Potentiation of TRPM7 Inward Currents by Protons

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TRPM7 is unique in being both an ion channel and a protein kinase. It conducts a large outward current at +100 mV but a small inward current at voltages ranging from −100 to −40 mV under physiological ionic conditions. Here we show that the small inward current of TRPM7 was dramatically enhanced by a decrease in extracellular pH, with an ∼10-fold increase at pH 4.0 and 1–2-fold increase at pH 6.0. Several lines of evidence suggest that protons enhance TRPM7 inward currents by competing with Ca^{2+} and Mg^{2+} for binding sites, thereby releasing blockade of divalent cations on inward monovalent currents. First, extracellular protons significantly increased monovalent cation permeability. Second, higher proton concentrations were required to induce 50% of maximal increase in TRPM7 currents when the external Ca^{2+} and Mg^{2+} concentrations were increased. Third, the apparent affinity for Ca^{2+} and Mg^{2+} was significantly diminished at elevated external H^+ concentrations. Fourth, the anomalous-mole fraction behavior of H^+ permeation further suggests that protons compete with divalent cations for binding sites in the TRPM7 pore. Taken together, it appears that at physiological pH (7.4), Ca^{2+} and Mg^{2+} bind to TRPM7 and inhibit the monovalent cationic currents; whereas at high H^+ concentrations, the affinity of TRPM7 for Ca^{2+} and Mg^{2+} is decreased, thereby allowing monovalent cations to pass through TRPM7. Furthermore, we showed that the endogenous TRPM7-like current, which is known as Mg^{2+}-inhibitable cation current (MIC) or Mg nucleotide–regulated metal ion current (MagNuM) in rat basophilic leukemia (RBL) cells was also significantly potentiated by acidic pH, suggesting that MIC/MagNuM is encoded by TRPM7. The pH sensitivity represents a novel feature of TRPM7 and implies that TRPM7 may play a role under acidic pathological conditions.

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INTRODUCTION

TRPM7 is a ubiquitously distributed ion channel that belongs to the long or melastatin-related transient receptor potential (TRPM) ion channel subfamily (Harteneck et al., 2000; Montell, 2001; Clapham, 2003; Fleig and Penner, 2004). It is unique in being both an ion channel and a protein kinase. Although the physiological functions of the kinase are not well understood, recent studies have suggested that TRPM7 plays important roles in cellular Mg^{2+} homeostasis (Schmitz et al., 2003), anoxic neuronal cell death (Aarts et al., 2003), cell proliferation and viability (Nadler et al., 2001; Hanano et al., 2004), and diseases caused by abnormal magnesium absorption (Schlingmann et al., 2002; Walder et al., 2002; Chubanov et al., 2004).

TRPM7 produces pronounced outward currents at nonphysiological voltages ranging from +50 to +100 mV and small inward currents at negative potentials between −100 to −40 mV when expressed heterologously in mammalian cells (Nadler et al., 2001; Runnels et al., 2001; Monteilh-Zoller et al., 2003; Schmitz et al., 2003). Unlike some other TRP channels that are gated or potentiated by activation of the PLC pathway (Clapham, 2003), TRPM7 is inhibited by depletion of PIP_2 mediated by PLC activation (Runnels et al., 2002; Aarts et al., 2003). The basal activity of TRPM7 is regulated by millimolar levels of intracellular MgATP and Mg^{2+}, so that TRPM7 is activated by depletion of intracellular MgATP and Mg^{2+}, and is inhibited by high concentrations of MgATP and Mg^{2+} with an IC_{50} of ∼0.6 mM (Nadler et al., 2001). The mechanism by which Mg^{2+} inhibits TRPM7, however, is not yet entirely clear (Nadler et al., 2001; Hermosura et al., 2002; Prakriya and Lewis, 2002; Runnels et al., 2002; Kerschbaum et al., 2003; Kozak and Cahalan, 2003; Monteilh-Zoller et al., 2003; Schmitz et al., 2003). Other divalent cations have also been reported to inhibit TRPM7 (Kozak and Cahalan, 2003).

Although inactivation of TRPM7 has been extensively investigated, the activation mechanism of TRPM7 under physiological conditions remains unknown. Intracellular Mg^{2+} levels (0.5–1 mM) under physiological conditions can inactivate >50% of TRPM7 channel activities (Nadler et al., 2001; Kozak and Cahalan, 2003). Thus, the inward current amplitude, which is usually 1/30 to 1/10 of the outward current amplitude measured at +100 mV (Nadler et al., 2001; Runnels et al., 2001; Schmitz et al., 2003), may be very small under

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Abbreviations used in this paper: DVF, divalent-free solution; MagNuM, Mg nucleotide–regulated metal ion current; MIC, Mg^{2+}-inhibitable cation; RBL, rat basophilic leukemia; TRPM, melastatin-related transient receptor potential.

physiological internal Mg^{2+} levels in the native cells. Given the potential important physiological functions (Nadler et al., 2001; Runnels et al., 2002; Schlingmann et al., 2002; Walder et al., 2002; Aarts et al., 2003; Ryazanova et al., 2004; Schmitz et al., 2003; Chubanov et al., 2004; Hanano et al., 2004), it is likely that TRPM7 inward current may be potentiated by physiological or pathological stimuli. A recent study showed that TRPM7 is up- and down-regulated in a cAMP- and PKA-dependent manner (Takezawa et al., 2004), with the changes assessed by outward current amplitude. H_2O_2 was reported to increase TRPM7 inward currents by one- to twofold after prolonged (30–50 min) incubation (Aarts et al., 2003). However, the mechanism by which H_2O_2 regulates TRPM7 remains unclear.

In the present study, we demonstrate that protons markedly potentiate TRPM7 inward currents. Lowering extracellular pH increases the inward current by ~10-fold, whereas the outward current is only changed by <30%. This transforms the normal outward rectification of TRPM7 to rectification in both the inward and outward directions. Further, our data suggest that protons enhance TRPM7 inward currents by competing with external Ca^{2+} and Mg^{2+} for binding sites in the TRPM7 pore. Therefore, the effect of acidic pH on TRPM7 is more pronounced when extracellular Ca^{2+} concentration is decreased. Importantly, we show that the well-characterized endogenous TRPM7-like current MIC (Mg^{2+}-inhibitable cation)/MagNuM (Mg nucleotide–regulated metal ion) in rat basophilic leukemia (RBL) cells is similarly potentiated by a decrease in extracellular pH. As high proton concentrations (pH < 6) can be generated during various forms of injury, including infection, inflammation, and ischemia (Jacobus et al., 1977; Stevens et al., 1991; Steen et al., 1992), the significant increase in TRPM7 inward currents by protons suggests that TRPM7 may play a role under acidic pathological conditions.

MATERIALS AND METHODS

Cell Culture

HEK-293 cells stably transfected with a FLAG-tagged murine TRPM7 in pCDNA4/TO vector were provided by A. Scharenberg (University of Washington, Seattle, WA). Cells were grown with DMEM medium supplemented with 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin, blasticidin (5 μg/ml), and zeocin (0.4 mg/ml). Expression of TRPM7 was induced 12–24 h before experiments by adding 1 μg/ml tetracycline to the culture medium (Nadler et al., 2001; Schmitz et al., 2003). Unless otherwise stated, experiments were conducted using TRPM7 expressing HEK-293 cells after tetracycline induction.

RBL-2H3 cells were provided by D. Clapham (Harvard Medical School, Boston, MA). Cells were cultured in DMEM supplemented with 10% FBS and 100 U/ml penicillin and 100 mg/ml streptomycin. For electrophysiological experiments, cells were plated onto glass coverslips and used 12 h thereafter.

Electrophysiology

Whole-cell currents were recorded using an Axopatch 200B (Axon Instruments, Inc.) amplifier. Data were digitized at 20 kHz and low-pass filtered at 2 kHz. pClamp9 software was used for data acquisition and analysis. Patch electrodes were pulled from borosilicate glass and fire polished to a resistance of ~3 MΩ when filled with internal solutions. Series resistance (R_s) was compensated up to 90% to reduce series resistance errors to <5 mV. Cells in which R_s was >10 MΩ were discarded (Yue et al., 2002). All experiments were conducted at 22 ± 2°C.

Voltage stimuli lasting 250 ms were delivered at 1- to 5-s intervals, with either voltage ramps or voltage steps ranging from −120 to +100 mV. Unless otherwise stated, 3–5 min were allowed to let TRPM7 current develop and reach a steady state after break-in. A fast perfusion system was used to exchange extracellular solutions. A complete solution exchange can be achieved in ~1–3 s.

The internal pipette solution (P1) for whole cell current recordings in HEK-293 cells stably expressing TRPM7 contained (in mM) 145 Cs-methanesulfonate, 8 NaCl, 10 EGTA, and 10 HEPES, pH adjusted to 7.2 with CsOH. In some experiments, Mg^{2+} was added to the pipette solution and the free Mg^{2+} concentration was titrated to 3 mM (calculated with the MaxChelator software, available at http://www.stanford.edu/~cpatton/webmaxcS.htm). For current recordings in RBL cells, pipette solution (P2) contained (in mM) 145 Cs-methanesulfonate, 8 NaCl, 1 EGTA, 0.084 Ca^{2+}, 1 $MgCl_2$, 5 ATP-Na_2, 10 HEPES, pH adjusted to 7.2 with CsOH. Free Ca^{2+} and Mg^{2+} concentrations were estimated at ~15 nM and 26 μM, respectively (MaxChelator). In experiments designed to diminish outward currents, pipette solution (P3) contained (in mM) 120 NMDG, 108 glutamic acid, 10 HEPES, 10 EGTA, 10 CsCl, pH adjusted 7.2 with NMDG.

The standard extracellular Tyrode's solution contained (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 20 HEPES, and 10 glucose, pH adjusted to 7.4 (NaOH). HEPES (20 mM) was used in the solutions at pH 7.0 and 7.4, and was replaced by 10 mM HEPES and 10 mM MES for the solutions at pH ≤ 6 (Jordt et al., 2000; Askwith et al., 2004; Yermolaieva et al., 2004). Divalent-free solution (DVF) contained (in mM) 145 NaCl, 20 HEPES, 5 EGTA, 2 EDTA and 10 glucose, with estimated free $[Ca^{2+}] < 1$ nM at pH 7.4 and free $[Mg^{2+}] \approx 10$ nM at pH 7.4 (MaxChelator). HEPES (20 mM) was replaced by 10 mM HEPES and 10 mM MES in DVF solutions at pH 4.0, and the estimated free $[Ca^{2+}]$ was 7.7 μM and free $[Mg^{2+}]$ was 9.9 μM in DVF at pH 4.0 (MaxChelator). Appropriate Ca^{2+} or Mg^{2+} was added to the DVF at pH 7.4 to prepare solutions containing ≤10 μM Mg^{2+} or Ca^{2+} (Fig. 6). Solutions containing 1, 2, and 10 mM Mg^{2+} or Ca^{2+} at both pH 4.0 and 7.4 were prepared by omitting EDTA and EGTA in the DVF solution, and by adding the appropriate concentrations of Mg^{2+} or Ca^{2+}. Isotonic Ca^{2+} or Mg^{2+} solution contains 120 mM Ca^{2+} or Mg^{2+}, 10 mM HEPES, 10 mM glucose, with pH adjusted to pH 7.4 or pH 4.0. Anomalous mole fraction behavior of Ca^{2+} permeation (Fig. 3 F) was evaluated in a series of external solutions, including isotonic Ca^{2+} (120 mM), 10 mM Ca^{2+}, 2 mM Ca^{2+}, 1 mM Ca^{2+}, 100 μM Ca^{2+}, and nominally Ca^{2+}-free solution in which free Ca^{2+} concentration was estimated at 10 μM (Vennekens et al., 2000; Yue et al., 2001). The solutions containing 100 μM to 10 mM Ca^{2+} were prepared from normal Tyrode's solution by adding appropriate concentration of Ca^{2+}, with reductions in Na^+ concentration when necessary to keep the constant osmolarity. The same method was used to prepare a series of solutions containing various Mg^{2+} concentrations for anomalous mole fraction behavior of Mg^{2+} permeation experiment shown in Fig. 3 F. Cells were usually exposed to acidic solutions for ~30 s to avoid desensitization unless otherwise stated. Current amplitude was measured at −120 or +100 mV. Amiloride was added to the perfusate as indicated in the text. All the chemicals for electrophysiological experiments were from Sigma-Aldrich.

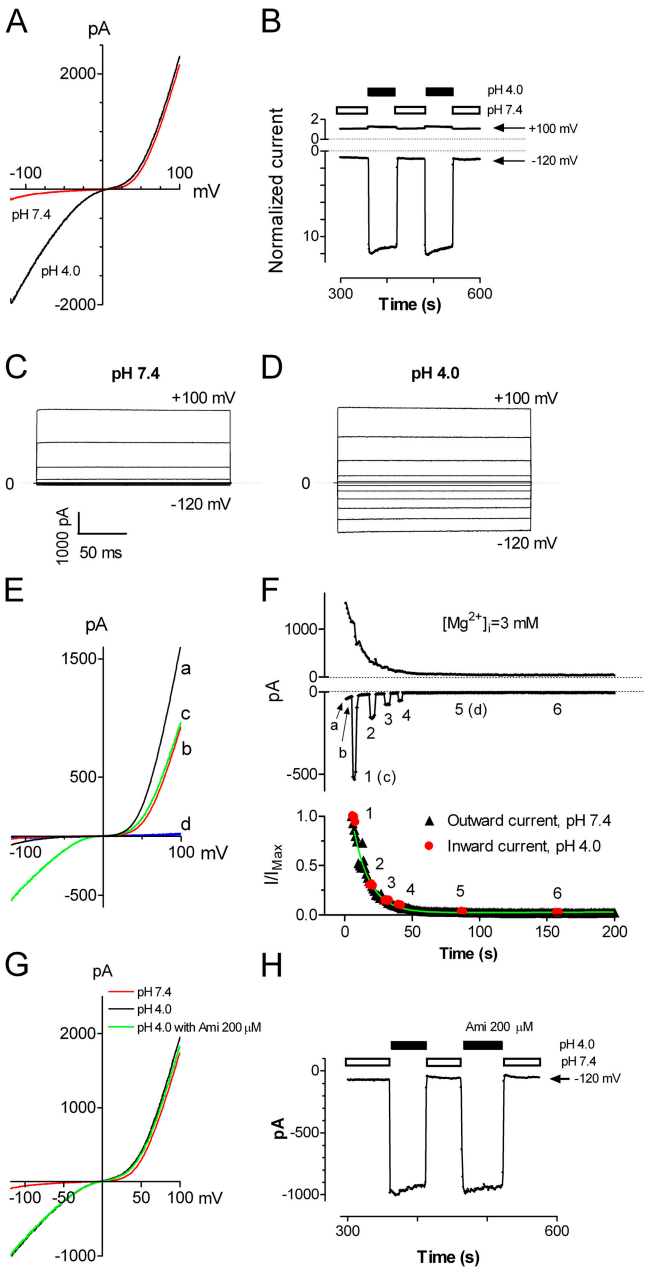

Figure 1. Potentiation of TRPM7 inward currents by external protons in HEK-293 cells stably expressing TRPM7. (A) Representative TRPM7 currents evoked by voltage ramps ranging from −120 to +100 mV in the external Tyrode's solutions at pH 7.4 and pH 4.0. (B) Normalized outward (+100 mV) and inward currents (−120 mV) at pH 4.0 and pH 7.4. The acidic external solution (pH 4.0) was applied after the current amplitude reached a steady-state (∼5 min after break-in). Repetitive application of acidic solution produced a similar increase in inward and outward currents. (C and D) TRPM7 currents elicited by voltage steps ranging from −120 to +100 mV with an increment of 20 mV at pH 7.4 (C) and pH 4.0 (D). Only inward current was significantly increased at pH 4.0 (D). (E) Currents recorded at various time points in a cell dialyzed with the pipette solution containing 3 mM free Mg^{2+}. a, immediately after formation of whole-cell configuration; b, before the external solution was changed from pH 7.4 to pH 4.0; c, the first time application of external solution at pH 4.0 induced an increase in inward current; d, the fifth time application of external solution at pH 4.0 could not induce any change because TRPM7 was completely blocked by intracellular Mg^{2+}. (F) In the same cell as shown in E, continuous changes in inward (top) and outward (middle) currents measured at +100 mV and −120 mV were plotted as a function of time. The inward currents labeled 1, 2, 3, 4, 5, and 6 (middle) represent the time points when the cell was exposed to the acidic external solution (pH 4.0). (Bottom) Normalized inward current at pH 4.0 (filled circle, red) superimposed with the normalized outward current at pH 7.4 (filled triangle, black). The green line obtained by fitting the normalized outward current represents currents decay. (G) TRPM7 currents recorded at pH 7.4 and pH 4.0 with and without 200 μM amiloride (Ami), respectively. (H) Time-dependent changes of the inward current induced by pH 4.0 in the presence and absence of 200 μM amiloride (Ami).

Data Analysis

Pooled data are presented as mean ± SEM. Concentration–response curves were fitted by an equation of the form: $E = E_{max}\{1/[1 + (EC_{50}/C)^n]\}$, where E is the effect at concentration C, E_{max} is maximal effect, EC_{50} is the concentration for half-maximal effect and n is the Hill coefficient (Yue et al., 2000). EC_{50} is replaced by IC_{50} if the effect is an inhibitory effect. Statistical comparisons were made using two-way analysis of variance (ANOVA) and two-tailed t test with Bonferroni correction; $P < 0.05$ indicated statistical significance.

RESULTS

Potentiation of TRPM7 Inward Currents by Protons

TRPM7 currents were elicited by voltage ramps or voltage steps ranging from −120 to +100 mV from a holding potential of 0 mV (Runnels et al., 2001). As previously reported, TRPM7 produced large outward currents and small inward currents (Fig. 1 A, red trace) (Nadler et al., 2001; Runnels et al., 2001; Schmitz et al., 2003). After break-in, 3–5 min was allowed to let TRPM7 current amplitude reach a steady state (see MATERIALS AND METHODS) before changing external solutions. As the measurable outward current occurs at nonphysiological range, we investigated whether the TRPM7 inward current can be potentiated by pathological stimuli. As shown in Fig. 1 A, the inward current of TRPM7 was dramatically increased by a decrease in the extracellular pH to 4.0. In the same cell as shown in Fig. 1 A, a similar degree of increase in inward current was observed upon a second application of the external solution at pH 4.0, indicating that the effect of pH 4.0 on TRPM7 was reversible and reproducible (Fig. 1 B). While the inward currents were increased by ∼10-fold (at −120 mV) at pH 4.0, the outward currents measured at +100 mV were only mildly changed, showing an ∼30% increase (Fig. 1 B, top). The large increase in inward current compared with the small change in outward current induced by acidic pH was also evident in the currents elicited by voltage steps ranging from −120 to +100 mV (Fig. 1, C and D).

To test whether the changes elicited by low pH were mediated by TRPM7, we studied the effects of acidic pH solution on control cells. In HEK-293 cells without tetracycline induction of TRPM7 expression, small endogenous TRPM7-like currents were recorded. The endogenous inward currents were also enhanced by ~10-fold at pH 4.0 (n = 6; unpublished data). Although the outwardly rectifying I-V curve suggests that the endogenous current in the HEK-293 cells is TRPM7-like current, we cannot exclude the possibility that the current may be from some leaky expression. To further confirm that the pH 4.0–elicited increases in inward current are through TRPM7, we did the following experiments. As TRPM7 is ubiquitously expressed in various cell types (Nadler et al., 2001; Hermosura et al., 2002; Kozak et al., 2002; Prakriya and Lewis, 2002; Runnels et al., 2002; Aarts et al., 2003; Jiang et al., 2003; Kozak and Cahalan, 2003), including the HEK-293 cells used to create the stable TRPM7 expressing cell lines (Nadler et al., 2001; Takezawa et al., 2004), it is difficult to find a cell type that does not have endogenous TRPM7 expression. Therefore, we used a pipette solution containing 3 mM free Mg^{2+} to suppress TRPM7 currents and then measured the response of the cells to acidic pH. TRPM7 currents were apparent immediately after formation of the whole-cell configuration (Fig. 1 E, a and b), but then the current amplitude gradually decreased (Fig. 1 F). Application of external solution at pH 4.0 induced dramatic increases in inward current with a small increase in outward currents early after forming the whole cell configuration (Fig. 1 E, c, and Fig. 1 F, 1(c)). Before the current was completely inhibited by intracellular Mg^{2+}, exposing the cell to the external solution at pH 4.0 for a second, third, and fourth time repeatedly induced significant increases in TRPM7 inward currents (Fig. 1 F, 1, 2, 3, and 4). After TRPM7 was totally blocked by intracellular Mg^{2+}, pH 4.0 failed to induce any change in current amplitude (Fig. 1 E, d, and Fig 1 F, 5 and 6). Similar results were observed in five other cells, suggesting that pH 4.0–elicited increases in current amplitude are through TRPM7. In addition, the normalized inward current at pH 4.0 plotted as a function of time superimposed with that of normalized outward current at pH 7.4, indicating that the inward current at pH 4.0 and the outward current at pH 7.4 decay at the same rate when the pipette solution contains 3 mM free Mg^{2+} (Fig. 1 F, bottom), further indicating that the pH 4.0–induced increase in inward current is mediated by TRPM7.

To rule out the possibility that proton-activated Na channels (Waldmann and Lazdunski, 1998) were involved in the increased inward current elicited by low pH, amiloride (200 μM) was added to the external solution. No significant difference was observed in inward currents elicited by pH 4.0 in the presence and absence of 200 μM amiloride (Fig. 1, G and H), suggesting that the pH 4.0–induced inward currents were not due to acid-sensitive channels of the degenerin family (Waldmann and Lazdunski, 1998). Similarly, contamination by a proton-activated Cl^- current (Cherny et al., 1997) was excluded because acidic pH–induced increases in TRPM7 currents were not affected by replacement of NaCl with $NaSO_3CH_3$ in the external solution (see Fig. 2). All the above-mentioned results suggest that the marked increases in inward currents evoked by acidic pH in TRPM7-overexpressing cells were mediated by TRPM7 channels.

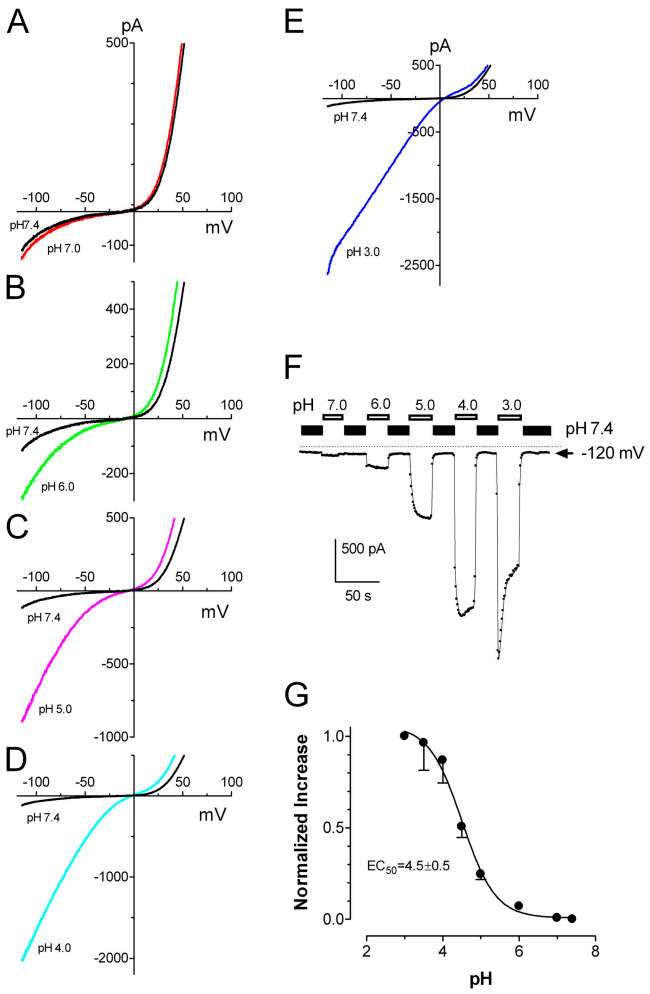

Figure 2. Concentration-dependent effects of protons on TRPM7 currents. (A–E) Potentiation of TRPM7 inward currents by protons at pH 7.0 (A), pH 6.0 (B), pH 5.0 (C), pH 4.0 (D), and pH 3.0 (E). Representative traces were elicited by voltage ramps ranging from −120 to +100 mV in the external solutions with NaCl replaced by $NaSO_3CH_3$. The y axis in each panel was scaled to illustrate the changes in inward currents. (F) Concentration-dependent changes in inward current amplitude measured at −120 mV from the recordings elicited by voltage ramps at indicated pH. The dashed line represents zero current levels. (G) The increase in current amplitude at the indicated pH was normalized to the value at pH 3.0. Best fit of the normalized data yielded an EC_{50} = 4.5 ± 0.5 pH unit (mean ± SEM, n = 8; Hill coefficient was 1).

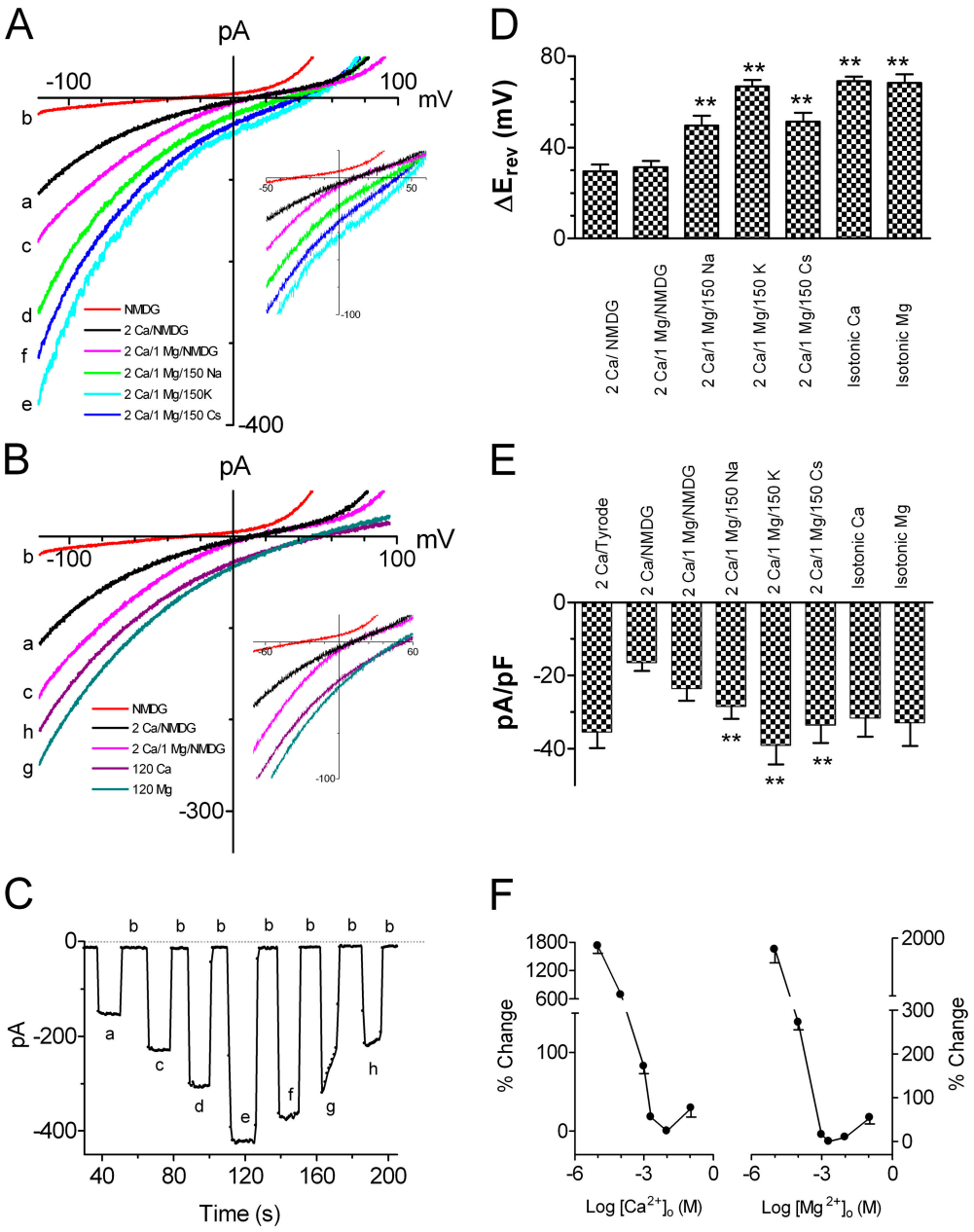

Figure 3. Monovalent permeability of TRPM7 at pH 7.4. (A and B) Original recordings of TRPM7 currents obtained using P3 pipette solution in the following external solutions at pH 7.4 (mM): a, 2 Ca^{2+}/NMDG; b, isotonic NMDG; c, 2 Ca^{2+}/1 Mg^{2+}/NMDG; d, 2 Ca^{2+}/1 Mg^{2+}/150 Na^{+}; e, 2 Ca^{2+}/1 Mg^{2+}/150 K^{+}; f, 2 Ca^{2+}/1 Mg^{2+}/150 Cs^{+}; g, isotonic Mg^{2+} (120 mM); h, isotonic Ca^{2+} (120 mM). The insets show an expanded section of the graph to illustrate changes in reversal potential in different solutions. (C) Inward current amplitude in a representative cell in the indicated external solutions. (D) Changes in reversal potential obtained by subtracting reversal potential in isotonic NMDG from those in test solutions (mean ± SEM, $n = 6$). **, $P < 0.01$ in comparison with the value obtained in 2 Ca^{2+}/1 Mg^{2+}/NMDG. (E) Averaged TRPM7 current amplitude in various test solutions. Note that the current amplitude in 2 Ca^{2+}/1 Mg^{2+}/150 Na^{+}, 2 Ca^{2+}/1 Mg^{2+}/150 K^{+}, and 2 Ca^{2+}/1 Mg^{2+}/150 Cs^{+} is significantly larger than that in 2 Ca^{2+}/1 Mg^{2+}/NMDG. (F) Ca^{2+}- and Mg^{2+}-dependent anomalous mole-fraction effects. In the left panel, changes in inward current measured at −120 mV were normalized in reference to the value at 10 mM extracellular Ca^{2+} (extracellular solutions were nominally Mg^{2+} free). In the right panel, changes in inward current measured at −120 mV were normalized in reference to the value at 2 mM extracellular Mg^{2+} (extracellular solutions were nominally Ca^{2+} free). Note that the current amplitude is minimal in the external solutions with 10 mM Ca^{2+} (left) or 2 mM Mg^{2+} (right) (mean ± SEM, $n = 6$).

We next studied concentration-dependent effects of protons on TRPM7. A small increase in the inward current was seen at pH 7.0 (Fig. 2 A); the increase was significant at pH 6.0 and reached a maximum at pH 3.0 (Fig. 2, B–E). The concentration-dependent increase of TRPM7 inward currents from the same cell is shown in Fig. 2 F. At pH 3.0, the average inward current amplitude measured at −120 mV was increased by 10.2 ± 1.3–fold compared with the current amplitude at pH 7.4. The pH required for inducing half-maximal increase in inward current was 4.5 (Fig. 2 G). Changes in the outward current (measured at +100 mV) were much smaller than those of the inward current. For example, 13.1 ± 1.1%, 23.1 ± 2.5%, 23.3 ± 3.1%, and 24.8 ± 3.4% increases were observed at pH 7.0, 6.0, 5.0, and 4.0 ($P < 0.05$), respectively; whereas a 26.5 ± 4.0% ($P < 0.05$) decrease was seen at pH 3.0.

Protons Increase Monovalent Cation Permeability through TRPM7

We previously reported that TRPM7 is a nonselective cation channel that is permeable to both monovalent

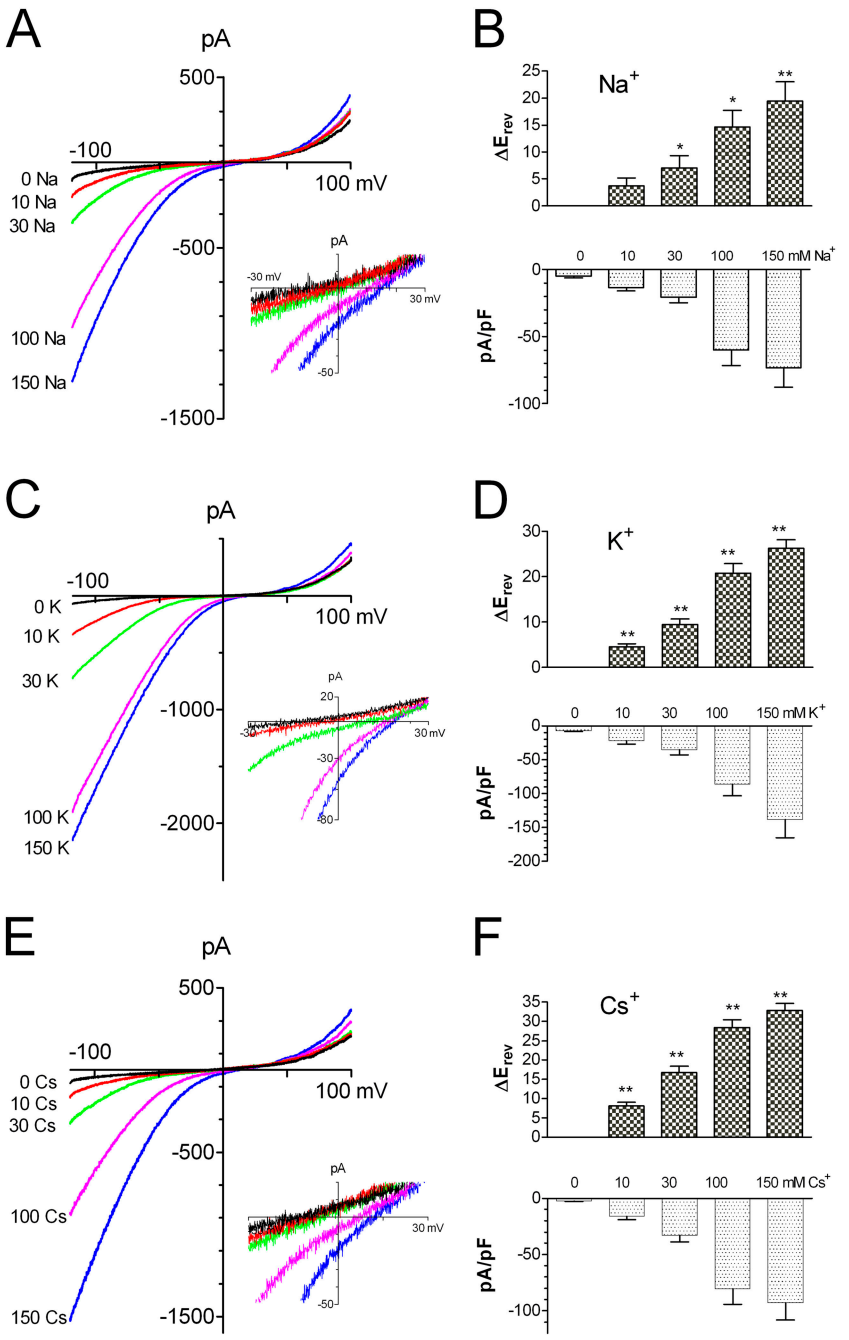

Figure 4. Protons enhance TRPM7 inward currents by increasing monovalent cation permeability. (A, C, and E) Representative recordings of TRPM7 in 0, 10, 30, 100, and 150 mM Na^+ (A), K^+ (C), and Cs^+ (E) in the presence of 10 mM Ca^{2+} at pH 4.0, respectively. The external solution containing 10 mM Ca^{2+}/150 mM NMDG (pH 4.0) was used as a control solution. When monovalent ion concentrations were increased, equal molar NMDG was omitted from the solution to keep the osmolarity constant. Note the concentration-dependent increase of TRPM7 conductance in A, C, and E. The insets show expanded section of the graphs to illustrate changes in reversal potential in various monovalent ion concentrations. (B, D, and F) Average changes in reversal potential (top) and inward current density (bottom) measured at −120 mV in the external solutions with various concentrations of monovalent cations. The changes in reversal potential were obtained by subtracting reversal potential in the control solution (10 mM Ca^{2+}/NMDG) from those in test solutions with various monovalent cation concentrations (mean ± SEM; $n = 6$ [B]; $n = 7$ [D]; $n = 9$ [F]). *, $P < 0.05$; **, $P < 0.01$ in comparison with E_{rev} in 10 mM Ca^{2+}/NMDG.

and divalent cations (Runnels et al., 2001). Monovalent permeability in the presence of divalent cations was also reported for the MIC channel in Jurkat cells (Prakriya and Lewis, 2002). Other studies suggested that TRPM7 is selective for divalent ions, and the inward current was carried exclusively by divalent cations in external solutions containing 10 mM Ca^{2+} and 2 mM Mg^{2+} (Nadler et al., 2001). To test whether monovalent cations contribute to TRPM7 inward currents under physiological Ca^{2+} (2 mM) and Mg^{2+} (1 mM) concentrations, we compared the TRPM7 current amplitude and reversal potentials in extracellular solutions containing different monovalent cations, with those in the isotonic divalent cation solutions (Fig. 3 B). The P3 pipette solution containing lowered Cs^+ concentration (10 mM, see MATERIALS AND METHODS) was used to minimize outward current amplitude (Fig. 3). In the presence of 2 mM Ca^{2+} and 1 mM Mg^{2+}, changing the external solution from nonpermeable NMDG (Jiang et al., 2003; Kozak and Cahalan, 2003) to the solutions containing 150 mM Na^+, K^+, or Cs^+ significantly increased inward current amplitude and shifted reversal potentials (Fig. 3, A, C, D, and E), indicating that under physiological divalent cation concentrations,

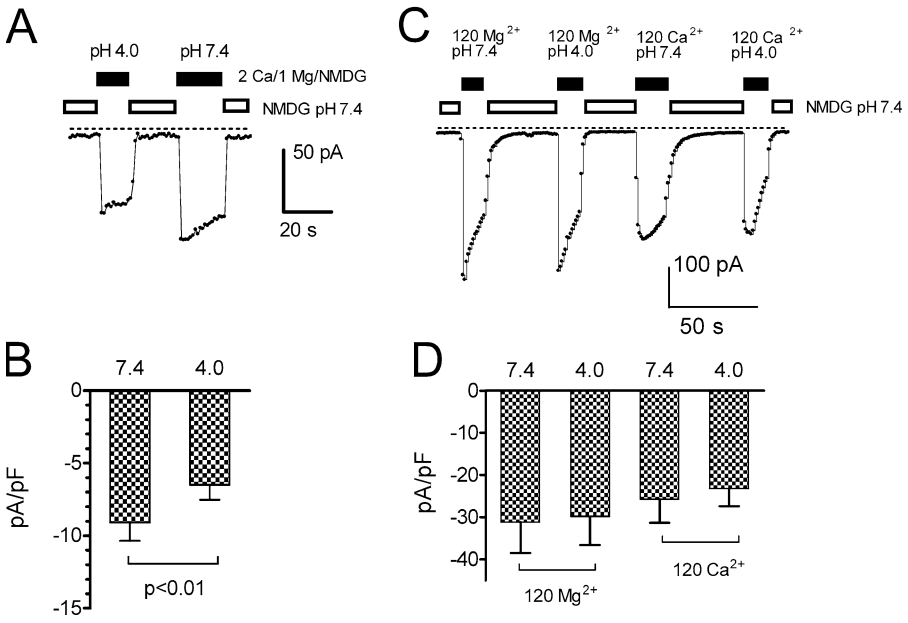

Figure 5. Acidic pH does not increase TRPM7 divalent conductance. Currents were elicited by voltage ramps (−120 to +100 mV) with P1 pipette solution. (A) Divalent current amplitude measured at −120 mV in the external solutions containing 2 mM Ca^{2+}/1 mM Mg^{2+}/NMDG at pH 7.4 and pH 4.0, respectively. Dash line represents zero current level. (B) Averaged divalent current density of TRPM7 measured at −120 mV in the external solutions as shown in A (mean ± SEM, $n = 8$). Current density at pH 4.0 was significantly smaller than that at pH 7.4. (C) Current amplitude of TRPM7 measured at −120 mV in the isotonic Mg^{2+} and isotonic Ca^{2+} solutions at pH 7.4 and pH 4.0, respectively. Dashed lines represent zero current level. (D) Mean current density at −120 mV in the isotonic Mg^{2+} or Ca^{2+} solutions at pH 7.4 and pH 4.0, respectively (mean ± SEM, $n = 6$).

monovalent cationic currents contribute to TRPM7 inward currents. We further studied the relative effects of divalent cations on TRPM7 monovalent currents. Fig. 3 F shows anomalous mole fraction behavior of Mg^{2+} and Ca^{2+} permeation (Fig. 3 F). The smallest current amplitude was observed in the external solutions containing 10 mM Ca^{2+} or 2 mM Mg^{2+}, respectively.

We proceeded to study the monovalent conductance under acidic conditions by using external solutions containing different concentrations of Na^+, K^+, or Cs^+ in the presence of 10 mM Ca^{2+} (Fig. 4). The P3 pipette solution was used in this experiment. Whole-cell configuration was established in the 2 mM Ca^{2+} Tyrode's solution at pH 7.4, and cells were then exposed to 0, 10, 30, 100, and 150 mM Na^+, K^+, or Cs^+ solutions in the presence of 10 mM Ca^{2+} at pH 4.0 for 30–60 s. Concentration-dependent increases in current amplitude and shift of reversal potentials at high monovalent cation concentrations at pH 4.0 were observed (Fig. 4, A, C, and E, also see insets). These results (Fig. 4) indicate that the proton-evoked increases in TRPM7 inward currents were attributed to the increased Na^+, K^+, and Cs^+ conductance.

To ensure that protons enhance monovalent conductance without increasing divalent conductance, we evaluated the effects of acidic pH on divalent currents. At physiological concentrations of Ca^{2+} (2 mM) and Mg^{2+} (1 mM) in the presence of NMDG solution, protons significantly decreased current amplitude measured at −120 mV (Fig. 5, A and B). This result suggests that protons may compete with divalent cations for binding sites in the pore, so that low concentration of divalent cations were outcompeted by protons, therefore the divalent current amplitude at pH 4.0 was smaller than that at pH 7.4. In agreement with this notion, we found that the isotonic Ca^{2+} and Mg^{2+} currents at pH 4.0 were not significantly different from those at pH 7.4 (Fig. 5, C and D). Thus, the acidic external solution–induced increase in inward currents was mediated by increasing monovalent cation permeability through TRPM7.

Protons Increase TRPM7 Currents by Competing with Ca^{2+} and Mg^{2+} for Binding Sites

To investigate the mechanism by which protons potentiate TRPM7 monovalent inward currents, we studied the effects of protons on TRPM7 currents in the presence of various external Ca^{2+} or Mg^{2+} concentrations. At pH 4.0, protons induced a maximal increase in inward current in the external solution containing 0.5 mM Ca^{2+}, but only ∼30% of maximal response in the external solution containing 10 mM Ca^{2+} (Fig. 6 A). The EC_{50} for protons was changed from pH 5.1 to 4.5 and 3.4 when the external Ca^{2+} concentration was increased from 0.5 mM to 2 and 10 mM (Fig. 6 B), respectively. Similarly, pH 3.5 elicited a maximal increase in inward current in the external solution containing 1 mM Mg^{2+}, but only ∼50% maximal increase in TRPM7 inward current in the external solution containing 10 mM Mg^{2+}. The EC_{50} was changed from pH 4.6 to pH 3.6 when the external Mg^{2+} concentration was increased from 1 to 10 mM (Fig. 6, C and D). The 1.1 and 1.7 pH unit change in EC_{50} when external Ca^{2+} was changed from 0.5 to 2 and 10 mM and 1 pH unit change in EC_{50} when external Mg^{2+} was increased from 1 to 10 mM indicate that increasing external Ca^{2+} or Mg^{2+} concentrations decreases the affinity of TRPM7 for protons, and suggests

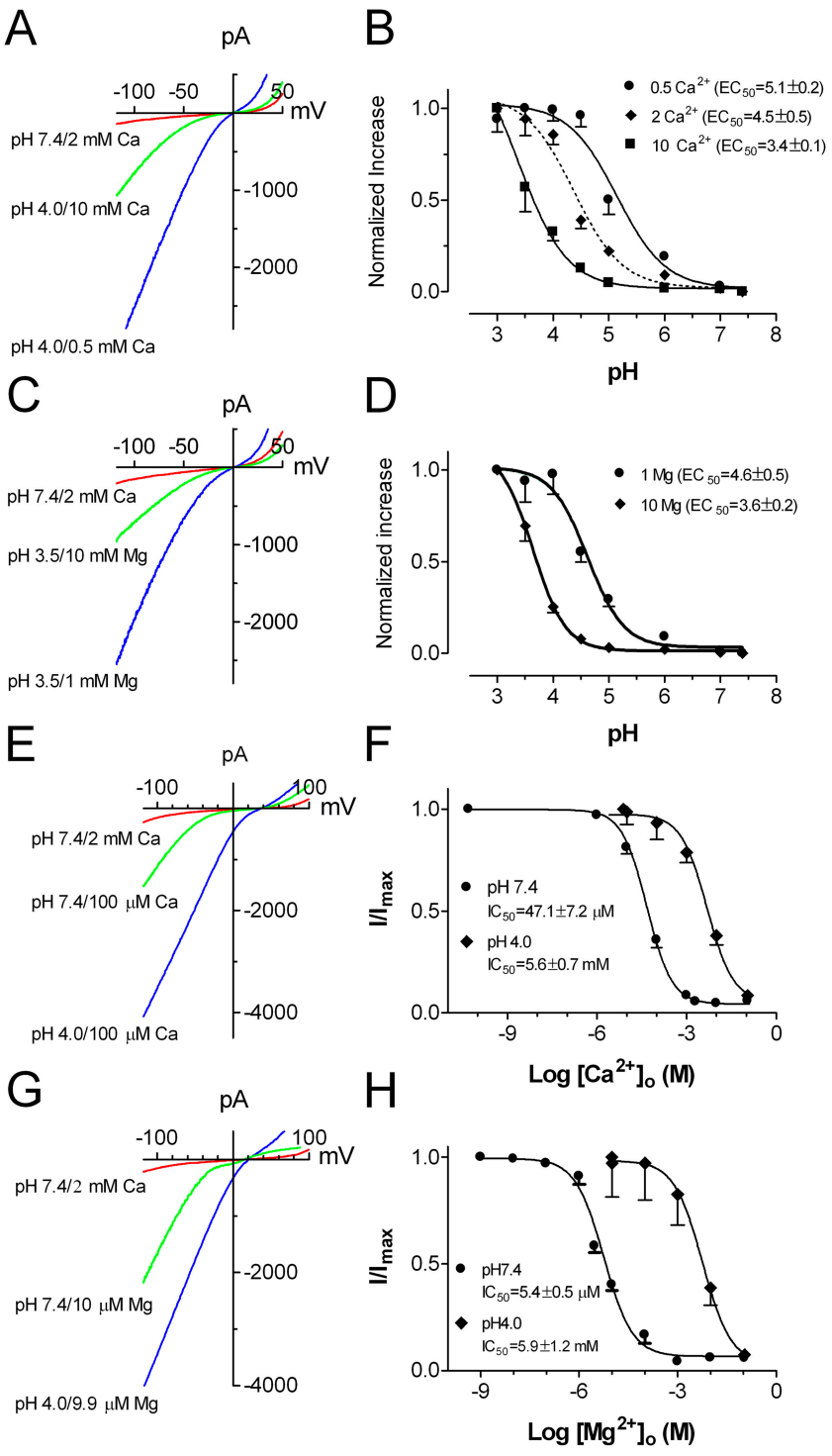

Figure 6. Protons increase TRPM7 inward currents by competing with Ca^{2+} and Mg^{2+} for binding sites. Current recorded in 2 mM Ca^{2+} Tyrode's at pH 7.4 (red) is shown in A, C, E, and G as a reference. (A) Representative traces of TRPM7 currents recorded in 0.5 and 10 mM external Ca^{2+} at pH 4.0. (B) Normalized increase in TRPM7 inward currents at indicated pH in the external solutions containing 0.5 and 10 mM Ca^{2+}. The dotted lines represent the results obtained in 2 mM external Ca^{2+} as shown in Fig 2. The pH values for 50% maximal response were 5.1 ± 0.2, 4.5 ± 0.5, and 3.4 ± 0.1 in 0.5, 2, and 10 mM external Ca^{2+} (mean ± SEM, n = 6, 8, and 6), respectively. The Hill coefficient factors were 1.0 for the dose–response curves in B, D, F, and H. (C) Representative TRPM7 currents elicited by voltage ramps in 1 and 10 mM external Mg^{2+} at indicated pH values. (D) Normalized increase in TRPM7 inward current amplitude at indicated pH in the external solutions containing various Mg^{2+} concentrations. The pH values for 50% maximal response were pH 4.6 ± 0.5 and pH 3.6 ± 0.2 in 1 and 10 mM external Mg^{2+}, respectively (mean ± SEM, n = 6 for each group). (E) Representative currents elicited by voltage ramps in 100 μM external Ca^{2+} at pH 7.4 and pH 4.0. (F) Normalized current amplitude at pH 4.0 and pH 7.4 was plotted as a function of external Ca^{2+} concentrations. The Ca^{2+} concentration required to block 50% of the monovalent currents was 47.1 ± 7.2 μM at pH 7.4 and 5.6 ± 0.7 mM at pH 4.0 (n = 6). (G) Original recordings of TRPM7 in 9.9 and 10 μM external Mg^{2+} at indicated pH values. (H) Normalized current plotted as a function of Mg^{2+} concentrations at pH 7.4 and pH 4.0. The IC_{50} was 5.9 ± 1.2 mM at pH 4.0, and 5.4 ± 0.5 μM at pH 7.4 (mean ± SEM, n = 6), respectively.

that protons compete with Ca^{2+} and Mg^{2+} for the binding sites in the TRPM7 pore.

We observed that protons produced different effects on outward currents under different external divalent cation conditions. For example, compared with the currents at pH 7.4 in 2 mM external Ca^{2+}, the outward current amplitude at pH 4.0 measured at +100 mV was changed by +41.1 ± 5.4% (n = 6), +276.7 ± 35.3% (n = 6), −22.0 ± 2.2% (n = 6), +24.8 ± 3.4% (n = 8), and +26.2 ± 8.9% (n = 6) in the external solutions containing 1 mM Mg^{2+}, 10 mM Mg^{2+}, 0.5 mM Ca^{2+}, 2 mM Ca^{2+}, and 10 mM Ca^{2+}, respectively (see Fig. 6, A and C, but the entire outward currents were not shown). The increase in outward current by low pH was larger in the external solutions with higher divalent concentrations; and the effect of protons on outward

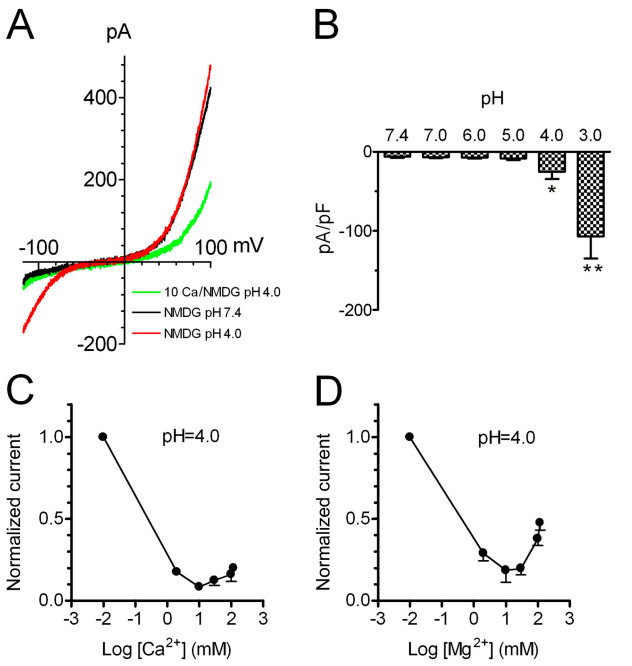

Figure 7. Currents carried by protons through TRPM7 channels. (A) Original recordings in isotonic NMDG solution at pH 7.4 and pH 4.0 using P3 pipette solution (note the inward current induced at pH 4.0, red), and in 10 Ca^{2+}/NMDG solution at pH 4.0 (green) in a HEK-293 cell overexpressing TRPM7. The proton current was largely blocked by 10 mM Ca^{2+}. (B) Averaged proton current at various test pH. Protons carry measurable currents at pH 5.0, and pronounced currents at pH 4.0 and 3.0 were observed (mean ± SEM, $n = 6$). *, $P < 0.05$; **, $P < 0.01$. (C and D) Anomalous mole fraction behavior of H^+ permeation at pH 4.0. Current amplitude measured at −120 mV at indicated Ca^{2+} or Mg^{2+} concentration was normalized in reference to the current amplitude at 10 μM extracellular Ca^{2+} or Mg^{2+} (mean ± SEM, $n = 7$).

TRPM7 current was more pronounced in Mg^{2+}-containing than in Ca^{2+}-containing external solutions. This is presumably due to the fact that, at normal pH (7.4), Mg^{2+} exhibits a stronger block on TRPM7 outward current than that of Ca^{2+} (Kerschbaum et al., 2003; Monteilh-Zoller et al., 2003). Therefore, when the Mg^{2+} block is removed by protons, larger changes in TRPM7 outward current are observed. These results are in agreement with the notion that protons compete with Ca^{2+} and Mg^{2+} for binding sites in the TRPM7 pore.

If protons and divalent cations compete for binding sites, and the binding of protons to the external sites of the channel pore allows monovalent ions to pass through TRPM7, one would expect that the inhibitory effects of Ca^{2+} and Mg^{2+} on monovalent currents should be influenced by proton concentrations. We therefore studied the inhibitory effects of Ca^{2+} and Mg^{2+} on TRPM7 monovalent currents at pH 7.4 and pH 4.0. At pH 4.0, the free Ca^{2+} concentration was 7.7 μM and the free Mg^{2+} concentration was 9.9 μM, although the same concentrations of EDTA and EGTA can decrease free Ca^{2+} and Mg^{2+} concentrations to <1 or 10 nM at pH 7.4 (see MATERIALS AND METHODS). Fig. 6 E shows representative recordings of TRPM7 in the presence of 100 μM Ca^{2+} at pH 4.0 and 7.4, respectively. IC_{50} was 47.1 μM Ca^{2+} at pH 7.4 and 5.6 mM Ca^{2+} at pH 4.0. Similarly, 10 μM Mg^{2+} produced more inhibition on TRPM7 at pH 7.4 than that at pH 4.0 (Fig. 6 G). The IC_{50} of Mg^{2+} at pH 4.0 (5.9 mM) is about 1,000-fold different from that at pH 7.4 (5.4 μM) (Fig. 6 H). The significantly decreased Ca^{2+} and Mg^{2+} affinities (Fig. 6, F and H) to TRPM7 channels in the acidic external solutions further indicate that protons compete with divalent Ca^{2+} and Mg^{2+} for binding sites, thereby allowing monovalent cations to pass through TRPM7 channels.

Anomalous-mole Fraction Behavior of H^+ Permeation

As protons compete with divalent cations for binding sites in the channel pore, we tested whether protons pass through TRPM7 channels at low external divalent cation concentrations. No current was observed in the isotonic NMDG solution at pH 7.4, 7.0, or 6.0 (Fig. 7, A and B). However, inward currents were observed in isotonic NMDG solutions at pH 5.0, 4.0, and 3.0. Since NMDG is nonpermeant (Jiang et al., 2003; Kozak and Cahalan, 2003), it seems that the inward current is carried by protons. This notion is supported by the fact that current amplitude increased with increasing proton concentrations (Fig. 7 B). A high intracellular free Mg^{2+} concentration (3 mM), which inactivates TRPM7 channels, prevented the development of proton currents in TRPM7-expressing cells (unpublished data), suggesting that the proton-carried current was mediated by TRPM7 channels. Fig. 7 (C and D) shows anomalous mole fraction behavior of H^+ permeation. The largest current amplitude was observed at 10 μM extracellular Ca^{2+} or Mg^{2+}, whereas the smallest current amplitude occurred at 10 mM extracellular Ca^{2+} or Mg^{2+}. These anomalous-mole fraction effects further indicate that protons compete with Ca^{2+} and Mg^{2+} for binding sites in the external pore of TRPM7, consistent with the results shown in Fig. 6.

Effects of Protons on the Endogenous TRPM7-like Current MIC/MagNuM in RBL Cells

Endogenous TRPM7-like currents MIC/MagNuM have been identified in a variety of cells (Nadler et al., 2001; Hermosura et al., 2002; Kozak et al., 2002; Prakriya and Lewis, 2002; Runnels et al., 2002; Aarts et al., 2003; Jiang et al., 2003). Since MIC/MagNuM in RBL cells have been well characterized (Hermosura et al., 2002; Kozak et al., 2002; Kozak and Cahalan, 2003, 2004), we chose to use RBL cells to study whether protons regulate endogenous MIC/MagNuM channels. As high concentration of intracellular Ca^{2+} blocks TRPM7 (Monteilh-Zoller et al., 2003), a pipette solution containing 15 nM

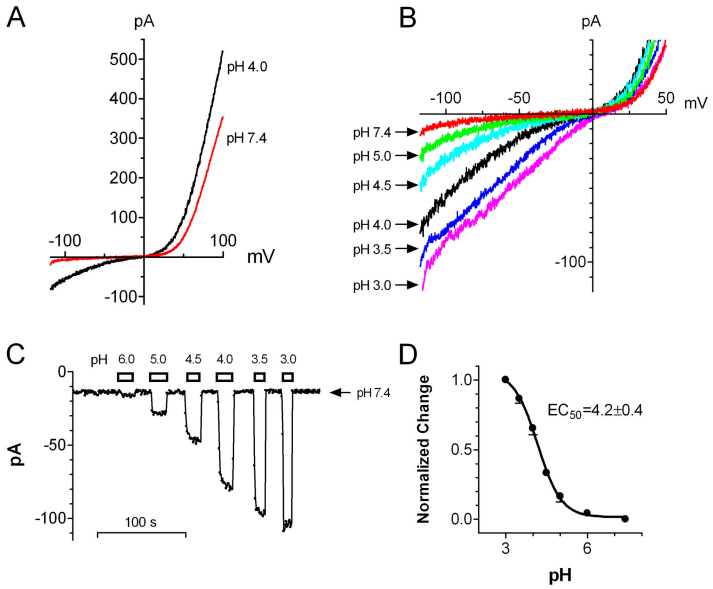

Figure 8. Effects of protons on MIC/MagNuM in RBL cells. Experiments were performed in external solutions with NaCl replaced by $NaSO_3CH_3$. (A) Representative MIC currents elicited by voltage ramps ranging from −120 to +100 mV in the external solutions at pH 7.4 (red) and 4.0 (black) in a RBL cell. (B) Concentration-dependent effects of protons on MIC inward currents in a representative RBL cell. The y axis was scaled to illustrate inward currents. (C) Inward current amplitude measured at −120 mV at different test pH. External solution was changed to Tyrode's solution at pH 7.4 after each application of low pH external solution. (D) Concentration-dependent effects of protons on MIC currents. Best fit of the normalized change yielded an EC_{50} = 4.2 ± 0.4 (mean ± SEM, n = 6; Hill coefficient factor was 1).

Ca^{2+} with weak buffering (1 mM EGTA) was used in this experiment. A low intracellular Ca^{2+} buffering condition (1 mM EGTA) was reported to be able to deactivate I_{CRAC} after its activation (Zweifach and Lewis, 1995) and was used for recording of TRPM7-like MIC/MagNuM currents in Jurkat cells (Prakriya and Lewis, 2002). We also included 5 mM CsCl in the external solution to eliminate the endogenous potassium current. NaCl was replaced by $NaSO_3CH_3$ in the Tyrode's solution at pH 4.0 to prevent contamination from proton-activated Cl^- currents. After break-in, a voltage ramp protocol was used to monitor current for 10 min to ensure the complete deactivation of I_{CRAC} (Prakriya and Lewis, 2002) and full activation of TRPM7 (Kozak et al., 2002). Similar to TRPM7 currents in the heterologous expression system, the MIC/MagNum currents in RBL cells were increased dramatically when the pH of the external Tyrode's solution changed from 7.4 to 4.0 (Fig. 8 A). The increase elicited by low pH was not affected by adding 200 μM amiloride (unpublished data), suggesting that the increase in MIC/MagNuM current was not due to proton-activated Na^+ channels. A best fit of normalized concentration-dependent increases in inward current (Fig. 8, B and C) yielded an EC_{50} of pH 4.2 ± 0.4 (Fig. 8 D), which is similar to the pH value required to induce 50% of maximal response in the heterologously expressed TRPM7 currents (see Fig. 2 G). The similar response of TRPM7 and MIC/MagNuM to acidic pH provides further evidence that MIC/MagNuM is encoded by TRPM7.

DISCUSSION

We have shown that low extracellular pH significantly enhanced TRPM7 inward current by increasing the monovalent cation permeability. The mechanism by which protons increase monovalent currents is likely to be through competition with divalent cations for binding sites in the external pore of TRPM7, thereby removing the divalent cation block on the monovalent currents. The pH sensitivity of TRPM7 and native MIC/MagNuM channels suggests that TRPM7 may play an important role under acidic pathological conditions.

TRPM7 Is an Acid-sensitive Ion Channel

Previous studies have shown that TRPM7 exhibits many unique features (Nadler et al., 2001; Runnels et al., 2001; Monteilh-Zoller et al., 2003; Schmitz et al., 2003), including the observation that it conducts small inward current at physiological voltages (−100 to −40 mV) and large outward current at +100 mV, producing the characteristic outwardly rectifying I-V. The present study extends our understanding about TRPM7 by showing that TRPM7 is also a pH-sensitive ion channel. We show that a decrease in external pH evokes a marked increase in TRPM7 inward currents, with an ∼10-fold increase at pH 4.0 and ∼1–2-fold increase at pH 6.0 in the presence of a physiological external Ca^{2+} concentration (2 mM). The dramatic increase in TRPM7 inward current by pH 4.0 transforms the normally outwardly rectifying I-V curve to a double-rectifying I-V shape. We found that the increase in TRPM7 current by protons was more pronounced when the external Ca^{2+} and Mg^{2+} concentrations were decreased. Although the physiological function of TRPM7 is not completely understood, it has been shown that TRPM7 plays an important role in neuronal cell death caused by anoxia (Aarts et al., 2003). Given that native TRPM7 is only active to a small degree in the presence of physiological intracellular Mg^{2+}, an approximately one- to

twofold increase in TRPM7 inward current at pH 6.0 suggests that TRPM7 may play an important role under acidic pathological conditions in which extracellular pH may decrease to pH 6.0 (Jacobus et al., 1977; Stevens et al., 1991; Steen et al., 1992).

Potentiation of TRPM7 inward currents by external protons is a novel feature of TRPM7. A previous study showed that a decrease in intracellular pH (pH_i) inhibits its monovalent Na^+ currents through MIC channels in Jurkat T lymphocytes (Kerschbaum and Cahalan, 1998) and RBL cells (Braun et al., 2001). It is proposed that inhibition of MIC or TRPM7 by internal Mg^{2+}, other polyvalent cations, and H^+ represents a general electrostatic cationic screening process (Kozak and Cahalan, 2003, 2004). We did not study how intracellular low pH affects TRPM7 in the present study, because the intracellular pH should not be changed due to the high concentration of HEPES buffering under our experimental conditions. The marked increase in TRPM7 inward current by external acidic pH shown in the present study and the inhibitory effects on TRPM7 outward currents by pH_i as previously reported (Kerschbaum and Cahalan, 1998; Braun et al., 2001; Kozak and Cahalan, 2003, 2004) indicate that TRPM7 is an acid-sensitive ion channel.

Potential Mechanism by which Protons Potentiate TRPM7 Inward Currents

We showed that both Ca^{2+} and Mg^{2+} exhibit anomalous mole-fraction effects at normal physiological pH. The apparent affinity of TRPM7 is 47.1 μM for Ca^{2+} and 5.4 μM for Mg^{2+}, similar to the previously reported Ca^{2+} (20 μM, at −120 mV) (Fomina et al., 2000) and Mg^{2+} affinity (3 μM, at −120 mV) to native MIC/MagNuM channels (Kerschbaum et al., 2003). Under normal physiological Ca^{2+} (2 mM) and Mg^{2+} (0.7–1.1 mM) concentrations (Konrad et al., 2004), we showed that monovalent cations contribute to the inward currents of TRPM7 (Fig. 3), and the contribution of monovalent currents becomes more pronounced under acidic conditions (pH 4.0, Fig. 4).

Several lines of evidence shown in the present study indicate that external protons increase TRPM7 inward currents by competing with divalent cations for binding sites in the TRPM7 pore, thereby enhancing monovalent cation permeability. First, there was a concentration-dependent increase in monovalent cation conductance and reversal potential for Na^+, K^+, and Cs^+ at pH 4.0, indicating that the enhanced inward TRPM7 currents resulted from an increased monovalent cation permeability. Second, the half-maximal pH was changed toward acidic pH direction by 0.6 and 1.7 pH units when the extracellular Ca^{2+} was increased from 0.5 to 2 and 10 mM, respectively; similarly, an increase of external Mg^{2+} concentration from 1 to 10 mM shifted the half-maximal pH toward acidic pH direction by 1.0 pH unit (Fig. 6). The decreased proton affinity to TRPM7 at higher extracellular divalent concentrations indicate that protons compete with Ca^{2+} and Mg^{2+} for the same binding sites, therefore, at higher concentrations of divalent cations, more protons are required to induce 50% of maximal response. Third, high proton concentrations significantly decreased the affinity of Ca^{2+} and Mg^{2+} to TRPM7. The Ca^{2+} affinity to TRPM7 was decreased by ∼100-fold and the Mg^{2+} affinity was decreased by ∼1,000-fold when the external pH was changed from 7.4 to 4.0. Fourth, anomalous mole fraction permeation of protons indicate that protons compete with Ca^{2+} and Mg^{2+} for the binding sites in the external pore of TRPM7 (Fig. 7). Taken together, it seems that at physiological pH, TRPM7 only permeates a small inward current due to the Ca^{2+} and Mg^{2+} block on the monovalent current; whereas at acidic pH, Ca^{2+} and Mg^{2+} are outcompeted by protons, which relieves the block on the monovalent current and elicits a large inward current carried by monovalent cations.

Where are the binding sites for divalent cations and protons? A high affinity site for binding Mg^{2+} within the electric field and two low-affinity sites have been proposed for MIC channel by Kerschbaum et al. (2003) using the Eyring rate model. Kerschbaum et al. also proposed that the internal Mg^{2+} inhibits MIC in a voltage-independent manner, suggesting that internal Mg^{2+} is unable to access the pore from the inside (Kerschbaum et al., 2003). Our results show that external protons elicit a marked increase in TRPM7 currents at hyperpolarized potentials but only a small increase at positive potentials. These voltage-dependent effects suggest that protons can access the TRPM7 pore and compete with Mg^{2+} and Ca^{2+} for binding. The anomalous mole fraction behavior of Ca^{2+}, Mg^{2+} (Fig. 3 F), and H^+ permeation (Fig. 7, C and D) indicates that they can bind to the TRPM7 pore, and compete for binding sites within the pore (Fig. 6).

Proton competition for Ca^{2+} binding sites and the consequent channel opening has been proposed as the gating mechanism for ASIC3 (Immke and McCleskey, 2001, 2003), a proton-activated Na^+ channel of the degenerin family (Waldmann and Lazdunski, 1998; Immke and McCleskey, 2003). Immke and McCleskey (2003) showed that the Ca^{2+} affinity was changed from K_d = 12 μM at pH 7.4 to K_d = 100 μM at pH 7.0, such that Ca^{2+} is released from a binding site and Na^+ can pass through the channel at millimolar Ca^{2+} concentrations. The authors predict that, like the Ca^{2+} chelator EGTA (with four titratable acid groups), the titratable Ca^{2+} binding site of ASIC3 is able to bind four protons (Hill coefficient is 4). Our data suggest that protons enhance TRPM7 current by a similar competing mecha-

nism, such that binding of protons to TRPM7 relieves the blockade of Ca^{2+} and Mg^{2+}, thereby allowing Na^+ to pass through TRPM7. Since the dose–response curves can be best fitted by sigmoidal does–response equation (see MATERIALS AND METHODS) with the Hill coefficient factor of 1, we assume that there is probably one binding site. We do not know if the binding site is a specific amino acid residue or a site formed by several amino acid residues. It was reported that protonation of voltage-gated Ca^{2+} channels requires multiple carboxylates of the glutamic acid (Glu) residues to form a single high affinity site (Chen and Tsien, 1997). The Glu residues are also important for proton regulation on TRPV1 (Tominaga et al., 1998; Jordt et al., 2000; Ryu et al., 2003) and TRPV5 (Yeh et al., 2003). There are seven negatively charged Glu and Aspartic acid (Asp) residues between transmembrane domain 5 (TM5) and TM6 of TRPM7, which may be involved in Ca^{2+}, Mg^{2+}, or H^+ binding. The half-maximum pH (pH 4.5) for TRPM7 is also close to the pKa of free Glu ($\sim$pH 4.0) and Asp ($\sim$pH 3.8). Thus, it is possible that Glu or Asp in the TRPM7 pore may serve as the binding sites for external Ca^{2+} and Mg^{2+}, and are also able to bind to protons, so that monovalent cations can readily pass through when the binding sites are occupied by protons. Alternatively, instead of competitive binding, protons may titrate away the block of Ca^{2+} and Mg^{2+} on monovalent current by causing conformational changes. Further studies are required to elucidate the proton binding sites and detailed mechanisms by which protons increase TRPM7 inward monovalent currents.

Potential Significance

It has been suggested that TRPM7 plays an important physiological role in Mg^{2+} homeostasis, neuronal cell death, and cell viability (Nadler et al., 2001; Schlingmann et al., 2002; Walder et al., 2002; Aarts et al., 2003; Schmitz et al., 2003; Chubanov et al., 2004). We demonstrated here that acidic pH 6.0 increases TRPM7 inward current by approximately one- to twofold. Such an acidic condition (pH $<$ 6) can occur during various forms of tissue injury (Jacobus et al., 1977; Stevens et al., 1991; Steen et al., 1992), or during repetitive nerve activities, ischemia, and seizures (Siesjo, 1988; Chesler and Kaila, 1992), suggesting that TRPM7 may play a role under acidic pathological conditions. However, without knowing the real physiological functions of TRPM7, it is difficult to predict a potential role of TRPM7 under acidic conditions. We have investigated if there are other factors that may change the pH sensitivity of TRPM7 closer to physiological pH. With 20 μM PIP_2 in the pipette solution, the EC_{50} was pH 4.7 ± 0.7 (mean ± SEM, n = 6, Hill coefficient 1.0; unpublished data), which is not significantly different from the EC_{50} obtained without PIP_2 in the pipette solution (Fig. 2). In addition, with normal physiological intracellular Mg^{2+} concentrations in a native system, the MIC/MagNuM channel is only active to a small degree. Further studies are required to elucidate the physiological and pathological roles of TRPM7 under normal and acidic conditions.

Like TRPM7, the native MIC/MagNuM current in RBL cells showed a similar acidic potentiation to that seen for TRPM7 currents in the heterologous expression system (Fig. 8), indicating that MIC/MagNuM is encoded by TRPM7. A recent study by Gwanyanya et al. (2004) showed that MIC in cardiac myocytes and RBL cells were inhibited by acidic pH. It is not clear why there is a discrepancy between our and their results. One difference is that they evaluated pH effects on MIC in divalent-free solutions, whereas we used physiological external solutions containing divalent cations in the present study.

Protons have been reported to regulate channel activities in different channel superfamilies (Hille, 2003; Holzer, 2003), including TRP channel superfamily. It has been shown that TRPV1 and TRPV4 are enhanced by low pH, whereas TRPV5 (Yeh et al., 2003) and TRPM5 (Liu et al., 2005) are inhibited by protons. We show here that protons dramatically potentiate TRPM7 inward current by competing with divalent cations for binding sites. The pH sensitivity is a novel feature of TRPM7, and the results in the present study not only provide a clue as to the potential functions of TRPM7 in vivo, but also may help to identify the amino acid residues that are important in the ion selectivity of TRPM7 channels.

Potential Limitations

Proton permeation has been observed for voltage-gated Na^+ channels when the external solution is free of Na^+ (Mozhayeva and Naumov, 1983; Hille, 2003). We showed anomalous mole fraction behavior of H^+ permeation in the external solutions free of permeant monovalent cations (Fig. 7). In 2 mM Ca^{2+}/NMDG/pH 4.0 solution, the proton carried current is 4.4 pA/pF, corresponding to 17% of the current amplitude obtained in 10 μM Ca^{2+}/NMDG/pH 4.0 (Fig. 7 C). This proton current amplitude is similar to the value shown in Fig. 5 B (6.5 pA/pF, pH 4.0), indicating that the current at pH 4.0 (Fig. 5 B) is mainly carried by protons, and consistent with the notion that protons compete with divalent cations and therefore almost blocked all the divalent cation current under the conditions shown in Fig. 5 (A and B).

It is possible that in the normal Tyrode's solution at pH 4.0, protons may pass through TRPM7 along with Na^+ or K^+. If this were the case, proton-carried current may have contributed to the inward monovalent cur-

rents at pH 4.0. However, given that the current amplitude (6.5 pA/pF, Fig. 5 B) in NMDG solution at pH 4.0 is only ~2.5% of the inward current amplitude (247 pA/pF, Fig. 2) obtained in normal Tyrode's solutions at pH 4.0, contribution of proton-carried inward currents in the normal Tyrode's solutions at pH 4.0 should be <2.5%, and should not contaminate the experimental results.

At pH 3.0, the I-V curve of TRPM7 elicited by voltage ramps seems different from those at pH >3.0 (Fig. 2 E), and the TRPM7 outward currents were inhibited by pH 3.0. In addition, a strong inactivation or desensitization was observed (Fig. 2 F) when cells were continuously exposed to the external solution at pH 3.0. The following potential mechanisms may account for the above observations. First, TRPM7 is desensitized at pH 3.0; second, protons may pass through TRPM7, resulting in low intracellular pH, which inhibits TRPM7 outward current (Kozak and Cahalan, 2004); and third, protons may exhibit complex effects on TRPM7. Further studies are required to reveal the detailed mechanisms.

Conclusion

In conclusion, we have demonstrated that acidic pH significantly increases TRPM7 inward monovalent currents by competing with divalent cations for binding sites. The pH sensitivity represents a novel feature of TRPM7. We showed that MIC/MagNuM currents were similarly potentiated by protons, suggesting that MIC/MagNuM is encoded by TRPM7. The large TRPM7 inward current elicited by low pH suggests that TRPM7 may play a role under acidic pathological conditions. Further studies are required to elucidate the mechanism by which protons potentiate TRPM7, as well as the potential significance of TRPM7 under acidic pathological conditions.

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