On Potential Interactions between Non-selective Cation Channel TRPM4 and Sulfonylurea Receptor SUR1*

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Background: SUR1, the regulatory subunit of KATP channels, was hypothesized to associate with TRPM4 to form novel channels, implicated in cell death following neurovascular trauma.

Results: The properties of heterologously expressed TRPM4 channels are not modified by SUR1.

Conclusion: The coupling between SUR1 and TRPM4 is unlikely.

Significance: The roles of TRPM4 and KATP channels in the pathogenesis of brain edema and hemorrhage should be reassessed.

The sulfonylurea receptor SUR1 associates with Kir6.2 or Kir6.1 to form KATP channels, which link metabolism to excitability in multiple cell types. The strong physical coupling of SUR1 with Kir6 subunits appears exclusive, but recent studies argue that SUR1 also modulates TRPM4, a member of the transient receptor potential family of non-selective cation channels. It has been reported that, following stroke, brain, or spinal cord injury, SUR1 is increased in neurovascular cells at the site of injury. This is accompanied by up-regulation of a non-selective cation conductance with TRPM4-like properties and apparently sensitive to sulfonylureas, leading to the postulation that post-traumatic non-selective cation currents are determined by TRPM4/SUR1 channels. To investigate the mechanistic hypothesis for the coupling between TRPM4 and SUR1, we performed electrophysiological and FRET studies in COSm6 cells expressing TRPM4 channels with or without SUR1. TRPM4-mediated currents were Ca2+-activated, voltage-dependent, underwent desensitization, and were inhibited by ATP but were insensitive to glibenclamide and tolbutamide. These properties were not affected by cotransfection with SUR1. When the same SUR1 was cotransfected with Kir6.2, functional KATP channels were formed. In cells cotransfected with Kir6.2, SUR1, and TRPM4, we measured KATP-mediated K+ currents and Ca2+-activated, sulfonylurea-insensitive Na+ currents in the same patch, further showing that SUR1 controls KATP channel activity but not TRPM4 channels. FRET signal between fluorophore-tagged TRPM4 subunits was similar to that between Kir6.2 and SUR1, whereas there was no detectable FRET efficiency between TRPM4 and SUR1. Our data suggest that functional or structural association of TRPM4 and SUR1 is unlikely.

The cation channel superfamily consists of a very large number of protein subunits, each of which contains one, two, or four pore-forming domains that interact in a tetrameric association to generate a mature transmembrane protein that surrounds a central ion conducting pathway (1). Depending on the detailed structure of the narrow selectivity filter, ion specificity of mature channels is variable, from highly specific for K+, Na+, or Ca2+, to nonspecific for monovalent cations, to non-specifically permeable to any small organic and inorganic cations. The members of the transient receptor potential (TRP)2 channel family are structurally similar to the voltage-gated K+ (Kv) channels, each being formed as a tetramer of six-transmembrane (TM) helix subunits, each subunit containing a voltage sensor in TM4, and a pore-lining domain between TM5 and TM6 (see Fig. 1) (1). TRP channels are non-selective cation channels implicated in signal transduction, by means of Ca2+ permeation and/or membrane depolarization (2). They underlie many sensory and metabolic processes and are expressed in brain, tongue, pancreas, liver, adipose tissue, kidney, skeletal, and cardiac muscle and cells of the immune system, among others (2, 3). TRP channels are modulated by factors as diverse as voltage, Ca2+, phosphoinositide 4,5-bisphosphate, diacylglycerol, mechanical stretch, and temperature (2, 4).

The ATP-binding cassette protein superfamily is encoded by one of the largest gene families in the mammalian genome (5). These proteins are all characterized by a core structure of two major six-TM domains each associated with a nucleotide binding fold (see Fig. L4). The two nucleotide binding folds dimerize to generate two nucleotide binding sites at the interface, and nucleotide hydrolysis then provides the power stroke for activation of multiple processes. Typically, these involve the transport of specific substrates against a transmembrane gradient (5), but in two well studied systems, this involves the activation of electrodiffusive ion channels. In one of these, the cystic fibrosis transmembrane receptor, the channel is formed by the

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2 The abbreviations used are: TRP, transient receptor potential; TM, transmembrane; SUR, sulfonylurea receptor; KATP, ATP-sensitive K+; NMG, N-methyl-D-glucamine; EGFP, enhanced green fluorescent protein; ECFP, enhanced cyan fluorescent protein; EYFP, enhanced yellow fluorescent protein.
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Heterologous Expression—COSm6 cells were plated on coverslips in six-well plates and cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 10^5 units/liter penicillin, and 100 mg/liter streptomycin. At 60% confluency, cells were transfected with the relevant plasmids using FuGENE 6 transfection reagent (Roche Diagnostics) at a 1 µg of DNA to 3 µl of FuGENE ratio, and the amount of DNA per well was kept constant at 2 µg. Multiple transfections were made in parallel.

Electrophysiology—For most experiments (Figs. 2–4), cells were either transfected with 1) mouse TRPM4b (the long splice variant of TRPM4 in pEGFP-N1, hereafter referred to as TRPM4); 2) empty pEGFP vector (GFP, or mock-transfected); 3) equal amounts of TRPM4 and GFP; or 4) equal amounts of TRPM4 and hamster SUR1 in pECE (SUR1). The same SUR1 DNA was cotransfected with mouse Kir6.2 (in pEGFP) for K_{ATP} channel expression, at a 1:0.6 ratio (see Fig. 5). For experiments entailing coexpression of K_{ATP} and TRPM4 subunits (see Fig. 6), the transfection mixture contained SUR1, Kir6.2 and GFP (K_{ATP}) or SUR1, Kir6.2 and TRPM4 (K_{ATP} + TRPM4) at a 1:0:6:1 ratio. Cells expressing the different EGFP fusion proteins fluoresced under ultraviolet light and were selected for patch clamp analysis. Experiments were performed at room temperature 24–72 h after transfection using a chamber that allowed rapid changes between four different solutions as described (19). Micropipettes were prepared from nonheparinized hematocrit glass (Kimble-Chase) on a horizontal puller (Sutter Instrument) and filled to a typical electrode resistance of 1.5 megohms with pipette solution containing 140 mM NaCl, 10 mM KCl, 1 mM MgCl2, 5 mM CaCl2, and 10 mM Hepes (pH adjusted to 7.4 with NaOH). The composition of the different experimental bath solutions is given in the figure legends. Unless otherwise noted, all buffers were adjusted to pH 7.4 with NaOH. 100 mM stocks of glibenclamide, tolbutamide, and diazoxide (in dimethyl sulfoxide), or K^+–ATP (in a 150 mM KCl solution, with the pH adjusted to 7.4 with KOH), were kept at −20 °C and diluted into working concentrations immediately before use. All chemicals were purchased from Sigma. Membrane patches were voltage-clamped using an Axopatch 1D amplifier (Molecular Devices), and currents were recorded at 0 mV or +100 mV (pipette voltage, −100 mV) in on-cell or inside-out excised patch configuration. In other experiments, a pulse protocol was applied in which membrane potential (V_m)
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was held at 0 mV for 50 ms and stepped to a test value for 500 ms before returning to the holding potential for an additional 150 ms. The test potential varied from −100 to +100 mV in 20-mV increments. Currents were measured at the end of the 500-ms voltage pulse. Data were filtered at 2 kHz, and signals were digitized at 5 kHz with a Digidata 1322A (Molecular Devices). pClamp and Axoscope software (Molecular Devices) was used for pulse protocol application and data acquisition.

Macroscopic Rb⁺ Efflux Assays—GFP-transfected cells and cells cotransfected with Kir6.2 and SUR1 were pre-incubated
for 6 h in culture medium containing 1 μCi/ml ^86^RbCl (PerkinElmer Life Sciences), and then incubated for 30 min in Ringer’s solution containing 118 mM NaCl, 2.5 mM CaCl\_2, 1.2 mM KH\_2PO\_4, 4.7 mM KCl, 25 mM NaHCO\_3, 1.2 mM MgSO\_4, and 10 mM Hepes (pH 7.4); plus metabolic inhibitors, i.e. 2.5 μg/ml oligomycin and 1 mM 2-deoxy-D-glucose (Sigma). Subsequently, at selected time points, the medium was collected and replaced with fresh solution, in the absence or presence of 10 μM glibenclamide from the same batch as that used in the experiments described above. Upon completion of the assay, cells were lysed with 2% SDS and collected, and radioactivity in the samples was measured by liquid scintillation. Raw data are presented as mean±S.E. Where indicated, one-way analysis of variance and Kruskal-Wallis test, paired or unpaired Student’s t test, were applied to evaluate statistical differences between groups.

**RESULTS**

Properties of TRPM4 Channels Expressed in COSm6 Cells Are Not Altered by Coexpression with SUR1—Figs. 2–4 summarize the essential biophysical properties of TRPM4 channels expressed heterologously in COSm6 cells alone (when applicable, white symbols and bars) and cotransfected with SUR1 subunits (gray symbols and bars). In experiments shown in Fig. 2, membrane potential (V_m) was first held at 0 mV, and patches were excised into a Ca\(^{2+}\)-free Na\(^+\) buffer before V_m was stepped to +100 mV (see representative traces in Fig. 2, A and B). In TRPM4 and TRPM4 + SUR1, currents on cell and in inside-out excised patches in absence of intracellular Ca\(^{2+}\) were indistinguishable from those measured in mock-transfected GFP cells (Fig. 2A, bottom panel), e.g. 0.013 ± 0.002 nA on cell, and 0.024 ± 0.003 nA in excised patches at +100 mV (n = 8 each). When the internal surface of the patch was exposed to 300 μM Ca\(^{2+}\), outward currents in TRPM4-transfected cells were activated rapidly (Fig. 2, A and B) and peaked at 3.4 ± 0.7 nA (Fig. 2C); individual current measurements varied from 0.7 to 8 nA but were at least 15-fold higher than in GFP cells (0.05 ± 0.01 nA; Fig. 2C, black symbols). The activation time constant τ_on was 2.4 ± 0.7 s (Fig. 2D). In cells cotransfected with TRPM4 and SUR1, Ca\(^{2+}\)-activated currents peaked at 2.7 ± 0.7 nA (Fig. 2C; p = 0.43 when compared with TRPM4 cells, unpaired Student’s t test), with τ_on = 2.1 ± 0.4 s (Fig. 2D; p = 0.72). Although not significantly different, peak currents in TRPM4 + SUR1 were on average 20% smaller than in TRPM4

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E = \frac{F_{430(525)} - F_{480(525)}}{F_{480(525)}},
\]

where \(F_{430(525)}\) and \(F_{480(525)}\) are the emissions at 525 nm following excitation at 430 nm and 480 nm, respectively, and \(F_{ECFP}\) is the emission at 525 nm of cells transfected with ECFP fusion constructs.

Data Analysis—Data were analyzed using Clampfit (version 10.1, Molecular Devices) and Excel (Microsoft). SigmaPlot 10.0 (Systat Software) and CorelDRAW X3 13.0 (Corel Corp.) were used for curve fitting, statistics, and figure preparation. Results are presented as mean±S.E. Where indicated, one-way analysis of variance and Kruskal-Wallis test, paired or unpaired Student’s t test, were applied to evaluate statistical differences between groups.
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In the next series of experiments, presented in Fig. 4, patches from TRPM4 and TRPM4 + SUR1 cells were excised as usual in a Ca$^{2+}$-free solution, and outward currents were activated at +100 mV by exposure to 300 μM Ca$^{2+}$ (representative data shown in Fig. 4A). After desensitization, we tested the effect of 10 μM free ATP, as reported (10). Steady-state currents were significantly inhibited by ATP (Fig. 4A), but the extent of inhibition was again similar in TRPM4 and TRPM4 + SUR1 (Fig. 4B), respectively 41 ± 4% and 50 ± 4% in (p = 0.13). The time constant of ATP inhibition $\tau_{\text{ATP}}$, best estimated by fitting the time course of current decay to a single exponential relationship, was 1.8 ± 0.2 s in TRPM4 and 2.3 ± 0.4 s in TRPM4 + SUR1 (n = 6 each; p = 0.1). The $K_{\text{ATP}}$ channel opener diazoxide did not counter this inhibition, instead, in both cases, it increased it slightly and reversibly by ~10%. Currents recovered 80–95% upon removal of ATP (Fig. 4, A and B, washout); inactivated in Ca$^{2+}$-free buffer (Fig. 4, A and B, background) with similar kinetics, i.e. $\tau_{\text{off}} = 14 ± 2$ s in TRPM4 and 9 ± 3 s in TRPM4 + SUR1 (p = 0.3); and reactivated again upon re-exposure to Ca$^{2+}$ (Fig. 4A).

Same SUR1 Subunits Form Functional $K_{\text{ATP}}$ Channels—To validate the SUR1 cDNA used in all previous experiments, we cotransfected it with Kir6.2; the properties of the resulting $K_{\text{ATP}}$ channels are summarized in Fig. 5. When the same voltage pulse protocol as in Fig. 3 was applied on-cell to patches from cells cotransfected with Kir6.2 and SUR1 (Fig. 5A, top left), we observed that currents were small and indistinguishable from those measured in mock-transfected cells (Fig. 5A, bottom left). Voltage-dependent K$^+$ currents of up to 4 nA rapidly activated upon excision into a Ca$^{2+}$-free, 150 mM K$^+$ buffer (Fig. 5B, top right). Accordingly, $K_{\text{ATP}}$ channels are inhibited by intracellular ATP, and spontaneously open when their cytoplasmic side is exposed to an ATP-free solution (7). In GFP-transfected cells, excision into K$^+$ buffer did not elicit any significant current (Fig. 5B, bottom right). Data from five $K_{\text{ATP}}$ channel-expressing cells were normalized as described in the legend and represented in Fig. 5B as means ± S.E.; note that, in our experimental conditions, i.e. [K$^+$]$_{\text{out}} = 10$ m$m$ and [K$^+$]$_{\text{in}} = 150$ m$m$, $K_{\text{ATP}}$-mediated K$^+$ currents reversed very close to $E_X$ (−68 mV) (Fig. 5B, circles). In other experiments (Fig. 5, C and D), exposure of cells cotransfected with Kir6.2 and SUR1 to metabolic inhibitors (oligomycin and 2-deoxy-d-glucose) led to the activation of time-dependent $^{86}\text{Rb}^+$ efflux that was 1) several-fold higher than in mock-transfected cells and 2) significantly inhibited by glibenclamide (Fig. 5C), i.e. the rate of $K_{\text{ATP}}$-specific $^{86}\text{Rb}^+$ efflux $k_2$, proportional to the $K_{\text{ATP}}$-mediated K$^+$ conductance, dropped 90% in presence of the drug (Fig. 5D). Again, this was consistent with the well understood properties of cloned $K_{\text{ATP}}$ channels (7, 24, 25).

When Expressed in Same Cells, SUR1 Subunits Control Kir6.2 Channel Activity, without Modifying TRPM4 Channel Properties—In experiments shown in Fig. 6 (see representative traces in Fig. 6A), patches from cells transfected with Kir6.2 and SUR1 ($K_{\text{ATP}}$), Kir6.2, SUR1, and TRPM4 ($K_{\text{ATP}} + $TRPM4), or mock-transfected (GFP), were first held at 0 mV and excised into a Ca$^{2+}$-free, K$^+$ buffer. In $K_{\text{ATP}}$ and $K_{\text{ATP}} + $TRPM4, this elicited instantaneous outward $K_{\text{ATP}}$ currents, 0.45 ± 0.09 nA in $K_{\text{ATP}}$ ($n = 5$) and 0.23 ± 0.04 nA in $K_{\text{ATP}} + $TRPM4 ($n = 12$; p < 0.05); individual measurements varied (Fig. 6B) but were at least 10-fold higher than in mock-transfected cells (0.02 ± 0.001 nA; n = 5). Subsequently, the patches were exposed to a Na$^+$ buffer, and this abolished the $K_{\text{ATP}}$ currents (Fig. 6A). Then, the voltage was stepped to +100 mV, and the patches were exposed to 300 μM Ca$^{2+}$, which induced small outward currents in both $K_{\text{ATP}}$ (0.06 ± 0.02 nA) and GFP (0.07 ± 0.02 nA; Fig. 6B). In $K_{\text{ATP}} + $TRPM4, large TRPM4-like currents
were activated that peaked at 3.5 ± 0.8 nA, relaxed to the steady-state (2.5 ± 0.6 nA, Fig. 6B), and were inactivated in the absence of Ca2+ with kinetics in the same range as those described in the legends to Figs. 2 and 4. Despite the presence of SUR1 subunits, as evidenced by the formation of functional KATP channels, the TRPM4-like currents in KATP + TRPM4 patches were not inhibited by tolbutamide (Fig. 6B), consistent with the hypothesis that SUR1 does not

FIGURE 3. Voltage dependence of TRPM4 current properties is not modified by SUR1 or glibenclamide. A, currents measured in excised patches from representative cells transfected with TRPM4 (left) or TRPM4 and SUR1 (right). Data were acquired sequentially upon excision in a Ca2+-free Na+ buffer (background) (1); at the steady-state after desensitization of the currents activated by 300 μM Ca2+ (control) (2); following 1-min exposure to 10 μM glibenclamide (3); and after replacement of Na+ with NMG (4). The composition of the bath solutions is the same as described in the legend to Fig. 2. The pulse protocol is shown in the top left panel. The dotted lines indicate zero current. B, current-voltage relationships of background (triangles), steady-state Ca2+-activated (circles), and NMG-inhibited (squares) currents, from the same individual experiments as described in A. Currents at each voltage were measured at the end of the 500-ms pulse and are normalized to the current measured at +100 mV in control conditions (indicated by arrowheads in A, panel 2), i.e. 2.5 nA in TRPM4 and 1.5 nA in TRPM4 + SUR1. C and D, voltage dependence of steady-state currents in cells transfected with TRPM4 (C) or TRPM4 + SUR1 (D), before and after exposure to 10 μM glibenclamide, from experiments as described in A. Data are means ± S.E. from seven patches per transfection type, and in each experiment, currents were normalized to their own control at +100 mV. On average, control currents at +100 mV were 2.2 ± 0.8 nA (TRPM4) and 1.7 ± 0.5 (TRPM4 + SUR1) (non-significant, p = 0.54; unpaired Student’s t test).
modulate TRPM4 channels. To highlight this fact, individual current measurements taken before and after exposure to the drug in the same experiment have been represented in Fig. 6B with the same symbol, in light and dark shades of gray, respectively.

**DISCUSSION**

Sulfonylurea receptor subunits (e.g. SUR1) couple to inward-rectifying K⁺ channel subunits (e.g. Kir6.2) to form ATP-sensitive K⁺ (K<sub>ATP</sub>) channels (Fig. 1), which are central to numerous physiological processes, from insulin secretion to the generation and maintenance of cardiac rhythm and vascular tone (26). The archetypal pancreatic β-cell K<sub>ATP</sub> channel, for example, is composed of four Kir6.2 and four SUR1 subunits (27), and the link between these two proteins has proven essential for K<sub>ATP</sub> channel gating, modulation, and pharmacology (25, 28, 29). In the K<sub>ATP</sub> channel octamer, the Kir6.x subunits define the ATP-sensitive pore, whereas the SURx subunits confer sensitivity to sulfonylureas, K⁺ channel openers, and NDPs (7). Only the fully assembled octameric channel complex can reach the plasma membrane, as SUR1 and Kir6.2 contain endoplasmic reticulum retention motifs that must be shielded mutually to allow trafficking (30). This tight and required structural and functional association also seems to be exclusive; whereas one report suggested that SUR1 is capable of coupling with Kir1.1 (8), these findings have not been confirmed by independent research, and Kir1.1 subunits are well known to assemble in fully functional tetrameric channels, indispensable to the regulation of ion homeostasis in the kidney (31).

TRPM4 subunits are distantly related members of the cation channel superfamily that also assemble into tetrameric functional channels (Fig. 1) (32) which are constitutively expressed in vascular smooth muscle, pacemaker cells, mast cells, and lymphocytes and play key roles in vasoconstriction, cerebral
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FIGURE 5. Cotransfection of SUR1 with Kir6.2 results in functional K<sub>ATP</sub> channels. A, currents measured in a representative cell cotransfected with Kir6.2 and the same SUR1 plasmid DNA used in the experiments shown in Figs. 2–4 (top panels) or in a representative mock-transfected cell (GFP; bottom); on cell (left) and after excision into a K<sup>+</sup> buffer (K<sup>+</sup>) containing 150 mM KCl, 1 mM K<sup>+</sup>-EDTA, 1 mM EGTA, and 10 mM Hepes (pH 7.4 with KOH) (right). The pulse protocol is the same as in Fig. 3A. The dotted lines indicate zero current. B, voltage dependence of currents measured on cell (triangles) and in inside-out excised patches (circles), from experiments in K<sub>ATP</sub>-expressing cells as in A. Currents at each voltage were measured at the end of the 500-ms pulse and are normalized to the current measured at +100 mV in the excised patch configuration (indicated by an arrowhead in the top right panel of A) on average 3.6 ± 0.8 nA. Data are means ± S.E. from five patches. C, time course of 86Rb<sup>+</sup> efflux in mock-transfected cells (GFP; white symbols) or in cells cotransfected with Kir6.2 and SUR1 (K<sub>ATP</sub>; black symbols) under metabolic inhibition conditions, in absence (M<sub>, circles</sub>) or presence of 10 μM glibenclamide (M<sub>, + Glib</sub>; squares). D, flux data were fit to Equation 1 to estimate the rate constants for K<sub>ATP</sub>-dependent 86Rb<sup>+</sup> efflux k<sub>1</sub> (black bars). *, p < 0.05 as compared with k<sub>2</sub> in the absence of glibenclamide (paired Student’s t test). Nonspecific k<sub>1</sub> (white bars) were estimated from GFP-transfected cells. C and D, results are means ± S.E. from four to six experiments.

blood flow, the generation of heart rhythm, and the immune response (33–39). TRPM4-mediated currents are up-regulated in cardiomyocytes from spontaneous hypertensive rats (40), and gain-of-function mutations in TRPM4 have been associated with familial heart disease (41, 42). TRPM4 channel kinetics are finely tuned by diverse cellular factors, including Ca<sup>2+</sup>-calmodulin, adenine nucleotides, and phosphoinositide 4,5-bisphosphate (15).

Several recent studies have led to the suggestion that SUR1 may also couple with TRPM4 to form novel channels critical to the outcome of injuries to the central nervous system. In particular, it has been postulated that de novo expression of a putative TRPM4/SUR1 channel complex in neurovascular tissues after traumatic brain injury, spinal cord injury, ischemic stroke, or traumatic brain injury and spinal cord injury (11, 12, 43–45). The effects of trauma appear to be reduced by administration of glibenclamide (46, 47); clinical trials to test the potential of this antidiabetic drug as a treatment for brain edema are under way (48), but the subject remains controversial (49, 50). On the other hand, fragmentation and hemorrhaging in capillaries correlate with an increase in TRPM4 expression in rats after spinal cord injury and are significantly mitigated in TRPM4-null mice (17).

Although these studies provide very provocative interpretations, the TRPM4-SUR1 association hypothesis remains untested experimentally. Therefore, we performed functional and structural studies in COSm6 cells expressing TRPM4 in the absence and presence of SUR1 (Figs. 2–7). Na<sup>+</sup><sup></sup> currents measured in excised inside-out patches from cells transfected with TRPM4 were 1) activated by cytoplasmic Ca<sup>2+</sup> (Figs. 2, 4, and 6); 2) voltage-dependent and outward-rectifying (Fig. 3); 3) reversibly inhibited by ATP (Fig. 4); and 4) underwent desensitization (Figs. 2, 4, and 6). These were in agreement with the biophysical properties of TRPM4 channels established by several independent groups (15, 16) and were not modified by the presence of SUR1 (Figs. 2–4 and 6). Co-expression with SUR1 did not confer sensitivity to glibenclamide, tolbutamide, or diazoxide (Figs. 2, 4, and 6). When coexpressed with Kir6.2, the same SUR1 subunits assembled in fully functional, glibenclamide-sensitive K<sub>ATP</sub> channels (Fig. 5) that were not affected by the presence of TRPM4 (Fig. 6). FRET analysis confirmed the strong physical association between TRPM4 subunits and between Kir6.2 and SUR1 subunits, while showing no evidence for the structural coupling of TRPM4 and SUR1 (Fig. 7).

Our data thus strongly suggest that either functional or physical interaction between TRPM4 and SUR1 is improbable.
Structurally such association seems unlikely because SUR1 probably packs tightly against the two inner helices of the Kir6.2 subunit (51), whereas the S1–S4 “voltage sensor” domain (52) will likely pack against the inner helices in TRPM4 (Fig. 1).

FIGURE 7. FRET analysis demonstrates no structural association between TRPM4 and SUR1. A, representative emission spectra from COSm6 cells transfected with TRPM4-EYFP or TRPM4-ECFP. Cells expressing TRPM4-EYFP were excited at 480 nm (solid orange line) or 430 nm (dashed orange line), and emission between 490–550 and 450–550 nm, respectively, was measured by means of fluorescence spectroscopy. Cells expressing TRPM4-ECFP were excited at 430 nm, and emission between 450–550 nm was measured (cyan). B, representative emission spectra from cells expressing Kir6.2, TRPM4, and SUR1 with C-terminal ECFP or EYFP tags. Cells were excited at 430 nm, and emission between 450–550 nm was measured. A remarkable emission peak at 525 nm, which is due to ECFP/EYFP FRET, was detected in cells cotransfected with Kir6.2-ECFP plus SUR1-EYFP (red) or TRPM4-ECFP plus TRPM4-EYFP (green), but not for those cotransfected with TRPM4-ECFP plus SUR1-EYFP (black) or Kir6.2-ECFP (cyan). C, apparent FRET efficiencies between the C-terminal ECFP and EYFP tags of Kir6.2, TRPM4, and SUR1. The apparent FRET efficiency due to the unspecific excitation of EYFP was 0.09 ± 0.03 in cells transfected with TRPM4-EYFP and 0.09 ± 0.01 in cells transfected with SUR1-EYFP. Coexpression of TRPM4-ECFP and TRPM4-EYFP, or Kir6.2-ECFP and Sur1-EYFP, resulted in apparent FRET efficiencies of 0.28 ± 0.03 and 0.27 ± 0.03, respectively. In cells cotransfected with TRPM4-ECFP and SUR1-EYFP, the apparent FRET efficiency was 0.06 ± 0.01, i.e., within the range of that measured in cells transfected with EYFP fusion constructs alone. FRET data are presented as mean ± S.E. from three to six measurements, each from an independent transfection. a.u., arbitrary units.

Structurally such association seems unlikely because SUR1 probably packs tightly against the two inner helices of the Kir6.2 subunit (51), whereas the S1–S4 “voltage sensor” domain (52) will likely pack against the inner helices in TRPM4 (Fig. 1).
Here, we used the long splice variant of TRPM4, but other variants have been identified that lack different portions of the N terminus (16). In most, however, the transmembrane segments remain intact (22, 23, 53) and thus are likely to undergo similar packing to the full-length protein and hence still unlikely to physically couple to SUR1. The TRPM4 mRNA detected in neurovascular cells of rodents subjected to spinal cord injury is actually equivalent to the long splice variant (17), and this suggests that shorter orthologs are not likely to play a relevant role. We provide compelling evidence that in a recombinant system, TRPM4 channels are not modulated by SUR1; although we cannot discard the possibility that interaction may occur in a different cellular environment, our data do not support the hypothesis that the cationic currents measured in astrocytes and endothelial cells from rodent models for neurovascular trauma are due to TRPM4 channels under direct modulation by SUR1 subunits (9). At the foundation of this hypothesis lies the observation that these currents are apparently inhibited by sulfonylureas (11, 12, 45, 54). It is unclear whether the effect of these drugs was tested once desensitization of TRPM4-like currents was complete and a steady-state had been reached, as done in our study (Figs. 2, 4, and 6) and essential to prevent artifacts due to ongoing inactivation (23, 55–57). One possibility is that both TRPM4 and K_ATP channels are up-regulated at the site of injury and contribute to different aspects of the pathogenesis of brain edema and microvascular failure. TRPM4-mediated currents are known to be up-regulated in certain pathological situations (40–42), and it is thus plausible that TRPM4 channels are responsible for the cationic currents that lead to post-traumatic neurovascular cell swelling and necrosis. On the other hand, Kir6.1 and Kir6.2 subunits are abundant in tissues where SUR1 is up-regulated following a stroke (54), and the possible concomitant increase in K_ATP currents remains to be addressed.

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