The Selectivity Filter of the Cation Channel TRPM4*

Received for publication, February 14, 2005, and in revised form, April 13, 2005
Published, JBC Papers in Press, April 21, 2005, DOI 10.1074/jbc.M501686200

Bernd Nilius‡§, Jean Preenen‡, Annelies Janssens‡, Grzegorz Owsianik†, Chunbo Wang‡, Michael X. Zhu‡, and Thomas Voets‡||

From the ‡Department of Physiology, Campus Gasthuisberg, KU Leuven, B-3000 Leuven, Belgium and %Department of Neuroscience and Center for Molecular Neurobiology, Ohio State University, Columbus, Ohio 43210

Transient receptor potential channel melastatin subfamily (TRPM) 4 and its close homologue, TRPM5, are the only two members of the large transient receptor potential superfamily of cation channels that are impermeable to Ca\(^{2+}\). In this study, we located the TRPM4 selectivity filter and investigated possible structural elements that render it Ca\(^{2+}\)-impermeable. Based on homology with known cation channel pores, we identified an acidic stretch of six amino acids in the loop between transmembrane helices TM5 and TM6 (981EDMDVA986) as a potential selectivity filter. Substitution of this six-amino acid stretch with the selectivity filter of TRPV6 (TIIDGP) resulted in a functional channel that combined the gating hallmarks of TRPM4 (activation by Ca\(^{2+}\), voltage dependence) with TRPV6-like sensitivity to block by extracellular Ca\(^{2+}\) and Mg\(^{2+}\) as well as Ca\(^{2+}\) permeation. Neutralization of Glu\(^{981}\) resulted in a channel with normal permeability properties but a strongly reduced sensitivity to block by intracellular spermine. Neutralization of Asp\(^{982}\) yielded a functional channel that exhibited extremely fast desensitization (\(\tau < 5\) s), possibly indicating destabilization of the pore. Neutralization of Asp\(^{984}\) resulted in a non-functional channel with a dominant negative phenotype when coexpressed with wild type TRPM4. Combined neutralization of all three acidic residues resulted in a functional channel whose voltage dependence was shifted toward very positive potentials. Substitution of Glu\(^{977}\) by a glutamate, the corresponding residue in divalent cation-permeable TRPM channels, altered the monovalent cation permeability sequence and resulted in a pore with moderate Ca\(^{2+}\) permeability. Our findings delineate the selectivity filter of TRPM channels and provide the first insight into the molecular basis of monovalent cation selectivity.

TRPM4\(^1\) is a Ca\(^{2+}\)- and voltage-dependent non-selective cation channel belonging to the melastatin subfamily of transient receptor potential (TRP) membrane proteins (1, 2). It has been proposed to be the molecular correlate of Ca\(^{2+}\)-activated non-selective cation channels in several excitable and non-excitable cell types, and it has been implicated in important physiological processes including T-cell activation, myogenic vasconstriction, and cardiac function (3–5).

TRPM4 and its close homologue, TRPM5, exhibit two salient features that are unique within the TRP superfamily. First, they represent the only known TRP channels that are directly gated by increases in intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{i}\)) (1, 2, 6–11). The Ca\(^{2+}\) sensitivity of TRPM4 activation is strongly modulated by several cellular factors, including protein kinase C phosphorylation, calmodulin binding, and ATP (11, 12). Second, both channels are impermeable to Ca\(^{2+}\) (1, 6–8). This contrasts with all other functionally expressed TRPs, which form either Ca\(^{2+}\)-permeable non-selective cation channels or even highly Ca\(^{2+}\)-selective channels.

The structural basis of TRPM channel permeation has not yet been studied. The region between the fifth and sixth transmembrane helices (TM5 and TM6), which is known to form the pore in the other tetrameric cation channels, shows only limited sequence homology to other (TRP) cation channels but is highly conserved among members of the TRPM subfamily. This region consists of a conserved hydrophobic region, a putative pore helix, followed by a hydrophilic region that contains a fully conserved aspartate residue (residue 984 in TRPM4), which may be part of the selectivity filter (13). There are, however, some clear sequence differences. In TRPM4 and TRPM5, the putative selectivity filter is highly acidic, with a cluster of three (TRPM4) or four (TRPM5) aspartates or glutamates. The other TRPM channels, which all exhibit some degree of Ca\(^{2+}\)-permeability, have only one or two acidic residues in this region. Moreover, the spacing between the putative pore helix and selectivity filter is one amino acid shorter in TRPM2 and TRPM8 than in the other TRPM channels. We hypothesized that the lack of Ca\(^{2+}\)-permeability of the TRPM4 and TRPM5 pores may be related to these structural differences.

In this study, we used a site-directed mutagenesis approach to investigate how changes to the putative selectivity filter affect the pore properties of TRPM4. Our data indicate that this region determines the TRPM4 permeability properties and its sensitivity to block by intracellular spermine.

EXPERIMENTAL PROCEDURES

Cell Culture—HEK293 human embryonic kidney cells were grown in Dulbecco’s modified Eagle’s medium containing 10% (v/v) human serum, 2 mM l-glutamine, 2 units/ml penicillin, and 2 mg/ml streptomycin at 37 °C in a humidity-controlled incubator with 10% CO\(_2\).

Transient Expression of Human TRPM4 and Mutagenesis—We used NMDG, N-methyl-D-glucamime; HEDTA, N-(2-hydroxyethyl) ethylene-diamine-N,N’,N’-triacetic acid; GFP, green fluorescent protein; WT, wild type; IV, current-voltage.

---

*This work was supported by Human Frontiers Science Programme (HFSP Research Grant RGP 32/2004), the Belgian Federal Government, the Flemish Government, the Onderzoeksradi KU Leuven (Grants GOA 2004/07, F.W.O.G. 0214.99, F.W.O.G. 0136.00, and F.W.O.G. 0172.03 and the Interuniversity Poles of Attraction Program, Prime Minister’s Office IUAP), and National Institutes of Health Grant NS42183. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Laboratorium voor Fysiologie, Campus Gasthuisberg, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium. Tel.: 32-16-34-5937; Fax. 32-16-34-5991; E-mail: bernd.nilius@med.kuleuven.ac.be.

§ Both authors contributed equally to this work.

¶ This abbreviation was used: TRP, transient receptor potential channel, melastatin subfamily; TRP, transient receptor potential;
the recombinant bicistronic expression plasmid pdiTRPM4b, which carries the entire protein-coding region for the human TRPM4b (GenBank accession number AX443227) (2) and for the green fluorescent protein (GFP) coupled with an internal ribosomal entry site sequence. HEK293 cells were transiently transfected with the pdiTRPM4b vector using previously described methods, and successfully transfected cells were visually identified by their green fluorescence in the patch clamp set up (2). For all mutations and deletions we used the standard PCR overlap extension technique (14) with the human TrpM4 cDNA constructed in the pCAGGSM2/IresGFP. All constructs were verified by sequencing analysis.

**Cell Surface Biotinylation Assay**—GFP-tagged TRPM4 and mutants were expressed in HEK293 cells, labeled with biotin on ice, and analyzed after precipitation with streptavidin-agarose exactly as previously described (11).

**Solutions**—This study was mostly carried out in cell free inside-out patches. The standard pipette solution for inside-out patch clamp measurements contained 150 mM NaCl, 5 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES. The extracellular bath solution was adjusted with CsOH to pH 7.2. Intracellular solution in these experiments contained 100 mM CaCl₂ in the pipette solution was used, buffered with Ca(OH)₂, pH 7.4. Before patch excision, the extracellular bath solution was changed to an "internal solution" for inside-out patch clamp measurements contained 150 mM NaCl, 5 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, 5 mM EGTA, and 5.297 mM CaCl₂ (resulting in 300 mM free [Ca²⁺]) calculated by the CaBuf program (ftp://ftp.cc.kuleuven.ac.be/pub/droogmans/cabuf.zip). Note that we added 5 mM EGTA to have the same amount of EGTA at the inner site as charge carrier, 30 mM CaCl₂ was added with Glu981 and Val985, with Glu981 located at the inner entrance of the loop between TM5 and TM6 based on the KcsA crystal structure (Fig. 1A). The resulting three-dimensional structure suggests that the selectivity filter is located between residues Glu⁹⁸¹ and Val⁹⁸⁵, with Glu⁹⁸¹ located at the inner entrance of the pore.

**RESULTS**

**Putative Structure of the TRPM4 Pore**—Fig. 1A shows an alignment of the putative pore region of the TRPM subfamily and indicates the TRPM4 residues that were mutated in this study. Using the Swiss Model server, we modeled the structure of the loop between TM5 and TM6 based on the KcsA crystal structure (Fig. 1B). The resulting three-dimensional structure suggests that the selectivity filter is located between residues Glu⁹⁸¹ and Val⁹⁸⁵, with Glu⁹⁸¹ located at the inner entrance of the pore.

**Permeation Profile of TRPM4**—Before evaluating the effects of the various mutations, we characterized the permeation profile of wild type (WT) TRPM4. The relative permeability of monovalent cations compared with Na⁺ was measured in inside-out patches by equimolar substitution of intracellular Na⁺ by the respective cation X⁺ and calculated according to

\[
P_{X^+} \frac{F_{Na}}{P_{Na}} = \exp\left(\Delta V_{\text{rev}} \times F/RT\right) \tag{1}
\]

Before calculation, the reversal potentials were corrected for liquid junction potentials (19) by

\[
V_{\text{rev,corrected}} = V_{\text{rev,measured}} - V_{\text{LJ}} \tag{2}
\]

where \(V_{\text{LJ}}\) is the calculated liquid junction potential (−4.4 mV)
Complete absence of Ca$^{2+}$ putative selectivity filter of TRPM4 (981EDMDVA986). Whole-cell currents were analyzed (see also Fig. 2A). Using Ca$^{2+}$ as the only charge carrier, no inward current is measured up to $-80 \text{ mV}$ (Fig. 3A). In step protocols from positive potentials to $-100 \text{ mV}$ (the usually applied protocol to measure TRPV5/6 currents, Ref. 25), a fast deactivating current can be seen in WT TRPM4, but no apparent inward current is seen with Ca$^{2+}$ as the sole charge carrier (Fig. 3B). In the selectivity filter swap mutant, replacing Na$^{+}$ with Ca$^{2+}$ induces a shift of the reversal potentials toward positive potentials, and clear inward current can be measured (Fig. 3C). In step protocols, currents carried by monovalent cations deactivate very rapidly. However, a small inward current of $17 \pm 4 \text{ pA/pA}$ can also be measured when Ca$^{2+}$ is the only charge carrier (Fig. 3D, bath, 30 mM CaCl$_2$, 105 mM NMDG-chloride) indicating that this chimera is Ca$^{2+}$-permeable. In addition, switching to the divalent-containing solution always induced a remarkable inhibition of the outward current, which was not observed for WT TRPM4 (Fig. 3, A and B for WT and C and D for the selectivity filter swap mutant).

In inside-out patches with the pipette solution containing only monovalent cations, activation of the chimeric mutant only occurred after patch excision in Ca$^{2+}$-containing solutions. Current density increased when [Ca$^{2+}$]$_i$ was elevated (data not shown), indicating that Ca$^{2+}$ sensitivity is preserved.

As for WT TRPM4, activation of the chimera was followed by desensitization (Fig. 3, E and F). The current kinetics at negative and positive potentials are similar to those of WT TRPM4 (Fig. 3E). Moreover, the currents through the chimeric channel displayed voltage-dependent activation and deactivation kinetics similar to those of WT TRPM4.

However, when 5 mM Ca$^{2+}$ and 1 mM Mg$^{2+}$ were included in the pipette, currents through the chimeric channel were dramatically reduced (Fig. 3, F and H). Block by extracellular Ca$^{2+}$ of the outward current at $+100 \text{ mV}$ occurred with an IC$_{50}$ of 431 mM (Fig. 3H). These data indicate that, as is the case for TRPV6, divalent cations block the permeation of monovalent...
Additional support for the correct identification of the selectivity filter was deduced from the following experiment. We noticed that a short stretch of amino acids C-terminal of TM5, \(943AYGVA947\), displays significant homology to the conserved selectivity filter of K\(^+\) channels. To examine whether this region influences the permeability of TRPM4, we constructed two mutants (VA946/947DM and AVA943/946/947GDM), reproducing the KcsA-like pore sequence, GYGDM). Although both mutants were functional, currents in 300 \(\mu M\) Ca\(^{2+}\) were extremely small (at \(+100\) mV: 59 \(\pm\) 21 pA, \(n = 6\) for VA946/947DM and 58 \(\pm\) 31 pA, \(n = 4\) for AVA943/946/947GDM). We found no indication that this stretch could eventually be a part of the pore itself.

**Point Mutations in the Putative Selectivity Filter**—As shown in Fig. 1, the putative TRPM4 selectivity filter contains three negatively charged residues. We neutralized these residues to alanines, either individually or in all possible combinations, and studied the pore properties of these mutants.

Currents through E981A were large and showed a similar decay (desensitization) behavior as the wild type channel (Figs. 2A and 4D; see also Ref. 10). As measured from reversal potentials, E981A had a similar permeation profile as WT TRPM4 (\(Na^+ > K^+ > Ca^{2+} > Li^+\); Fig. 4A).

Because our modeling predicts that Glu\(^{981}\) is located at the pore entrance from the inner side, we tested the sensitivity of this mutant to intracellular spermine. WT TRPM4 was completely blocked by 1 mM spermine (12), whereas the same concentration induced only 30% block of the E981A mutant (Fig. 4, F and G). This indicates that Glu\(^{981}\) contributes to the blocking site for intracellular spermine.

Currents through D982A were also large immediately after excision in 300 \(\mu M\) Ca\(^{2+}\), but they decayed so fast that an analysis of permeation or spermine block was impossible (Fig. 4E).

Currents through the double mutant E981A/D982A decayed slower in the presence of 300 \(\mu M\) Ca\(^{2+}\), allowing further analysis. The selectivity sequence was not changed (data not shown), and block by 1 mM spermine was reduced to 27 \(\pm\) 9% \((n = 4)\), similar to the E981A mutation alone. Similar results were obtained for another double mutant, E981Q/D982N.

In contrast to the E981A and D982A mutants, no current could be measured for the D984A mutant or the E981A/D984A and D982A/D984A double mutants (Fig. 4H), suggesting that Asp\(^{984}\) is crucial for the integrity of the TRPM4 pore. Strongly reduced currents could be measured in cells coexpressing WT TRPM4 and D984A in a 1:1 cDNA ratio. Desensitization properties were similar to the expression of WT alone (Fig. 5, A and B). During the steady-state phase, cation substitutions were performed (Fig. 5, C and D). However, because it is difficult to measure reversal potentials, we have analyzed changes in conductance for Na\(^+\), K\(^+\), Ca\(^{2+}\), and Li\(^+\) (see the legend for Fig. 5C), which were not significantly different from the transfection with the WT alone (Fig. 5D). Average current amplitude immediately after application of 300 \(\mu M\) Ca\(^{2+}\) was 119 \(\pm\) 31 pA \((n = 8)\), which corresponds to \(7\%\) of the amplitude in cells expressing only the WT subunit (1559 \(\pm\) 197 pA; \(n = 7\); Fig. 5E).

Assuming random assembly of WT and the mutant subunits, we would expect that \(~6\%\) of the tetrameric complexes would consist of four WT subunits and that all other complexes would contain at least one mutant subunit. Thus, the most straightforward explanation of the above data is that the D984A subunit is dominant negative and that the remaining...
TRPM4 Pore and Permeation

As shown in Fig. 4I, D984A, E981A/D984A, and D982A/D984A are all expressed to similar levels as WT TRPM4, in terms of both total protein level and expression at the cell surface. Therefore, the loss of function of the D984A mutation is most likely due to a defect in permeation or gating, rather than a defect in the trafficking to the cell surface.

Surprisingly, combined mutation of all three negative charges, E981A/D982A/D984A, resulted in a functional channel. As shown in Fig. 6, depolarizing steps activate large currents in WT TRPM4 (10, 11). Current-voltage curves were plotted from the currents at the end of the step pulses (Fig. 6, A and B). From tail current analysis (see Refs. 2 and 10), activation curves were measured and fitted by the Boltzmann equation (Fig. 6C). From these fits, the open probability at +100 mV was calculated (Fig. 6G). Activation of the triple mutant was strongly shifted toward positive potentials, and currents were only detectable at potentials of >+100 mV (Fig. 6, D–F). To estimate the shift in voltage-dependent activation for this mutant, the open probability at +100 mV (P_{open} + 100 mV) was compared and showed a dramatic reduction relative to the WT channel (Fig. 6G). In addition to the dramatic shift, currents measured in inside-out patches immediately after excision were much smaller in the E981A/D982A/D984A mutant compared with the WT TRPM4 (1.9 ± 1.1 nA, n = 4 for WT as compared with 0.4 ± 0.2 nA, n = 5, for the triple mutant). Due to the fast desensitization of the triple mutant (data not shown), a complete permeation study was not possible. However, when all cations in the pipette were replaced by Ca^{2+}, again no inward current could be measured in this mutant, indicating that it remains Ca^{2+}-impermeable (Fig. 6H). Similar to the WT channel (Fig. 2F), with just Ca^{2+} in the pipette, switching the test pulse from +100 to −160 mV did not elicit any inward tail current (n = 4, data not shown).

Biotinylation experiments showed that the triple mutant was efficiently expressed on the plasma membrane as WT TRPM4 and other mutants that contain the D984A substitution (Fig. 4I).

Mutations in the Linker between Putative Pore Helix and Selectivity Filter—TRPM6 and TRPM7, which have relatively high permeability for divalent cations, contain a glutamate following the hydrophobic region, whereas TRPM4 and TRPM5 contain a glutamate residue at the corresponding site (Glu^{977} in TRPM4). To check whether this residue interferes with divalent cation permeation, we studied the Q977E mutant. This mutant produced large currents and showed a similar decay as WT TRPM4. The permeability for monovalent cations was changed to Eisenman type VI (K^{+} > Na^{+} > Ca^{2+} > Li^{+}, Fig. 7A and C). With Ca^{2+} as the only charge carrier in the pipette, small but significant inward currents could be measured (insets in Fig. 7, B and D). The permeability of Ca^{2+} relative to Na^{+} was calculated from the absolute reversal potential measured with the respective Ca^{2+} concentration of 100 mM in the extracellular solution, according to Ref. 27.

\[ P_{i} = \frac{1 + \text{exp}(V - V_{\text{rev}})}{[\text{Na}^{+}] \times \text{exp}(V_{\text{rev}})} \times \frac{4[X]}{4[X]} \]  

where \( P_{i} \) represents the permeability of the divalent cation, and \([X]\) is extracellular concentration. A relative Ca^{2+} permeability (\( P_{i}/P_{Na} \)) of 0.05 ± 0.02 (n = 6) was obtained. Another property of this mutant was the much more pronounced outward rectification. The ratio between the current at −100 and +100 mV was 0.03 ± 0.008 (n = 6) for Q977E and 0.22 ± 0.06 (n = 12) for the WT TRPM4 (p < 0.001). This rectification was independent of the presence of divalent cations in the pipette solution.

currents in the coexpressing cells are conducted by channels consisting of four WT subunits. In line with this, the conductance sequence of these channels as shown above was identical to that of WT TRPM4.

One reason for the lack of function of the D984A mutation might be that this amino acid substitution interfered with the expression of the channel protein on the plasma membrane. To examine this, we performed a cell surface biotinylation assay.
Fig. 5. Effect of coexpression of TRPM4 wild type and the pore mutant D984A. A, time course of currents activated by 300 μM Ca\(^{2+}\) and voltage ramps as shown in the inset. Currents were small and decayed but reached a steady state similar to the WT TRPM4 currents. B, current-voltage curves obtained from A showing desensitization without a change in the reversal potential. C, in the steady-state phase, cation substitutions were performed. For analysis, currents for different cations at +100 mV were normalized to that for Na\(^{+}\) (see data in D). D, data obtained from experiments shown in C were pooled for coexpression experiments (C, WT + D984A) and for experiments in which only WT TRPM4 (●) was expressed. Data are nearly identical for both series. E, currents were measured immediately after patch excision in 300 μM Ca\(^{2+}\) at +100 mV. Note the dramatic current reduction in the coexpression experiments (all cells were from the same batch).

In TRPM4/5, the linker between the end of the putative pore helix and the selectivity filter (Gly\(^{976}\)–Gln\(^{980}\) in TRPM4) is one amino acid longer than that in the Ca\(^{2+}\)-permeable TRPM2/8 channels. Surprisingly, deletion of Gln\(^{980}\) in TRPM4 to mimic the corresponding regions of TRPM2/8 resulted in a functional channel exhibiting extremely fast current decay after patch excision (Fig. 8). Fig. 8A shows the normal time course of TRPM4 current after patch excision in 300 μM Ca\(^{2+}\), as has been described in detail elsewhere (10). The first voltage steps after excision evoke large outward and inward currents (Fig. 8B). In contrast, currents through the ΔQ980 mutant channel decayed already during the first step (Fig. 8, C and D). When all cations in the pipette were substituted by Ca\(^{2+}\), no inward tail currents could be observed, even upon application of large driving forces after current activation at +180 mV, indicating that the mutant is not measurably Ca\(^{2+}\)-permeable (Fig. 8E).

**DISCUSSION**

The Ca\(^{2+}\)-activated non-selective cation channel TRPM4 represents a molecular candidate for a large number of functionally similar Ca\(^{2+}\)-activated cation channels found in native cells types with, as a typical fingerprint, its lack of permeability for Ca\(^{2+}\). Together with TRPM5, this permeation feature is unique in the TRP superfamily. In this study, we localized the selectivity filter and identified amino acids that are crucial for cation permeation and spermine block in TRPM4.

The strongest evidence that the region between residues Glu\(^{981}\) and Ala\(^{986}\) forms the selectivity filter was obtained by replacing the putative selectivity filter of TRPM4 with the previously identified selectivity filter of TRPV6. This chimeric channel gives rise to large monovalent currents, but only in the absence of extracellular divalent cations. This is reminiscent of the potent block of monovalent TRPV5/6 currents by Ca\(^{2+}\) and Mg\(^{2+}\) (see Ref. 28 for a review). Importantly, the pore chimera showed no change in activation by Ca\(^{2+}\), desensitization, and voltage dependence, indicating that the molecular determinants of these gating parameters lie elsewhere in the TRPM4 protein (see also Ref. 11). Because of the fast current decay in the presence of extracellular Ca\(^{2+}\), a permeation analysis was difficult to perform. The inward rectification of the TRPV6 pore was not observed for the chimeric channel. However, this might indicate that rectification requires a larger part of the TRPV6 pore than only the selectivity filter. Consistent with this idea,
rectification properties of TRPV6 were also changed by mutations outside the selectivity filter (21, 29).

Mutating the three acidic residues in the putative TRPM4 selectivity filter (Glu 981, Asp 982, and Asp 984) had important functional consequences. Neutralization of Glu 981 did not change the permeation sequence, but it significantly reduced the sensitivity to block by spermine. Most likely, negative charges at the inner mouth of the channel pore provide a binding site for positively charged polyamines, inducing open channel block (30). Neutralization of Asp 982 resulted in a functional channel exhibiting extremely fast and complete current decay after activation. Because pore mutations are not expected to influence Ca\(^{2+}\)/H\(^{+}\) binding to the activation site(s) (11), we hypothesize that the stability of the pore might be deterio-
rated in this mutant such that the pore collapses rapidly after channel activation by Ca\(^{2+}\). A similar collapse may be caused by the D984A mutation, which apparently acts as a dominant negative subunit.

The putative pore loop is most strongly conserved among TRPM2, TRPM8, TRPM4, and TRPM5. These four channels share a conserved glutamine and proline in the span between the hydrophobic pore helix and the selectivity filter. A clear difference is that this span is one residue shorter in TRPM2 and TRPM8 than in TRPM4 and TRPM5, which contain an additional glutamine. Deletion of this glutamine causes a rapid closure of the pore after channel activation by Ca\(^{2+}\). We hypothesize that an intact linker is essential for the stability of the pore.
The E977Q mutation, which introduces the corresponding residue of the divalent cation permeable TRPM6/7 channels, induced a small change in monovalent permeability, which changed from Eisenman sequence VII to VI. More importantly, this mutation rendered the TRPM4 pore permeable to Ca\textsuperscript{2+}, albeit to a relatively low extent (P_{Ca}/P_{Na} \ll 1).

In conclusion, we propose that the stretch between Glu 981 and Ala\textsuperscript{296} forms all or part of the selectivity filter of TRPM4. Given the strong sequence conservation, we hypothesize that the variable permeability properties of the TRPM channels are due to the subtle amino acid variations in this region.

Acknowledgments—We thank Drs. V. Flockerzi and M. Freichel for providing the TRPM4 clone and Dr. K. Talavera and F. Mahieu for helpful discussions.

REFERENCES

1. Launay, P., Fleig, A., Perraud, A. L., Scharenberg, A. M., Penner, R., and Kinet, J. P. (2002) Cell 109, 397–407
2. Nilius, B., Prenen, J., Droogmans, G., Voets, T., Vennekens, R., Freichel, M., Wissenbach, U., and Flockerzi, V. (2003) J. Biol. Chem. 278, 30813–30820
3. Guinamard, R., Chatelier, A., Lenfant, J., and Bois, P. (2004) J. Cardiovasc. Electrophysiol. 15, 1153–1158
4. Liu, D., and Liman, E. R. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 15160–15165
5. Prawitt, D., Monteilh-Zoller, M. K., Brixel, L., Spangenberg, C., Zabel, B., Fleig, A., and Penner, R. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 15166–15171
6. Thomas, R. D., Voets, T., Breuer, E., Droogmans, G., and Nilius, B. (2005) J. Gen. Physiol. 124, 245–260