TWIK1, a unique background channel with variable ion selectivity

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TWIK1 belongs to the family of background K^+ channels with two pore domains. In native and transfected cells, TWIK1 is detected mainly in recycling endosomes. In principal cells in the kidney, TWIK1 gene inactivation leads to the loss of a nonselective cationic conductance, an unexpected effect that was attributed to adaptive regulation of other channels. Here, we show that TWIK1 ion selectivity is modulated by extracellular pH. Although TWIK1 is K^+ selective at neutral pH, it becomes permeable to Na^+ at the acidic pH found in endosomes. Selectivity recovery is slow after restoration of a neutral pH. Such hysteresis makes plausible a role of TWIK1 as a background channel in which selectivity and resulting inhibitory or excitatory influences on cell excitability rely on its recycling rate between internal acidic stores and the plasma membrane. TWIK1$^{-/-}$ pancreatic β cells are more polarized than control cells, confirming a depolarizing role of TWIK1 in kidney and pancreatic cells.

Two-pore-domain potassium (K_{2P}) channels constitute a unique class of background channels comprising 15 members in humans. They share the same overall architecture with four membrane-spanning segments (M1–M4), two pore domains (P1 and P2), and a large extracellular M1P1 loop. Active as dimers, they produce almost time- and voltage-independent currents that oppose membrane depolarization and cell excitability. K_{2P} channels have been involved in physiological functions as diverse as cell-volume regulation, apoptosis, adrenal gland development and primary hyperaldosteronism, vasodilatation, neuronal excitability and altered motor performance, central O_2 chemoreception and breathing control, perception of pain, polyunsaturated fatty acid-mediated neuroprotection, and mood control. They constitute a major target of volatile anesthetics (for recent and extensive reviews on K_{2P} channels see refs. 1–4).

Among K_{2P} channels, TWIK1 (also termed "K2P1" or "KCNK1") is atypical. Originally cloned from a kidney cDNA library, TWIK1 is expressed at significant levels in other tissues including heart, brain, pancreas, lung, and placenta (5–7). Upon heterologous expression, it forms homodimers (8) that produce only modest currents in *Xenopus* oocytes and almost no currents in cultured mammalian cells. Another hallmark is the fast inactivation of the TWIK1 currents. Because of this inactivation, their steady-state current–voltage (I–V) relationships display inward rectification, unlike the other K_{2P} currents that produce outwardly rectifying I–V relationships in physiological K^+ conditions. No native currents with similar properties have yet been reported despite the broad distribution of TWIK1. Another puzzling observation is provided by gene inactivation of TWIK1 in the mouse: Rather than a decrease of K^+ conductance in TWIK1$^{-/-}$ kidney cells, a decrease of a nonselective cationic conductance was observed that was associated with an increase in the resting membrane potential (9). More recently, it has been suggested that TWIK1 is responsible for paradoxical depolarization of human heart during pathological hypokalemia (10).

Based on experiments from transfected cells, it has been proposed that TWIK1 is a channel present at the plasma membrane that is inhibited by sumoylation of a lysine residue (K274). Mutation of this site (K274E) was shown to produce more active channels (11, 12). However, our own results ruled out sumoylation as the major mechanism of TWIK1 silencing (13, 14). In transfected cells and also in native kidney cells, we found that TWIK1 is present mainly in recycling endosomes (RE), an intracellular compartment also called the "subapical recycling compartment," in polarized epithelial cells (14, 15). We have shown that TWIK1 is actively and constitutively internalized from the cell surface and then is addressed to RE. Mutation of a di-isoleucine repeat (I293A,I294A) in the cytoplasmic C-terminal of TWIK1 prevents this endocytosis, resulting in a stably expressed TWIK1 channel at the plasma membrane and in measurable TWIK1 currents (14). However, despite strong expression of the mutated TWIK1 protein at the plasma membrane, the currents were much smaller than the currents produced in the same conditions by the other K_{2P} channels. In the absence of specific TWIK1 blockers it was not possible to rule out an eventual effect of TWIK1 overexpression on endogenous conductances.

Here we used structural modeling and directed mutagenesis to gather data on TWIK1. By comparing sequences of TWIK1 with other K_{2P} channels, we were able to design TWIK1 pore mutants that produce very robust currents. Thanks to these mutants, we confirmed our original electrophysiological description of TWIK1 in oocytes and in transfected cells expressing TWIK1I293,I294A. We also confirmed the importance of recycling between endocytic compartments and the plasma membrane for expression of TWIK1 current. These mutations not only increased TWIK1 current but also, in some cases, converted the weakly inwardly rectifying TWIK1 into a robust and classical background K_{2P} channel. We also found that TWIK1 selectivity is altered by acidification and identified a series of residues that are necessary for this property. At acidic pHs typical of secretory and endocytic/recycling pathways, TWIK1 conducts Na^+. In RE, TWIK1 behaves as a nonselective cationic channel. Moreover, transitions from the Na^+-conductive state to the K^+-selective state are very slow, suggesting that TWIK1 delivered to the cell surface will remain nonselective for several minutes at the plasma membrane. This phenomenon could explain the loss of cationic conductance and the hyperpolarization of the resting membrane potential recorded in TWIK1$^{-/-}$ kidney cells. We show here that TWIK1$^{-/-}$ pancreatic β cells also are hyperpolarized compared with WT cells, particularly when ATP-sensitive K^+ channels are turned off by the presence of glucose in the medium.

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Results

Residues in the Pore Sequence Limit Expression of TWIK1 Current. In transfected cells, TWIK1 is internalized from the plasma membrane rapidly and constitutively (14). We have shown that the mutation of a di-isoleucine motif (I293A,I294A) in the cytoplasmic C terminus of TWIK1 stabilizes TWIK1 expression at the cell surface and leads to a simultaneous increase of measurable K^+ current in mammalian cells. This increase also is observed in *Xenopus* oocytes expressing TWIK1I293A,I294A (see Fig. 2*A*). However, the current amplitude is weak compared with that of other K_{2P} channels such as the TREK1 or KCNK0 channels (16, 17). In KCNK0, three glycine residues located in M2 have been shown to be very important for channel activity (Fig. 1*B*) (18). These glycines are conserved in TREK1 but not in TWIK1, where they are replaced by leucine (L146), alanine (A151), and valine (V153). In KCNK0, substitution of these glycines by larger hydrophobic residues decreased the probability of an open channel. On the other hand, replacement of the first glycine by an aspartate produced a constitutively open channel (18). When introduced in TWIK1I293,294A, this additional mutation (L146D) leads to a 16-fold increase in the current (10.5 ± 1.5 μA vs. 0.66 ± 0.04 μA at 0 mV; Fig. 2*B*). This massive increase is not related to a change in protein-expression levels (Fig. 2*C*). We took advantage of the L146D mutation to reexamine previous results and tested this mutation together with other mutations affecting TWIK1 trafficking (I293A,I294A) and TWIK1 activity (K274E) (12). The fourfold difference between TWIK1L146D and TWIK1I293A,I294A,L146D confirms the effect of retrieving TWIK1 from the cell surface on the TWIK1 current density (8.0 ± 0.5 μA vs. 1.8 ± 0.2 μA at 0 mV; Fig. 2*D*). The effect of the K274E mutation, which has been attributed to a lack of sumoylation (12) or to a charge effect (14), also is confirmed by a threefold increase in TWIK1L146D vs. TWIK1L146D,K274E (5.5 ± 0.6 μA vs. 1.8 ± 0.2 μA; Fig. 2*D*). As expected, introducing the glycine residues conserved in TREK1 and KCNK0 M2 segments into TWIK1 produces also an increase of TWIK1 currents (Fig. S1). The backbone channel in which these mutations were introduced is the TWIK1I293A, I294A,K274E mutant, noted herein as "TWIK1surf," "surf" indicating that this mutant is present and active at the cell surface (Fig. S1). As in TWIK1, the I–V relationship of TWIK1surf exhibits a weak inward rectification (6). For TWIK1surf3G, in which three glycines (L146G, A151G, and V153G) have been introduced in the M2 segment, this inward rectification is lost. The TWIK1surf3G I–V relationship is very similar to that of the classical K_{2P} channels TREK1 and KCNK0: In physiological K^+ conditions, the inward rectification is replaced by an outward rectification (Fig. S1). Taken together, these results demonstrate that our initial description of TWIK1 electrophysiological properties in *Xenopus* oocytes was accurate. TWIK1 behaves as a low-activity channel because of its constitutive endocytosis and because of pore specificities that hinder its functionality at the cell surface.

pH-Dependent Ion Selectivity of TWIK1. Using TWIK1surf, which gives good levels of current expression, we next revisited the pH sensitivity of TWIK1. A previous report (12) showed that TWIK1 is inhibited by extracellular acidification with a pK_a of 6.7. TWIK1 channels carrying an H122N mutation were insensitive to acidification, prompting the authors of that study to conclude that TWIK1 behaves like the TWIK1-related acid-sensitive K^+ channels TASK1 and TASK3. A histidine residue at equivalent positions in TWIK1 and TASK channels was considered to be the proton-sensing element (Fig. 1*B*). Here we show that TWIK1 and TASK1/3 behaviors regarding extracellular pH variations are completely different. Upon progressive acidification, TWIK1 first is activated and then is inhibited below pH 6.5 (Fig. 3). Histidine 122 is required for both activatory and inhibitory effects, because TWIK1surfH122N is insensitive to acidification. However, the current density of TWIK1surfH122N is much smaller than that of TWIK1surf, suggesting that TWIK1 is activated, rather than inhibited, when the lateral side of H122 is protonated upon acidification (Fig. 3 *A* and *B*). This activation is confirmed by the pH sensitivity of an H122K mutant mimicking a channel with protonated histidine 122. At pH 7.5, TWIK1surfH122K produces larger currents than TWIK1 and is not stimulated by a pH shift from 7.5 to 6.5. However, TWIK1surfH122K retains sensitivity to further acidification, with only 50% of the initial current remaining at pH 6 (Fig. 3 *A* and *B*). How can this successive activation and inhibition of TWIK1 be explained? In fact, the I–V rela-

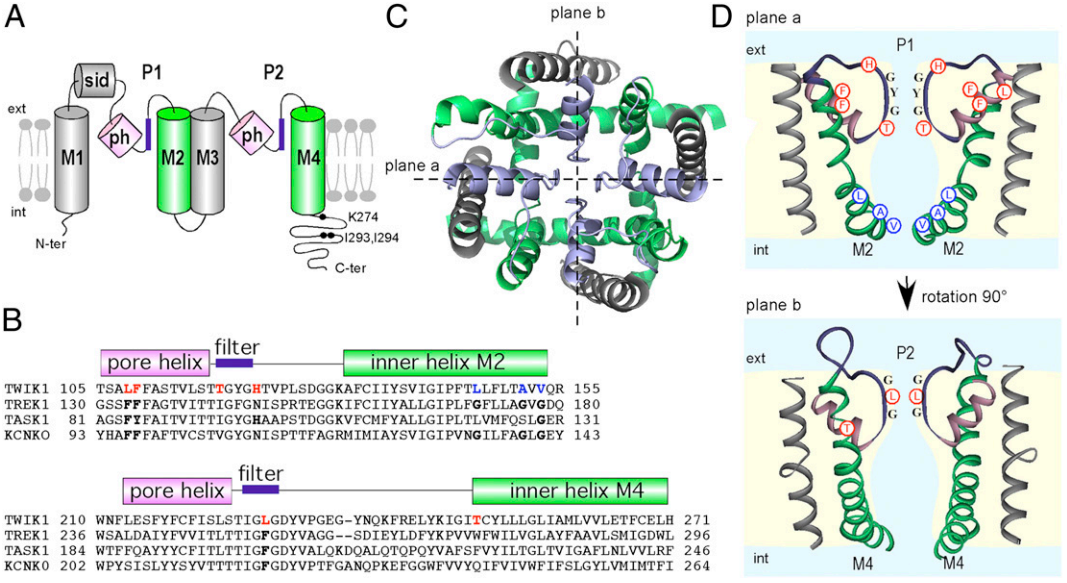

Fig. 1. TWIK1 sequence and topological organization. (*A*) Functional domains in a TWIK1 subunit: transmembrane segments (M1–M4), coiled-coiled self-interacting domain (SID), pore domains (P1 and P2), pore helices (ph), and selectivity filter sequences (purple bold line) are indicated. (*B*) Sequence alignment in which TWIK1 residues mutated in this article. Residues affecting ion selectivity are in red; residues affecting channel activity are in blue. (*C* and *D*) Structural modeling of a TWIK1 dimer. Cytoplasmic and extracellular domains are not depicted. (*C*) Top view of the TWIK1 extracellular side. (*D*) Side views of the TWIK1 membrane domain; out-of-plane loops and helices are not depicted. Mutated residues shown in *B* are depicted.

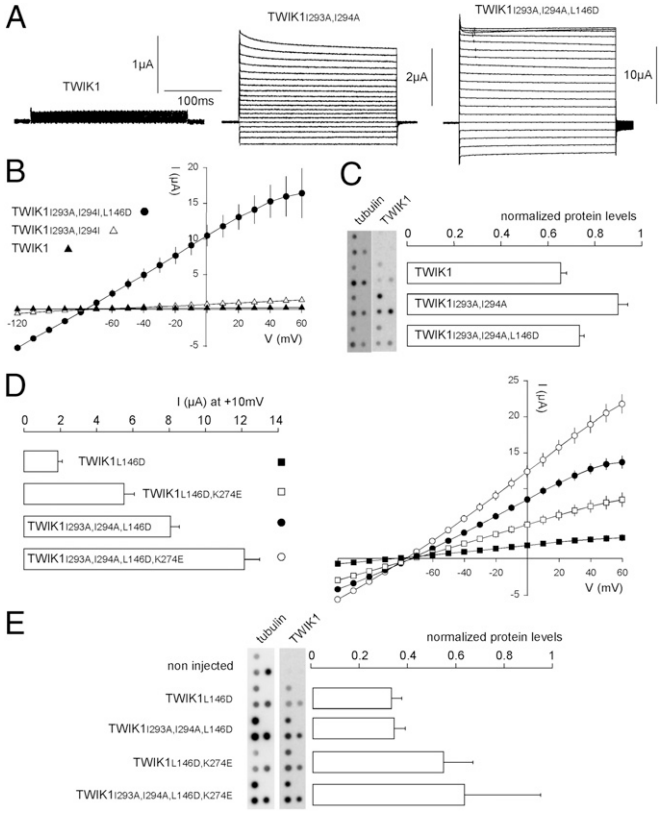

Fig. 2. Pore mutations stimulate TWIK1-channel activity. (*A*) Currents evoked during voltage pulses ranging from −120 mV to +60 mV in 10-mV steps from a holding potential of −80 mV. Recordings were obtained from *Xenopus* oocytes 3 d after cRNA injection for TWIK1 and TWIK1I293A,I294A and 1 d after cRNA injection for TWIK1I293A,I294A,L146D. (*B*) I–V relationships deduced from currents after 1 d of expression. Each value is the mean ± SEM; n = 8 cells for TWIK1; n = 7 cells forTWIK1I293A,I294A; n = 7cells for TWIK1I293A,I294A,L146D. (*C*) After recording, oocytes in *B* were solubilized, and lysates were spotted in triplicate on membrane before detection by anti-TWIK1 antibodies. (*D*) I–V relationships deduced from currents after 1 d of expression. Each value is the mean ± SEM; n = 7 cells for TWIK1L146D; n = 8 cells for TWIK1L146D,K274E; n = 10 cells for TWIK1I293A,I294AL146D; n = 13 cells for TWIK1I293A,I294A,L146D,K274E. (*E*) The same experiment as in *C* but using oocytes recorded in *D*.

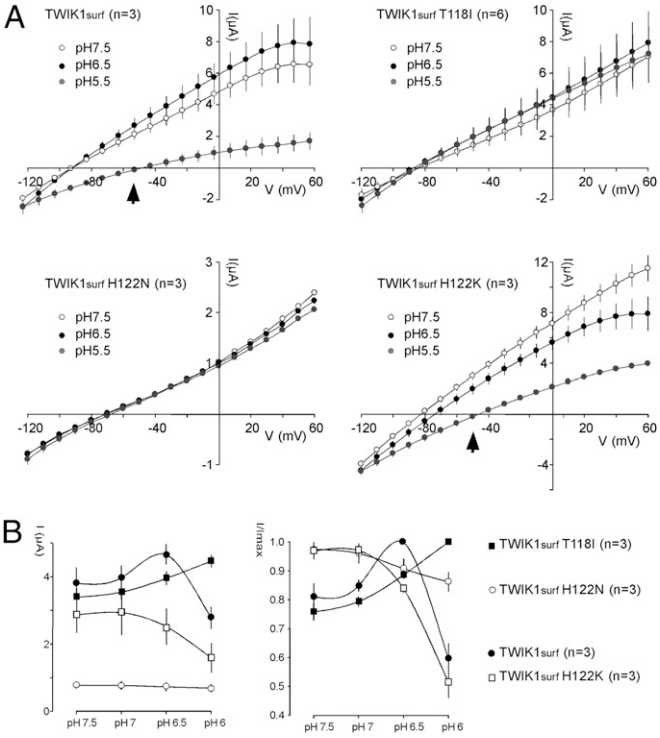

Fig. 3. TWIK1 sensitivity to pH variations. (*A*) Effect of progressive extracellular acidification. For I–V relationships, each value is the mean ± SEM (number of cells is indicated for each condition). The shift of the E_{rev} at pH 5.5 is indicated by an arrow. (*B*) Current variations at 0 mV. Each value is the mean ± SEM (number of cells is indicated for each condition).

tionships of TWIK1surf and TWIK1surfH122K currents display a marked shift of their reversal potential (E_{rev}) at pH 5.5 (−82.0 ± 1.7 mV vs. −48 ± 4 mV for TWIK1 and −88.3 ± 1.5 mV vs. −42.3 ± 8.2 mV for TWIK1surfH122K), indicating that upon acidification TWIK1 is less selective to K^+ (Fig. 3*A*). TWIK1 channels first are activated by acidification but, becoming less selective, they conduct inward Na^+ currents opposing outward K^+ currents during depolarization. This mechanism would explain the apparent inhibition of outward TWIK1 currents at pH 6 (Fig. 3*B*). A TWIK1surfTI118I mutant with a mutation previously reported to confer K^+ selectivity to TWIK1 in hypokalemic conditions (10) is activated only by progressive acidification without inhibition below pH 6.5 (Fig. 3*B*), also supporting the hypothesis that TWIK1 K^+ selectivity is lost in acidic conditions (Fig. 3*A*). The more active pore mutant TWIK1surf3G also shows a shift in E_{rev} at pH 6 (Fig. S1). Changing the extracellular pH from 7.4 to 6 shifted the E_{rev} of TWIK1surf3G from −94 ± 1 mV to −55 ± 2 mV (n = 2). There is no shift in channel-impermeable *N*-methyl-D-glucamine ($NMDG^+$)-based solutions, confirming that TWIK1surf3G is permeable to Na^+ at pH 6. At pH 7.4, removal of external Na^+ resulted in a decrease of the TWIK1surf3G current (Fig. S1). This finding can be explained by a stimulating effect of external Na^+, as previously shown for TREK1 (16).

Fig. 4 illustrates the change in TWIK1 ion selectivity at acidic pH. TWIK1surf currents were recorded in different external K^+ conditions (Fig. 4*A*), and the E_{rev} values were plotted against these concentrations (Fig. 4*B*). At pH 7.4, the relationships for TWIK1surf and TWIK1surfT118I are linear. The TWIK1surfT118I relationship is not affected by a shift to pH 6, but the TWIK1surf relationship is strongly modified. The E_{rev} are systematically more depolarized than expected. The effect of decreasing K^+ concentration is potentiated by acidification (Fig. 4*B*). At physiological K^+ concentration (5 mM K^+), the E_{rev} is more depolarized at pH 6 than at 7.4: −47.3 ± 2.8 mV vs. −62.8 ± 1.3 mV. The difference is even stronger at 2 mM K^+. Also, high external K^+ does not prevent the switch in ion selectivity induced by external acidification. At pH 6 and 50 mM K^+, the E_{rev} for TWIK1surf is more depolarized than the E_{rev} for the K^+-selective mutant TWIK1surfT118I (−16.1 ± 1.6 vs. −19.8 ± 1.0; Fig. 4*B*, *Right*). The relative Na^+ to K^+ permeability (P_{Na}/P_K) values were calculated with the Goldman–Hodgkin–Katz (GHK) equation using the E_{rev} values measured in Fig. 4*A*. Upon acidification from 7.4 to 6 pH, the P_{Na}/P_K of TWIK1surf shifts from 0.030 ± 0.008 to 0.19 ± 0.03. The P_{Na}/P_K of TWIK1surfT118I is not affected significantly by acidification (0.012 ± 0.004 vs. 0.020 ± 0.003). We also measured relative Na^+ to K^+ permeabilities of other TWIK1 mutants at pH 7.4 and 6 (Fig. 4*C*). Mutations are localized in the pore helices (L108 and F109), in the selectivity filter (L228), or in the N-terminal part of the M4 segment in a position (T250) that is important for channel gating (Fig. 1*C*) (19). Even if residues L108 and L228 are unique to TWIK1 and are not found in TREK, TASK, or KCNK0 channels, their mutation does not rescue K^+ selectivity at pH 6. When the L108F109 motif of TWIK1 is replaced by the FY motif found in TASK1, the corresponding TWIK1surfL108F,F109Y mutant shows an altered K^+ selectivity even at physiological pH (0.59 ± 0.09 at pH 7.4

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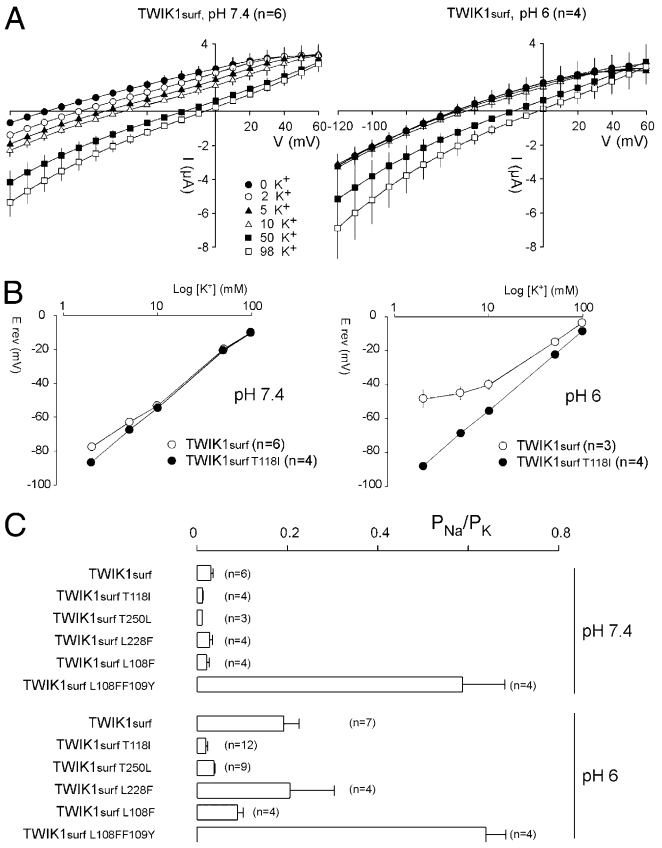

Fig. 4. Ion selectivity of TWIK1 is altered upon acidification. (*A*) I–V relationships obtained in different external K^+ conditions at pH 7.4 and 6. Na^+ is replaced by K^+. Each value is the mean ± SEM (n = 6 cells). (*B*) E_{rev} as a function of the extracellular K^+ concentration at pH 7.4 and 6. Each value is the mean ± SEM (n = 4 cells). (*C*) Relative P_{Na}/P_K of different TWIK1 mutants at pH 7.4 and 6. Each value is the mean ± SEM (n is indicated for each condition).

and 0.64 ± 0.04 at pH 6). Finally, TWIK1 mutated at position 250 (TWIK1surfT250L) shows only a moderate shift of its P_{Na}/P_K upon acidification, from 0.011 ± 0.001 at pH 7.4 to 0.037 ± 0.003 at pH 6. These results show that the modification of selectivity caused by acidification is highly dependent on the pore conformation of TWIK1.

Another element that links an alteration in ion selectivity to a pore conformational rearrangement is the kinetics of this modification (Fig. 5). Shifting the pH of the bath solution from 7.4 to 6 induced a two-phase effect on TWIK1surf. Within the first minute, there was a current increase (Fig. 5*A*) with a moderate shift of the E_{rev} in the hyperpolarizing direction, indicating that TWIK1surf channels maintained their K^+ selectivity. Then currents decreased, and the E_{rev} began to depolarize from −90 mV to reach −73 mV after 24 min, indicating that the channels altered their relative P_{Na}/P_K during this period (Fig. 5*A*). The time constant of the depolarizing phase is 266 ± 74 s (n = 4). This slowness reflects the process whereby the selectivity filter shifts from a K^+-selective to an Na^+-permeable conformation. The recovery then was evaluated by switching the pH of the bath solution back to 7.4 (Fig. 5*B*). The recovery is very slow, with a time constant of 366 ± 23 s (n = 4). Even after 25 min, the recovery is not complete (E_{rev} at −84 mV), suggesting that TWIK1 channels are blocked temporarily in a nonselective conformation.

Depolarizing Influence of TWIK1 in Pancreatic β Cells. The hysteresis of TWIK selectivity change upon pH shifts may explain why TWIK1 gene inactivation is associated with hyperpolarization

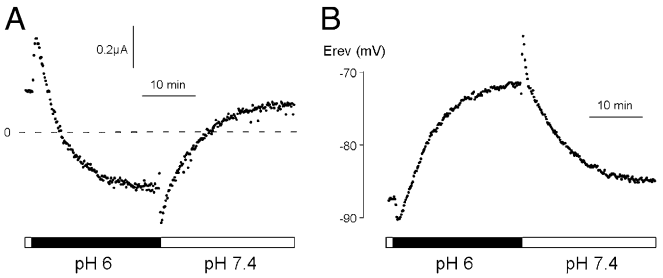

Fig. 5. Kinetics of the ion selectivity change. (*A*) Effect of changing extracellular pH on TWIK1 currents recorded at −80 mV at 10-s intervals. (*B*) Effect of changing extracellular pH on TWIK1 current E_{rev} recorded at 10-s intervals.

rather than depolarization in kidney principal cells (9). If TWIK1 is recycled actively between acidic endosomes and the plasma membrane, it may behave as a nonselective cationic channel in these cells and have a depolarizing influence. If this hypothesis is true, the depolarizing effect of TWIK1 also should be observed in other cell types. As shown in Fig. 6*A*, TWIK1 is coexpressed with insulin in native β cells from pancreatic islets. These cells were primary-cultured cells, and their resting membrane potential was measured by electrophysiology in the cell-attached perforated patch mode using nystatin (Fig. 6*B*). In basal conditions, the resting potential of $TWIK1^{-/-}$ cells tends to be lower than the potential of control (WT) cells (−79.7 ± 1.6 mV vs. −73.6 ± 2.8 mV); however the difference is not statistically significant (P = 0.07). ATP-sensitive K^+ channels (K_{ATP}) impose this very negative potential (20). When blood glucose increases, ATP rises inside β cells, promoting closure of K_{ATP} channels and depolarization of the membrane potential. This depolarization triggers calcium entry and insulin release. As expected, bathing β cells with a solution containing glucose leads to depolarization of their membrane potential. In these conditions, the difference between WT and $TWIK1^{-/-}$ cells becomes significant. In 20 mM glucose, $TWIK1^{-/-}$ cells have a resting potential that is 15 mV less depolarized than that of WT cells (−58.3 ± 3.1 mV vs. −43 ± 3.2 mV; n = 6 per group; Fig. 6*B*). These results support a depolarizing influence of TWIK1 channels in pancreatic β cells, as previously reported in kidney principal cells (9).

Discussion

Since its cloning in 1996, TWIK1 has been a matter of polemical discussions between laboratories that reported currents upon heterologous expression in *Xenopus* oocytes and those that failed to reproduce this result (6, 12, 21). In mammalian cells, TWIK1 expression has proven to be even more difficult, with no expression of current or with modest currents recorded in only in small subset of transfected cells (14, 22). This lack of expression and the absence of recordings of endogenous TWIK1 currents in native cells considerably slowed the characterization of TWIK1 functional properties and of its physiological roles. In 2005, it was proposed that TWIK1 is a plasma membrane channel silenced by the addition of a SUMO peptide to lysine 274. Inactivation of the sumoylation site by mutating lysine 274 (K274E) was associated with a strong increase in TWIK1 current (12). This report gained considerable attention, because sumoylation had not been known to act at the plasma membrane. However, this finding also implied that all TWIK1 channels were sumoylated in many different types of native and transfected cells that had been tested and in which TWIK1 had not produced currents, raising questions about the physiological relevance of this "universal" silencing and about its eventual modes of regulation. We have shown that the regulation of TWIK1 is a much more complex process. TWIK1 was detected mainly in RE in both transfected and native cells (15). Mutating a di-isoleucine motif (I293A,I294A) in the cytoplasmic C terminus of TWIK1 prevented its endocytosis and promoted currents at the plasma membrane (14). This result ruled out sumoylation as the principal mechanism of TWIK1 silencing. However, channel activity was not very robust,

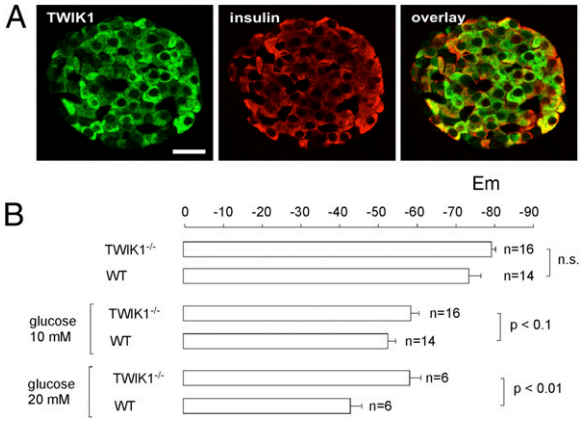

Fig. 6. Depolarizing influence of TWIK1 in pancreatic β cells. (*A*) In pancreatic islets, TWIK1 is immunodetected in insulin-expressing B cells. Cells that do not express TWIK1 are glucagon- or somatostatin-positive cells. (Scale bar, 5 μM.) (*B*) Membrane potential (Em) of β cells primary-cultured from TWIK1$^{-/-}$ or control (WT) mice in various external glucose concentrations.

despite the presence of TWIK1I193A,I194A at the cell surface. Here we show that this hindered channel activity is intrinsic to TWIK1. Mutations known to increase open probability of KCNK0 (L146D or L146G,A151G,V153G) (18) led to strong currents when introduced in the transmembrane segment M2 of TWIK1 (Fig. 2). Combining L146D with other mutations confirmed the importance of TWIK1 recycling as well as the stimulating effect of the K274E substitution. The effects of the mutations in M2 and in the cytoplasmic C terminus of TWIK1 were additive, indicating that they probably act at different levels. The L146G,A151G,V153G triple mutation not only produced more currents but also changed the channel kinetics. The I–V relationship of the corresponding mutant became similar to the I–V relationship of classical K_{2P} channels (e.g., the TASK, TREK, or KCNK0 channels) (Fig. S1). Taken together, these results confirm our original descriptions of the TWIK1 currents in both *Xenopus* oocytes and mammalian cells and demonstrate that the lack of TWIK1 current relies on a combination of low channel activity and low expression level at the plasma membrane.

Here we describe another property of TWIK1: the change in its ion selectivity upon acidification. This unique property is related to residues that are not conserved in other K_{2P} channels. When the pH value of the bathing solution was acidified progressively, TWIK1 currents first increased and then diminished after pH 6.5. This second phase was associated with a shift in the E_{rev} not observed when extracellular Na^+ was replaced by $NMDG^+$. This result suggested that TWIK1 becomes permeable to Na^+ upon acidification, resulting in a shift of the current E_{rev} toward more depolarized values and in less outward currents, with inward Na^+ currents opposing outward K^+ current. This suggestion was confirmed by measuring a significant shift of the relative P_{Na}/P_K of TWIK1 between pH 7.4 and 6 (Fig. 4). Histidine 122 acts as a proton sensor and is necessary for both the initial activation phase and the following phase of ion selectivity change that finally results in apparent current inhibition. H122K substitution mimicking protonation of the H122 side chain induced a suppression of the activation phase but did not alter the pH-sensitive shift of ion selectivity and the inhibition phase, indicating that acidification acts on additional sites to promote this change (Fig. 3). In the pore regions that are crucial for K^+ selectivity, TWIK1 has residues that are not conserved in other K_{2P} channels. TWIK1 has unusual sequences in the P1 and P2 domains. P1 contains a TGYG motif instead of IGY/FG, and P2 contains IGLG instead of IGFG (Fig. 1). Replacing IGLG by IGFG (mutant L228F) does not change TWIK1 sensitivity to acidification. However, replacing TGYG by IGYG (mutant T118I) improves K^+ selectivity at both neutral and acidic pH. Another unique motif in TWIK1 is LFF in the pore helix of its first P1 domain. In the other K_{2P} channels this motif is FFF (TREK1, KCNK0) or FYF (TASK1). This motif is important for pore conformation and ion selectivity of other K^+ channels (23). TWIK1 with FFF (TWIK1L108F) retains its sensitivity to acidification, albeit with a P_{Na}/P_K shift that is less important than for native TWIK1 (Fig. 4). TWIK1 with FYF (TWIK1L108F, F109Y) is much more affected, with altered ion selectivity at pH 6 and pH 7.4 (Fig. 4). A recent report has suggested that stimuli modulating TREK-channel activity converge on a common molecular gate comprising elements of the pore domains and the N terminus of M4 (19). This gating element is conserved among K_{2P} channels and is used whether the gating stimuli are inhibitory or activatory. In TWIK1, this element is a threonine located in the M4 transmembrane segment (T250). In terms of selectivity, TWIK1T250L is more similar to TWIK1T118I than to TWIK1 and retains K^+ selectivity at pH 6 (Fig. 4). The different types of K^+ channels, including K_{2P} channels, share a common molecular gate called "the C-type inactivation" or "upper" gate. Originally described in voltage-gated K^+ channels (K_v) that inactivate spontaneously upon depolarization, this gating involves conformational rearrangements of the upper pore region leading to a closed state (24). In many K^+ channels, the absence of external K^+ leads to a collapse of the pore in a closed state that is believed to be similar to the C-type inactivated state of K_v channels. Our results show that residues in P domains and M4 that are important for K^+ channel pore conformation and upper gating also are involved in the pH-dependent change in ion selectivity in TWIK1. Upon upper gate closure, TWIK1 would reach a Na^+-permeable state rather than a strictly closed state. TWIK1 ion selectivity also is affected in subphysiological K^+ concentrations (10). K^+ depletion or acidification precipitates a similar collapse of the pore in the Na^+-permeable state. This hypothesis is supported by the similarity of the the kinetics of the change in ion selectivity upon a decrease in K^+ concentration (373 ± 43 s) (10) or acidification (266 ± 73 s) (Fig. 5). Our results show that TWIK1 residues L108, T118, and T250 are required for this unique property.

What might be the physiological significance of this variable ion selectivity? Luminal pH of intracellular organelles along the secretory pathway becomes more acidic as they progress from the endoplasmic reticulum to the Golgi (pH 6.2–6.6) to the organelles that cycle between the plasma membrane and the cytosol (the trans-Golgi network, TGN; pH 5.9) and RE (pH 6.0–6.2) (for a recent review, see ref. 25). In transfected cells and also in native kidney and pancreatic cells, TWIK1 is present mainly in vamp8$^+$ and vamp3$^+$ RE that have a pH value close to 6 (26, 27). In this compartment, TWIK1 would not be K^+ selective but would conduct Na^+ also. TWIK1 channels addressed to the plasma membrane directly after synthesis from the TGN or indirectly after recycling from the RE also would be nonselective and are expected to elicit a depolarizing effect on the resting membrane potential. Because conformational rearrangements leading to K^+-selective pore recovery are very slow (Fig. 5), TWIK1 will remain depolarizing for several tens of minutes. If TWIK1 stays longer at the cell surface facing pH 7.4, it finally will conduct K^+ currents and have a hyperpolarizing influence. We have shown previously that in transfected mammalian cells TWIK1 endocytosis is rapid and constitutive, with an almost complete internalization occurring in less than 10 min (14). Together, these results explain why in kidney principal cells and pancreatic β cells TWIK1 gene inactivation leads not to depolarization but rather to hyperpolarization, reflecting the lack of a nonselective TWIK1 conductance. The electrical activity of pancreatic β cells consists of oscillations in membrane potential with successive electrically silent intervals and depolarized plateaus on which bursts of action potentials are superimposed. These oscillations are accompanied by changes in the cytoplasmic Ca^{2+} concentration, leading to brief pulses of insulin secretion. These oscillations reflect a balance between activities of voltage-gated Ca^{2+} channels (depolarization) and K^+ channels (repolarization). The depolarizing component predominates at the beginning of the burst, but the resulting influx of Ca^{2+} during the plateau leads to a progressive increase in K^+ channel activity. This increase in K^+ channel activity occurs both via

a direct effect on Ca^{2+}-activated K^+ channels (SK) and via an indirect effect on K^{ATP} channels by lowering of the cytoplasmic ATP:ADP ratio as the result of increased Ca^{2+}-ATPase activity. The burst ends when the increase in K^+ channel activity becomes large enough to repolarize the B cell. The slow pacemaker depolarization between two successive plateaus would result from the gradual restoration of $[Ca^{2+}]i$ and ATP:ADP ratio until the SK and K_{ATP} channels are closed again, the background depolarizing conductance becoming sufficiently large to trigger a new burst of action potentials (for a recent review see ref. 28). Our results suggest that TWIK1 may contribute to this depolarizing background conductance.

Conclusion

TWIK1 does not behave like the other K_{2P} channels. Its amino acid sequence contains several residues associated with unique features. These features are active intracellular recycling, low channel activity, and pH-modulated ion selectivity. Taken together, these properties explain the difficulty in obtaining measurable TWIK1 currents upon heterologous expression and also the lack of recording of their endogenous counterpart in native cells. TWIK1 constitutes a type of background channel for which the excitatory or inhibitory influence may be determined by its recycling rate between endosomes and plasma membrane. Very recently, the crystal structure of TWIK1 has been published (29). Crystals were grown at pH 8 and in 150 mM K^+. In these conditions, the selectivity filter of the dimeric TWIK1 channel approaches the fourfold symmetry of K^+ ion coordination previously observed in tetrameric K^+ channels, suggesting a universal mode of K^+ selectivity (Fig. S2). Growing crystals in acidic or low K^+ conditions should help elucidate the conformational changes associated with the ion-selectivity shift in TWIK1.

Materials and Methods

TWIK1 mutant preparation, electrophysiological and biochemical characterization in *Xenopus* oocytes, as well as primary culture of pancreatic islets, patch clamp, and cell immunofluorescence were carried out using standard methods described in *SI Materials and Methods*. TWIK1 homology modeling is based on the structure of the K^+ channel NaK and is described in *SI Materials and Methods*.

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