TWIK-1 K^+ channels. Thus, externalization of TWIK-1 fails to explain altered ion selectivity of TWIK-1 K^+ channels in lowered $[K^+]_o$. Instead, increases in detectable TWIK-1 currents may reflect the changes in single channel properties of TWIK-1 channels such as open probability and unitary current. Second, our previous report has shown that the K^+ selectivity of TWIK-1

channels could be restored ~77 min later, after $[K^+]_o$ was changed back from 0 mM to 5 mM (7). Because CHO cells are dialyzed by electrode solutions containing 10 mM EGTA for such a long time, most endogenous enzymes should be inactivated and biochemical processes should be terminated. It is unlikely that trafficking (externalization or internalization) of TWIK-1 K^+ channels could occur after >1 h of establishments of whole-cell configuration.

Silent TWIK-1 K^+ channels conduct extremely large inward Rb^+ and NH_4^+ currents

We have previously shown that in the transfected CHO cells, in which large TWIK-1 K^+ currents are detected, TWIK-1 K^+ channels are permeable to Rb^+ and NH_4^+ in both normal and lowered $[K^+]_o$ (7). Then we studied whether silent TWIK-1 K^+ channels heterologously expressed in CHO cells are permeable to Rb^+ and NH_4^+. In Na^+-based bath solutions with 5 mM $[K^+]_o$, TWIK-1 K^+ channels are basically silent, as whole-cell currents recorded at +80 mV are only 199 ± 52 pA ($n = 4$) (*black line*, Fig. 2 *A*) and 177 ± 38 pA ($n = 4$) (*black line*, Fig. 2 *D*), respectively. However, these silent TWIK-1 K^+ channels exhibit extremely large Rb^+ or NH_4^+ conductance in Rb^+ or NH_4^+-based solutions, and have a reversal potential of −11.7 ± 2.5 mV or −56.0 ± 1.0 mV in 5 mM $[K^+]_o$ (*pink line*, Fig. 2 *B*; *red line*, Fig. 2 *E*), respectively, in agreement with a previous report (7). Although the relative permeability of Rb^+ or NH_4^+ to K^+ of TWIK-1 K^+ channels is ~0.60 or ~0.1, respectively, at −140 mV these silent TWIK-1 K^+ channels conducted significantly 35-fold larger inward Rb^+ currents (*blue bar*, Fig. 2 *C*) or 40-fold larger inward NH_4^+ currents (*purple bar*, Fig. 2 *F*) in the bath solution containing 140 mM Rb^+ or NH_4^+ than inward K^+ currents (*orange bars*, Fig. 2, *C* and *F*) in the bath solution containing in 140 mM K^+. In contrast, these results are not observed in CHO cells transfected with GFP alone ($n = 15$; data not shown). Such large amounts of inward Rb^+ and NH_4^+ currents demonstrate that TWIK-1 K^+ channels are expressed on the surface of transfected CHO cells. This finding not only supports the hypothesis that silent TWIK-1 K^+ channels reside in the plasma membrane but also suggests that these silent TWIK-1 K^+ channels can be activated by extracellular Rb^+ and NH_4^+.

Because these results were obtained with rapid changes of bath solutions and the observed effects can be reversibly repeated in Na^+-based and Rb_4^+ (or NH_4^+)-based solutions with 5 mM K^+, externalization or internalization of TWIK-1 K^+ channels should not contribute to such a large amount of inward Rb^+ or NH_4^+ currents. Another potential challenge in interpreting large inward Rb^+ or NH_4^+ currents is due to the change in the TWIK-1 channel conductance. However, the change in the TWIK-1 channel conductance alone is unlikely to enhance inward currents by ~40-fold in response to extracellular ionic changes from 140 mM K^+ to 140 mM Rb^+ or NH_4^+. Also, the change in the TWIK-1 channel conductance alone should result in similar increases in both inward and outward currents, but the switches of K^+-based bath solutions to Rb^+ (or NH_4^+)-based bath solutions enhanced outward K^+ currents only by approximately three- or nine-fold (Fig. 2), inconsistent with increases in inward currents. It is possible that the changes in both open probability and conductance of TWIK-1 channels contribute to large inward Rb^+ or NH_4^+ currents.

We provided two pieces of evidence indicating that silent TWIK-1 K^+ channels are able to conduct large monovalent cation currents in transfected CHO cells when extracellular ionic conditions change. These results support the hypothesis that TWIK-1 K^+ channels are expressed in the cell surface but silent under physiological K^+ gradients. Our findings also suggest that the TWIK-1 gating behavior rather than the lack of cell surface expression of TWIK-1 results in nonmeasurable TWIK-1 K^+ currents in the heterologous expression systems.

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