

# MIC Channels Are Inhibited by Internal Divalent Cations but Not ATP

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**ABSTRACT** TRPM7 channels are nonselective cation channels that possess a functional  $\alpha$ -kinase domain. It has been proposed that heterologously expressed TRPM7 channels are activated (Runnels et al., 2001) or inhibited (Nadler et al., 2001) by dialyzing the cell with millimolar levels of ATP. The endogenous correlate of TRPM7 has been identified in T-lymphocytes and RBL (rat basophilic leukemia) cells and named MagNuM (for  $\text{Mg}^{2+}$ -nucleotide-inhibited metal) or MIC (for  $\text{Mg}^{2+}$ -inhibited cation). Here, we report that internal  $\text{Mg}^{2+}$  rather than MgATP inhibits this current. Cytoplasmic MgATP, supplied by dialysis at millimolar concentrations, effectively inhibits only when a weak  $\text{Mg}^{2+}$  chelator is present in the pipette solution. Thus, MgATP acts as a source of  $\text{Mg}^{2+}$  rather than a source of ATP. Using an externally accessible site within the pore of the MIC channel itself as a bioassay, we show that equimolar  $\text{MgCl}_2$  and MgATP solutions contain similar amounts of free  $\text{Mg}^{2+}$ , explaining the fact that numeric values of  $\text{Mg}^{2+}$  and MgATP concentrations necessary for complete inhibition are the same. Furthermore, we demonstrate that  $\text{Mg}^{2+}$  is not unique in its inhibitory action, as  $\text{Ba}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Mn}^{2+}$  can substitute for  $\text{Mg}^{2+}$ , causing complete inhibition. We conclude that MIC current inhibition occurs simply by divalent cations.

## INTRODUCTION

A member of the long TRP family of ion channels, TRPM7 (also known as ChaK1, Trp-PLIK, melanoma kinase and LTRPC7) has been recently cloned and characterized by several groups (Runnels et al., 2001; Ryazanova et al., 2001; Yamaguchi et al., 2001; Nadler et al., 2001). It contains a functional  $\alpha$ -kinase domain at the C terminus (Runnels et al., 2001; Ryazanova et al., 2001), the structure of which has been determined by x-ray crystallography (Yamaguchi et al., 2001). Heterologously expressed TRPM7 forms a functional nonselective cation channel that conducts divalent ions in the inward direction and monovalent ions outwardly with a pronounced outwardly rectifying I/V relation (Runnels et al., 2001; Nadler et al., 2001). Upon removal of external divalent ions, both  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , the current drastically changes its I/V shape, becoming linear and conducting monovalent cations inwardly. The channel requires  $\text{PIP}_2$  for its function (Runnels et al., 2002), but the activation mechanism of this current remains unknown.

The presence of the  $\alpha$ -kinase domain in the protein sequence led Runnels and co-workers to test whether inclusion of ATP in the internal solution would promote channel activity during whole-cell recording (Runnels et al., 2001). Dialysis of millimolar concentrations of NaATP led to activation of outwardly rectifying cation current, and ATP-dependent phosphorylation by the kinase domain was suggested to be a requirement for channel activity (Runnels et al., 2001). Subsequently, Nadler et al. showed that internal  $\text{Mg}^{2+}$  by itself (without ATP) inhibited TRPM7 currents (Nadler et al., 2001). This result provided an alternative

explanation for the apparent channel activation by ATP; addition of NaATP simply reduced the level of free  $\text{Mg}^{2+}$  in the pipette solution and allowed the channel to conduct by reducing cytoplasmic  $\text{Mg}^{2+}$  during whole-cell dialysis and recording. In addition, MgATP and MgGTP were reported to inhibit the channel in a similar fashion, but more potently than  $\text{Mg}^{2+}$  alone (Nadler et al., 2001). It was suggested that MgATP requires micromolar free  $\text{Mg}^{2+}$  as a “cofactor” to exert its inhibitory effect. Lack of the “ $\text{Mg}^{2+}$  cofactor” was taken as the explanation for why NaATP did not inhibit on its own.

Northern analysis has shown that TRPM7 has a widespread tissue distribution and is highly expressed in lymph nodes, thymus, and bone marrow, but not in brain (Ryazanova et al., 2001). A native conductance with properties similar to expressed TRPM7 was identified and characterized in Jurkat T lymphocytes and RBL cells (Nadler et al., 2001; Hermosura et al., 2002; Prakriya and Lewis, 2002; Kozak et al., 2002). The channel was named MagNuM (for magnesium-nucleotide-inhibited metal) to emphasize a role for magnesium nucleotides in regulating the channel. An alternative nomenclature was also proposed: MIC for magnesium-inhibited cation (Prakriya and Lewis, 2002). Regardless of the nomenclature issue, the mechanism by which  $\text{Mg}^{2+}$  or MgATP inhibits the channel is uncertain.

Here we present evidence that the native MIC (MagNuM) current in RBL cells is inhibited by internal free  $\text{Mg}^{2+}$  and not by MgATP. We compare the maximal current inhibition with varying  $\text{Mg}^{2+}$  and ATP levels in the presence of weak and strong  $\text{Mg}^{2+}$  chelators. To test computed levels of free  $\text{Mg}^{2+}$ , we made use of the fact that monovalent current through MIC channels can be blocked in a voltage-dependent manner by micromolar to millimolar levels of  $\text{Mg}^{2+}$  from the outside. We also used cells with preactivated MIC current in RBL cells and a rat T-lymphocyte cell line to assay and compare changes in current as dialysis with varying levels of  $\text{Mg}^{2+}$  (and ATP) progressed. Furthermore, internal

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$\text{Mg}^{2+}$  appears not to be unique in its inhibitory action, as millimolar amounts of  $\text{Ba}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Zn}^{2+}$  also completely eliminated the current.

## MATERIALS AND METHODS

### Cell culture

Rat basophilic leukemia cells (RBL-2H3) (Siraganian et al., 1982) were cultured in Eagle's MEM supplemented with 10% fetal bovine serum, in 5%  $\text{CO}_2$ -humidified atmosphere at  $37^\circ\text{C}$ . Cells were passaged twice weekly and plated on glass cover slips for recording. Rat PAS T cells were cultured as previously described (Beeton et al., 2001).

### Patch-clamp recording

Whole-cell patch-clamp recordings were performed as previously described (Kozak et al., 2002). Briefly, patch pipettes (1.5–4 M $\Omega$  resistance) were manufactured from soda lime glass capillaries (Beckton-Dickinson, Parsippany, NJ and Kimble, Vineland, NJ). Voltage ramps (–120 to +85 mV, 211 ms duration) were delivered at 0.5 Hz frequency and current/voltage relations obtained. The cells were held at 0 mV between the ramps. Data were analyzed using Pulse/Pulsefit, v. 8.11 (HEKA Elektronik, Lambrecht, Germany), Igor Pro (v. 3.1.2) (WaveMetrics, Lake Oswego, OR), and Microcal Origin (v. 6) (Microcal Software, Northampton, MA) software.

Pipette solutions were designed to vary free  $\text{Mg}^{2+}$  and MgATP levels independently. Maxchelator (v. 1.78) software, written by Chris Patton (Stanford University), was used to calculate free divalent concentrations. The low- $\text{Mg}^{2+}$  internal solution, with free  $\text{Mg}^{2+}$  of 230 nM, consisted of (mM): 128 Cs<sup>+</sup> glutamate, 8 NaCl, 10 EDTA, 1 mM  $\text{MgCl}_2$ , 10 HEPES, pH

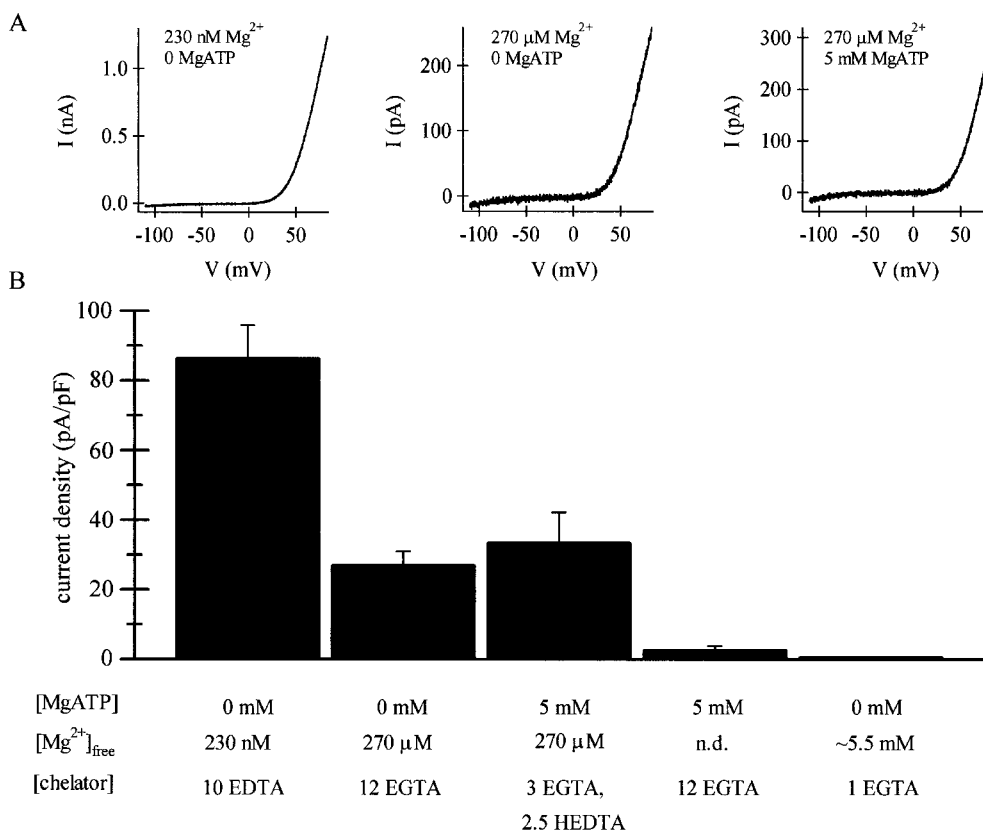
7.3. The intermediate- $\text{Mg}^{2+}$  internal solution, with free  $\text{Mg}^{2+}$  of  $\sim 270$   $\mu\text{M}$ , contained (mM): 130 Cs<sup>+</sup> glutamate, 8 NaCl, 12 EGTA, 0.5 mM  $\text{MgCl}_2$ , 10 HEPES, pH 7.3. The intermediate- $\text{Mg}^{2+}$  solution with MgATP had approximately the same level ( $\sim 270$   $\mu\text{M}$ ) of free  $\text{Mg}^{2+}$  and contained (mM): 128 Cs<sup>+</sup> glutamate, 8 NaCl, 3 EGTA, 2.5 HEDTA, 5 mM MgATP, 10 HEPES, pH 7.3. Solutions with the same  $\text{Mg}^{2+}$  and chelator concentrations were used as external solutions to test the calculated level of free  $\text{Mg}^{2+}$  experimentally. The divalent-containing external solution contained (mM): 2  $\text{CaCl}_2$ , 10 HEPES, 167 Na<sup>+</sup> aspartate, 2 mM Cs<sup>+</sup> methanesulfonate, pH 7.3. The divalent-free external solution contained (mM): 154 Cs<sup>+</sup> aspartate, 10 HEDTA, 10 HEPES, and 5 CsCl, pH 7.3. A total of 5 mM  $\text{BaCl}_2$ ,  $\text{SrCl}_2$ ,  $\text{MnCl}_2$ , or  $\text{ZnCl}_2$  were added to an internal solution containing Cs<sup>+</sup> glutamate, 1 mM EGTA, 10 mM HEPES, pH 7.3. The free  $\text{Ba}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$  concentrations were estimated at  $\sim 4$  mM.

MgATP (from a bacterial source) stock was stored at  $-20^\circ\text{C}$  and diluted in the recording solution before the experiment. MgATP and salts were purchased from Sigma (St Louis, MO). The MgATP used in this study contained 1.3 mmol  $\text{Mg}^{2+}$  per mmol of ATP.

## RESULTS

### $\text{Mg}^{2+}$ chelators distinguish the effect of internal $\text{Mg}^{2+}$ and ATP on MIC current amplitude

To test whether millimolar concentrations of MgATP inhibit MIC channels, we included 5 mM MgATP in the pipette and measured the current magnitude with different amounts of  $\text{Mg}^{2+}$  chelators present. Fig. 1 shows I/V curves and a comparison of MIC current amplitudes that develop with varying internal  $\text{Mg}^{2+}$  and MgATP concentrations. The



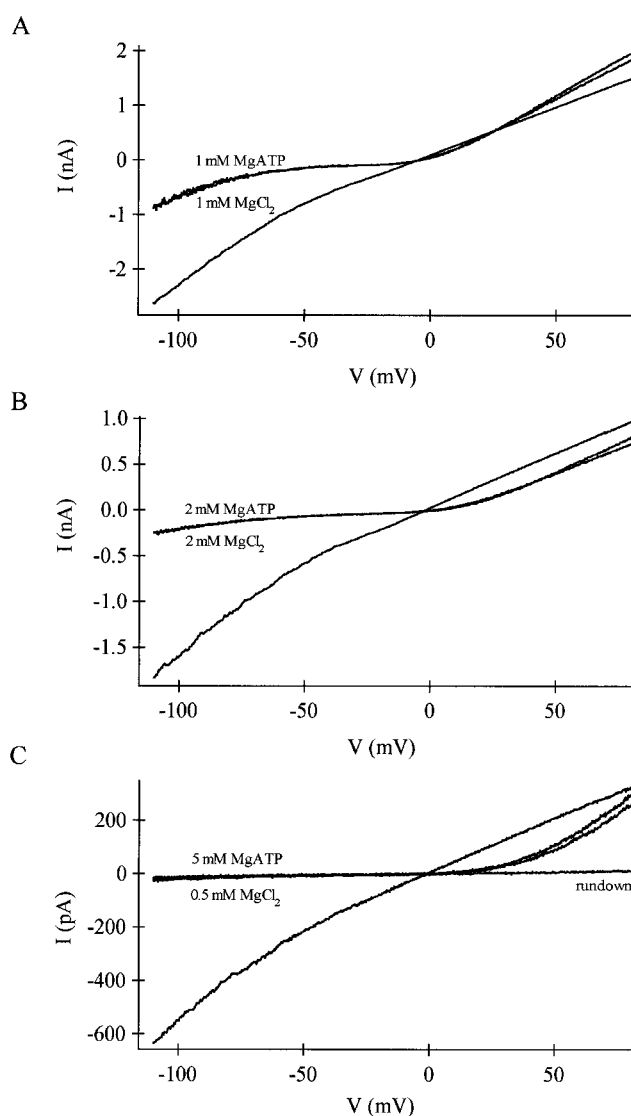
**FIGURE 1** Inhibition of MIC current by internal MgATP is dependent on the type and amount of chelator. (A) The I/V relations of MIC current activated by dialysis with internal solutions containing 1 mM  $\text{Mg}^{2+}$  (10 mM EDTA), 0.5 mM  $\text{Mg}^{2+}$  (12 mM EGTA), and 5 mM MgATP (3 mM EGTA/2.5 mM HEDTA). The I/V shape did not vary with the composition of the internal solution. Traces are leak subtracted. (B) Summary of MIC current amplitudes. RBL cells were dialyzed with  $\text{Mg}^{2+}$ - and MgATP-containing solutions; maximally activated MIC current amplitudes were measured at +80 mV. Maximal MIC current amplitudes were obtained by strongly chelating free  $\text{Mg}^{2+}$  with EDTA to an estimated 230 nM. An amount of 5 mM MgATP in the presence of 12 EGTA markedly inhibited the current, whereas the same amount of MgATP did not significantly inhibit when the internal chelator was 3 mM EGTA and 2.5 mM HEDTA. For comparison,  $\sim 5.5$  mM free  $[\text{Mg}^{2+}]$  and no added MgATP are shown.

characteristic outwardly rectifying current was allowed to develop to its maximal value, typically achieved 10–15 min after break-in and followed by a slow run-down of the current (Kozak et al., 2002). The largest MIC currents developed with low-Mg<sup>2+</sup> internal solution. In agreement with Nadler et al. (2001), we saw a drastic reduction of MIC current with 5 mM MgATP compared to the current recorded when the pipette contained intermediate levels of Mg<sup>2+</sup> (0.5 mM MgCl<sub>2</sub> with a calculated free [Mg<sup>2+</sup>] of 270  $\mu$ M; Fig. 1). MgATP did not change the I/V shape of MIC current. Also in agreement with Nadler et al. (2001), the inclusion of 6 mM MgCl<sub>2</sub> in the pipette (with 1 mM EGTA) completely inhibited MIC current and served as a positive control. Thus, dialysis of 5 mM MgATP mimics the effect of millimolar internal Mg<sup>2+</sup> in inhibiting the MIC current development.

When 5 mM MgATP was included in a different intermediate-Mg<sup>2+</sup> internal solution, containing 3 mM EGTA and 2.5 mM HEDTA (yielding a calculated free [Mg<sup>2+</sup>] of 270  $\mu$ M), the size of the current was not different from that achieved with the solution that contained 270  $\mu$ M free Mg<sup>2+</sup> without MgATP. MgATP inhibition, therefore, depends on the nature of the Mg<sup>2+</sup> chelator present. Inhibition is robust when a poor Mg<sup>2+</sup> chelator is present (12 EGTA) but absent when a stronger ( $\times 100$ ) Mg<sup>2+</sup> chelator is employed (2.5 HEDTA). These results suggest strongly that MgATP inhibition is mediated by free Mg<sup>2+</sup> ions rather than by ATP.

### Comparison of free Mg<sup>2+</sup> concentrations using the MIC current as a bioassay

Removal of external divalent ions enables inward monovalent currents in both expressed TRPM7 and native MIC channels, linearizing the I/V relationship. The monovalent inward current is highly sensitive to block by external Mg<sup>2+</sup> in the micromolar range (Nadler et al., 2001; Kozak et al., 2002). Specifically, external free Mg<sup>2+</sup> blocks the channel in a characteristic concentration- and voltage-dependent manner. We therefore decided to use the external Mg<sup>2+</sup> block of the MIC channel as a bioassay to estimate and compare free Mg<sup>2+</sup> content in various pipette and other test solutions simply by applying them from the outside and measuring the amount of monovalent current block. Since the common intracellular free Mg<sup>2+</sup> concentrations used are 1–5 mM MgCl<sub>2</sub> and 1–5 mM MgATP, we compared the extent of block of the monovalent MIC current by 1, 2, and 3 mM MgCl<sub>2</sub> to 1, 2, and 3 mM MgATP. As seen in Fig. 2, the block caused by 1 or 2 mM MgCl<sub>2</sub> was equal to that caused by corresponding concentrations of MgATP (Fig. 2, A and B). The same was true for 3 mM MgATP and MgCl<sub>2</sub> solutions (data not shown). Since the MIC channel was not affected by external ATP, the voltage-dependent block can be attributed only to free Mg<sup>2+</sup> acting from the outside. When the internal solutions containing no MgATP (with 0.5 mM MgCl<sub>2</sub>) or 5 mM MgATP (with EGTA/HEDTA) were applied externally, the block was equal in magnitude,



**FIGURE 2** Monovalent MIC current as a bioassay for determining free Mg<sup>2+</sup> concentrations. Solutions containing various amounts of MgCl<sub>2</sub> and MgATP were applied externally and the I/V relations compared. (A) MIC monovalent current with external Cs<sup>+</sup>-HEDTA solution was blocked to the same extent by 1 mM MgCl<sub>2</sub> (8 mM HEDTA) or by 1 mM MgATP (8 mM HEDTA). The calculated free [Mg<sup>2+</sup>] concentrations were 6.7  $\mu$ M and 8.2  $\mu$ M, respectively. (B) 2 mM MgCl<sub>2</sub> and MgATP (in the presence of 8 mM HEDTA) blocked the monovalent MIC current to the same extent. The calculated free [Mg<sup>2+</sup>] concentrations were 15.8  $\mu$ M and 17.5  $\mu$ M, respectively. (C) The internal solutions from the experiment described in Fig. 1 were applied externally to compare the degree of block: 0.5 mM MgCl<sub>2</sub> and 12 mM EGTA (calculated free [Mg<sup>2+</sup>] = 270  $\mu$ M); compared with 5 mM MgATP and 3 mM EGTA/2.5 mM HEDTA (calculated free [Mg<sup>2+</sup>] = 270  $\mu$ M). The current was allowed to run down completely to show the extent of block by 270  $\mu$ M Mg<sup>2+</sup>.

confirming the calculated free Mg<sup>2+</sup> concentration of  $\sim 270$   $\mu$ M in both solutions (Fig. 2 C). Therefore, we conclude that intracellular solutions with weak Mg<sup>2+</sup> chelators (such as BAPTA or EGTA) have close amounts of free Mg<sup>2+</sup> whether MgCl<sub>2</sub> or MgATP is used.

### The divalent metal cations $\text{Ba}^{2+}$ , $\text{Sr}^{2+}$ , and $\text{Mn}^{2+}$ mimic $\text{Mg}^{2+}$

We further tested whether the inhibitory effect of internal  $\text{Mg}^{2+}$  is unique for that ion. It was previously demonstrated that MIC current develops in RBL cells with 1 mM EGTA and no divalents in the pipette (Kozak et al., 2002). As shown in Fig. 3, inclusion of 5 mM  $\text{Ba}^{2+}$  or  $\text{Mn}^{2+}$  (no ATP) in the pipette caused complete inhibition of MIC current ( $<1\text{ pA/pF}$  remaining), comparable to 6 mM  $\text{Mg}^{2+}$ . For comparison, the control levels of MIC current without divalent cations were in the range of 30–50 pA/pF (data not shown).  $\text{Sr}^{2+}$  (5 mM) also inhibited the current substantially, but not completely; 6 mM was necessary for complete inhibition ( $n = 4$  cells).  $\text{Zn}^{2+}$  was also tested for its ability to prevent MIC current development. However, prolonged recordings with internal  $\text{Zn}^{2+}$  solutions proved difficult as the cells became leaky. Internal  $\text{Zn}^{2+}$  was tested on the preactivated current (see below) and was shown to be inhibitory. These results suggest that the channel is regulated by a metal-binding site with specificity for a high density of charge.

### Testing inhibition in cells with preactivated MIC channels

In the rat PAS T cell line, substantial endogenous MIC current is preactivated in the intact cell, as judged by current levels immediately after break-in to achieve whole-cell recording. These cells can be used to assess internal inhibition without waiting minutes for complete channel activation during dialysis. In the small fraction of RBL cells

(~5%) that also show preactivated MIC current, dialysis with millimolar free  $\text{Mg}^{2+}$  causes a gradual reduction of the current, leading to its disappearance within a few minutes (Kozak et al., 2002). Importantly,  $\text{Mg}^{2+}$  inhibition is voltage-independent, reducing the amplitude of the whole-cell current but leaving the I/V shape intact (Kozak et al., 2002). We used PAS T cells to test whether internal  $\text{Ba}^{2+}$  could inhibit the preactivated MIC current in the same fashion as  $\text{Mg}^{2+}$ . Fig. 4 A shows an I/V of MIC current immediately after breaking in with an internal solution containing ~4 mM  $[\text{Ba}^{2+}]_{\text{free}}$  (no ATP).  $\text{Ba}^{2+}$ , like  $\text{Mg}^{2+}$ , caused a gradual reduction of MIC current without affecting its I/V shape. The inhibition was complete by 79 s. Inclusion of ~4 mM  $\text{ZnCl}_2$  also inhibited the preactivated MIC current in RBL cells ( $n = 3$  cells, data not shown), without influencing the I/V shape before inhibition was complete. In contrast, when the pipette solution contained no divalents (12 mM EGTA), the current gradually *increased* in magnitude, as shown in Fig. 4 B; after 79 s of dialysis, MIC current was much larger than immediately after break-in. These experiments with pre-activated MIC current reinforce the conclusion that divalent metal cations inhibit nonspecifically.

### DISCUSSION

The TRPM subfamily of mammalian TRP cation channels has been identified recently by cloning and heterologous expression studies. Some TRPM members, TRPM2, TRPM6, and TRPM7, are remarkable in that they are “chanzymes” consisting of a channel domain and a

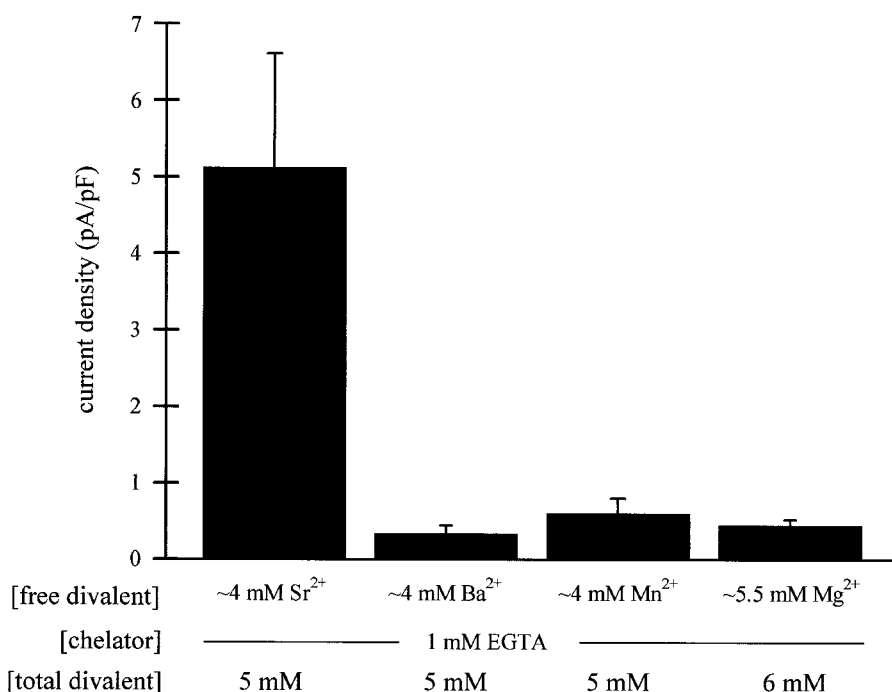


FIGURE 3 Internal  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$ , and  $\text{Mn}^{2+}$  can substitute for  $\text{Mg}^{2+}$  in inhibiting the MIC current in RBL cells. The maximal normalized current amplitudes (mean  $\pm$  SE) during dialysis with ~4 mM  $\text{Sr}^{2+}$  ( $n = 6$  cells),  $\text{Ba}^{2+}$  ( $n = 5$ ) and  $\text{Mn}^{2+}$  ( $n = 3$ ) (1 mM EGTA), obtained as in Fig. 1 B, are compared to current inhibition by 5.5 mM  $\text{Mg}^{2+}$  ( $n = 3$ ).

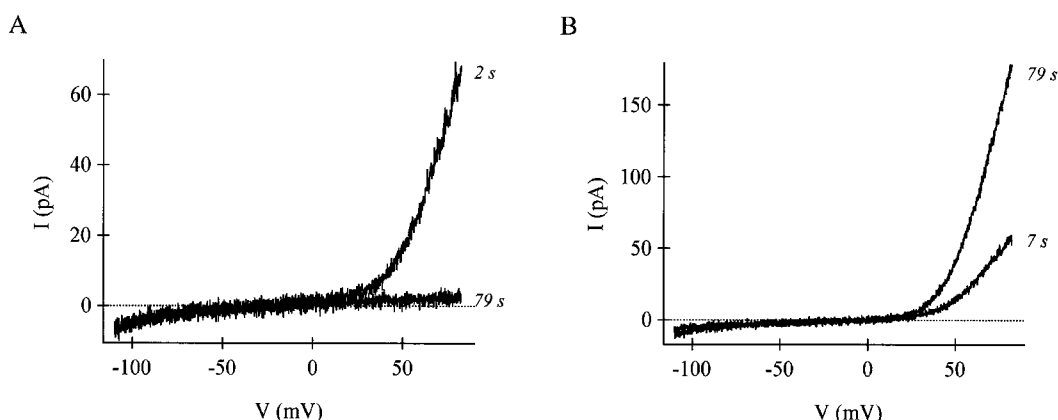


FIGURE 4 Millimolar concentrations of internal  $\text{Ba}^{2+}$  inhibit preactivated MIC current in rat PAS cells. (A) The MIC current I/V obtained 2 and 79 s after break-in with a pipette solution containing 5 mM  $\text{BaCl}_2$  and 1 mM EGTA. The inhibition is complete at 79 s. (B) The MIC current I/V obtained 7 and 79 s after break-in with a pipette solution containing no internal divalents (12 mM EGTA). The current was substantially increased after 79 s.

C-terminal enzyme domain (Montell et al., 2002). In the case of TRPM2, the enzyme is an ADP-ribose pyrophosphatase (Perraud et al., 2001; Sano et al., 2001), and in TRPM6 and TRPM7 it is an  $\alpha$ -kinase (reviewed in Ryazanov (2002)). The enzyme domains of TRPM2 and TRPM7 are functional in biochemical assays. When expressed by transfection, TRPM2 forms a  $\text{Ca}^{2+}$ -permeable channel that is activated by ADP-ribose, but not by ATP or other nucleotides, suggesting a direct involvement of its enzyme domain in channel gating (Perraud et al., 2001; Sano et al., 2001). In the case of TRPM6 and TRPM7, however, the situation may be more complex regarding a possible role of the kinase domain. The initial study by Runnels and colleagues suggested that ATP might activate TRPM7 and concluded that the  $\alpha$ -kinase domain was likely to be involved in channel gating (Runnels et al., 2001). Nadler et al. challenged this view and showed that  $\text{Mg}^{2+}$  and  $\text{MgATP}$  inhibit the current (Nadler et al., 2001). They also demonstrated that phosphorylation did not underlie  $\text{MgATP}$  action as other nucleotides, including nonhydrolyzable analogs, had similar effects in conjunction with  $\text{Mg}^{2+}$ . ATP hydrolysis would be unlikely in any case given that the inhibitory effect of  $\text{MgATP}$  was observed at millimolar concentrations, well above amounts used in biochemical reactions (Hilgemann, 1997). Thus, a possible physiological role for the  $\alpha$ -kinase domain of TRPM7 remains to be discovered.

In this study, we demonstrate that the inhibitory effect of millimolar  $\text{MgATP}$  concentrations on the native MIC current is dependent on the nature and amount of the  $\text{Mg}^{2+}$  chelator in the solution.  $\text{MgATP}$  (5 mM) indeed inhibits in the presence of EGTA (a weak  $\text{Mg}^{2+}$  chelator), but has no additional inhibitory effect when HEDTA (a strong  $\text{Mg}^{2+}$  chelator) is also present, reducing the free  $\text{Mg}^{2+}$  concentration. At the same concentration of free  $\text{Mg}^{2+}$  there is no additional inhibition by  $\text{MgATP}$ . Our results lead to the conclusion that TRPM7/MIC channels are not dependent on ATP levels within the cell. We also show that the mechanism

of inhibition is not unique to internal  $\text{Mg}^{2+}$ , as millimolar  $\text{Ba}^{2+}$  or  $\text{Sr}^{2+}$  exert similar effects.

The mechanism for internal divalent action on the MIC channel is not clear but may not involve direct channel blockade. It was previously shown that  $\text{Mg}^{2+}$  inhibition is voltage-independent and rather slow compared to dialysis of a blocker with a direct effect on the channel (Kozak et al., 2002). Prakriya and Lewis (2002) showed that micromolar and millimolar free  $\text{Mg}^{2+}$  concentrations were able to inhibit the single MIC channel in an inside-out patch, suggesting a membrane-delimited action. Interestingly,  $\text{Mg}^{2+}$  inhibited the MIC channel reversibly at 100  $\mu\text{M}$  but irreversibly at 2 mM. It is important to note that in the inside-out patch, MIC channels were already activated before exposure to  $\text{Mg}^{2+}$ , whereas in whole-cell recordings the number of functional channels is increased during dialysis and at the same time  $\text{Mg}^{2+}$  starts exerting its inhibitory effect. In an inside-out patch, the activation process has already occurred, and only inhibition is observed. In the case of macroscopic current on the other hand,  $\text{Mg}^{2+}$  may exert effects on both the activation process (addition of new functional channels) and on channels that are already opened. This experimental distinction, or possible factors lost during patch excision, may reconcile the observation that  $\text{MgATP}$  inhibition of TRPM7 was reversible even at millimolar concentrations (Nadler et al., 2001), whereas in the inside-out patch millimolar  $\text{Mg}^{2+}$  inhibited irreversibly (Prakriya and Lewis, 2002). In PAS T cells with MIC channels that are preactivated at break-in, nanomolar to micromolar concentrations of free internal  $\text{Mg}^{2+}$  did not inhibit the current, whereas 4–5 mM  $\text{Mg}^{2+}$  blocked both preactivated current and development of MIC current in RBL cells (Kozak et al., 2002). Preactivated MIC current provides a convenient assay for inhibitory ions at millimolar concentrations (Fig. 4) and may provide clues to physiological regulation of channel gating.

Most TRP channels have been investigated when the protein is overexpressed in a heterologous system. TRPM7

channels are unique among other TRP family members in that the native counterpart of TRPM7 (MagNum or MIC) is functionally expressed in T-lymphocytes and RBL cells, systems that have been described in great detail over the past 10 years. TRPM7 and native MIC (in fibroblasts) were shown recently to be inhibited by  $PIP_2$  depletion (Runnels et al., 2002). Consistent with this finding, MIC current in RBL cells runs down together with the endogenous  $PIP_2$ -sensitive IRK1 current (Huang et al., 1998; Kozak et al., 2002). It is likely that  $Mg^{2+}$  (or other divalent cations) prevents the electrostatic interaction between  $PIP_2$  and the channel by screening the negative charge on the lipid head group. Consistent with this idea, Fan and Makielski (1997) have demonstrated that polyvalent cations such as  $La^{3+}$  are able to abolish K-ATP channel activation by anionic phospholipids. Although divalent cations were not tested in that study, there may be a common mechanism through which polyvalent cations mediate inhibition by screening  $PIP_2$ . This interpretation is consistent with the common mode of action of  $Mg^{2+}$  and  $Ba^{2+}$ , divalent cations that exhibit very different binding characteristics and widely disparate biochemical activities as a result of differing charge coordination. Another possibility is the involvement of a low affinity  $Mg^{2+}$ -binding site either within the  $\alpha$ -kinase domain (Yamaguchi et al., 2001) or on another protein that can inhibit MIC current after binding divalent metal cations. Additional experiments are needed to understand the physiological and mechanistic basis for channel gating.

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