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ORIGINAL ARTICLE

Magnesium permeation through mechanosensitive channels: single-current measurements

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Compelling evidence shows that intracellular free magnesium $[Mg^{2+}]_i$ may be a critical regulator of cell activity in eukaryotes. However, membrane transport mechanisms mediating Mg^{2+} influx in mammalian cells are poorly understood. Here, we show that mechanosensitive (MS) cationic channels activated by stretch are permeable for Mg^{2+} ions at different extracellular concentrations including physiological ones. Single-channel currents were recorded from cell-attached and inside-out patches on K562 leukaemia cells at various concentrations of $MgCl_2$ when Mg^{2+} was the only available carrier of inward currents. At 2 mM Mg^{2+}, inward mechanogated currents representing Mg^{2+} influx through MS channels corresponded to the unitary conductance of about 5 pS. At higher Mg^{2+} levels, only slight increase of single-channel currents and conductance occurred, implying that Mg^{2+} permeation through MS channels is characterized by strong saturation. At 20 and 90 mM Mg^{2+}, mean conductance values for inward currents carried by Mg^{2+} were rather similar, being equal to 6.8 ± 0.5 and 6.4 ± 0.5 pS, respectively. The estimation of the channel-selective permeability according to constant field equation is obviously limited due to saturation effects. We conclude that the detection of single currents is the main evidence for Mg^{2+} permeation through membrane channels activated by stretch. Our single-current measurements document Mg^{2+} influx through MS channels in the plasma membrane of leukaemia cells.

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Introduction

Magnesium plays an important role in a large number of cellular processes acting as a cofactor in enzymatic reactions and transmembrane ion movements. With improved methods for measuring intracellular ionized Mg^{2+} concentration ($[Mg^{2+}]_i$), it has been established that $[Mg^{2+}]_i$ is maintained below electrochemical equilibrium in all cells investigated to date. Most eukaryotic cells appear to contain a Na^+/Mg^{2+} exchanger that use the transmembrane Na gradient to expel Mg^{2+}. There is also evidence for Mg^{2+}: $Cl^-$and Mg^{2+}: H^+ transporters [1, 2]. It has been accepted that Mg^{2+} content is kept constant at the level necessary for enzyme and channel function. Even small changes in internal levels of Mg^{2+} could have dramatic effects on cell activity. However, in the last decade, a number of new experimental observations have revealed that large fluxes of Mg^{2+} can cross the cell membrane in either direction following a variety of hormonal or non-hormonal stimuli [3]. Insulin, vasopressin, angiotensin and some other agonists induce cellular Mg^{2+} accumulation, at least partially, by stimulating Mg^{2+} entry from the extracellular fluid, while adrenergic agonists stimulate cellular Mg^{2+} release. It is unknown whether Mg^{2+} release and accumulation are accomplished by the same transporters operating in either direction or by distinct transport mechanisms in the plasma membrane. Little is known about membrane pathways mediating Mg^{2+} influx in eukaryotes. Voltage-clamp studies have provided evidence for the operation of a Mg^{2+}-specific current in *Paramecium* [4, 5]. Using single-current recording, a Mg^{2+}-permeable cation chan-

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nel in spinach thylakoid membranes has been described [6]. For mammalian cells, the data are scarce, especially on the single-channel level. The possibility that Mg^{2+} may cross the plasma membrane via a channel is appealing and indirectly supported by inhibitory effects of channel blockers on Mg^{2+} accumulation [3]. The examination of renal Mg^{2+} handling at the tubular and cellular levels is also consistent with the assumption that the Mg^{2+}-permeable channel functions in apical membranes [7]. Moreover, evidence has accumulated to suggest that extracellular Mg^{2+} concentration can rapidly regulate intracellular Mg^{2+} and has functional effects in a variety of tissues under physiological conditions and in pathophysiology. Specifically, dynamic changes in free Mg^{2+} have been shown to play a pivotal role in endothelial cell functions [8, 9] and in the brain following neurotrauma [10]. Now Mg^{2+} is considered a promising neuroprotective agent in the therapy of acute ischaemic stroke and vascular damages. A question arises whether cation-selective channels could be involved in the Mg^{2+}-transporting system in plasma membrane of mammalian cells. In recent years, data have appeared suggesting that TRP membrane proteins, specifically some members of TRPM subfamily, may function as Ca^{2+}- and Mg^{2+}-permeable cation channels [11–14]. It has been assumed that they are involved in cellular magnesium homeostasis. The aim of the present study is to directly estimate magnesium permeation through mechanosensitive (MS) channels that are considered to be ubiquitously present in various cell types. In our previous paper, gadolinium-blockable MS cationic channels have been identified and characterized in human leukaemia K562 cells [15]. Here, special attention has been paid to the detection of mechanogated single-channel currents carried by Mg^{2+} ions.

Materials and Methods

Cells

Human myeloid leukaemia K562 cells were obtained from Cell Culture Collection (Institute of Cytology, St Petersburg, Russia) and were maintained in glass flasks in RPMI-1640 containing 10% fetal bovine serum and antibiotics (100 mg/ml streptomycin and 100 units/ml penicillin or 80 mg/ml gentamycin) at 37 °C. Cells were plated on coverslips (0.4 × 0.4 cm) 1-3 days before experiment.

Electrophysiology

Single-channel currents were recorded using standard cell-attached mode of the patch-clamp technique [16]. Pipettes were pulled from soft glass capillaries to a resistance of 7~15 MΩ when filled with solution. Membrane currents were measured essentially as described earlier [15, 17]. Membrane voltage was the potential of the intracellular membrane side minus the potential of the extracellular one. Unless otherwise stated, data were filtered at 200 Hz and sampled at a rate of 1 kHz by 12-bit ADC for analysis and display. The recordings were performed at room temperature (22~23 °C) on the stage of an inverted microscope with Nomarsky optics (magnification of 256×). We used well-known method of mechanical stimulation of applying pressure to a patch pipette. A "gentle" seal was essential for the formation of the patch as well as for the application of stimulus to activate the channels. The pipette interior was connected to a manometer with a valve to allow either application of negative (positive) pressure or equilibration to atmospheric pressure. Mechanically gated ion channels were activated in response to the negative pressure application (suction). Pressure range was 1.333-2.666 kPa (10-20 mmHg).

Data analysis

Channel open probability (P_o) was determined using the following equation:

$$(1) \quad P_o = \frac{I}{iN},$$

where I is the mean current determined from the amplitude histograms, i is the unitary current amplitude and N is the number of functional channels.

Single-channel conductance values were determined by the slope of the current-voltage (I-V) curve, where the I-V data could be well fitted to a straight line. The relative permeability ratios of the channel (P_{Mg}/P_K) were defined as

$$(2) \quad \frac{P_{Mg}}{P_K} = \frac{[K]_i}{4[Mg]_o} \exp(E_{rev}F/RT)\{\exp(E_{rev}F/RT)+1\},$$

where the reversal potential E_{rev} was obtained by fitting the I-V curve. Subscripts i and o represent inside and outside of the membrane, and other parameters have their usual meanings. When free intracellular Mg^{2+} was also taken into account, we used modified Goldman-Hodgkin-Katz current equation [18]. The effect of the ionic strength of the solution on the activity of each ion is considered. Activity coefficients were calculated according to the extended Debye-Hückel equation:

$$(3) \quad -\log(\gamma) = \frac{Az^2\sqrt{\mu}}{1+Ba\sqrt{\mu}},$$

where γ is the activity coefficient, z is the charge of the ion, μ is the ionic strength of the aqueous solution, A and B are constants and α is an effective diameter of the hydrated ion. Averaged data are given as the mean ± s.e.m. (number of experiments).

Solutions

The bath solution for cell-attached measurements contained (in mM): 145 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES/KOH. In control experiments, pipettes were filled with normal external solution (in mM): 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES/Tris-OH. To measure Mg^{2+} currents, we used pipette solutions with different concentrations of Mg^{2+} supplemented with NMDG to maintain the tonicity and ionic strength. Solution of high Mg^{2+} contained (in mM): 90 $MgCl_2$ and 10 HEPES/Tris-OH. Solutions of lower Mg^{2+} concentrations contained (in mM): 20 $MgCl_2$ + 110 NMDGCl, 2 $MgCl_2$ + 137 NMDGCl and 1 $MgCl_2$ + 138 NMDGCl. In some experiments, 0.1-0.2 mM EGTA/Tris-OH was added to the magnesium pipette solutions to exclude any impurities by divalent or trivalent cations. Bath cytosol-like solution for inside-out measurements contained (in mM): 145 KAsp or KCl, 1 $MgCl_2$, 20 HEPES/KOH, 2 EGTA and

an appropriate quantity of $CaCl_2$ (0.175) to establish the final free ionized calcium concentration at the desired level of 0.01 mkM. pH of all solutions buffered with 10 HEPES/Tris-OH was set at 7.3. All chemicals were purchased from Sigma.

Results

Gadolinium-blockable MS channels in the plasma membrane of K562 leukaemia cells have been previously shown to be cation-selective and fully impermeable to large organic cations, such as $NMDG^+$ and $Tris^+$ [15]. To search for the magnesium permeability of MS channels, cell-attached measurements in the presence of 90, 20, 2 and 1 mM $MgCl_2$ in the pipette solution were performed. Mg^{2+} was the only available carrier for inward current; other cations in the pipette were impermeable $NMDG^+$ and $Tris^+$. It was specifically evaluated that there was no inward current through MS channels when the pipette contained only $NMDG^+$ and $Tris^+$ cations, in full agreement with the previous results [15]. MS channel activity was initiated by negative pressure application (suction, see also Materials and Methods). Representative recordings shown in Figure 1 demonstrate an activation of inward currents in response to suction in control experiment (Na^+-containing

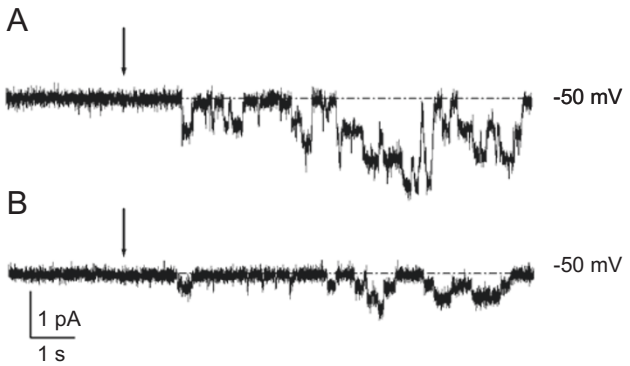

Figure 1 Mechanogated currents recorded from representative cell-attached patches on K562 cells: **(A)** in control experiment with Na^+-containing solution in the pipette and **(B)** in the presence of Mg^{2+} (20 mM) as the only permeant cation in the pipette solution. The application of suction is indicated by the arrows.

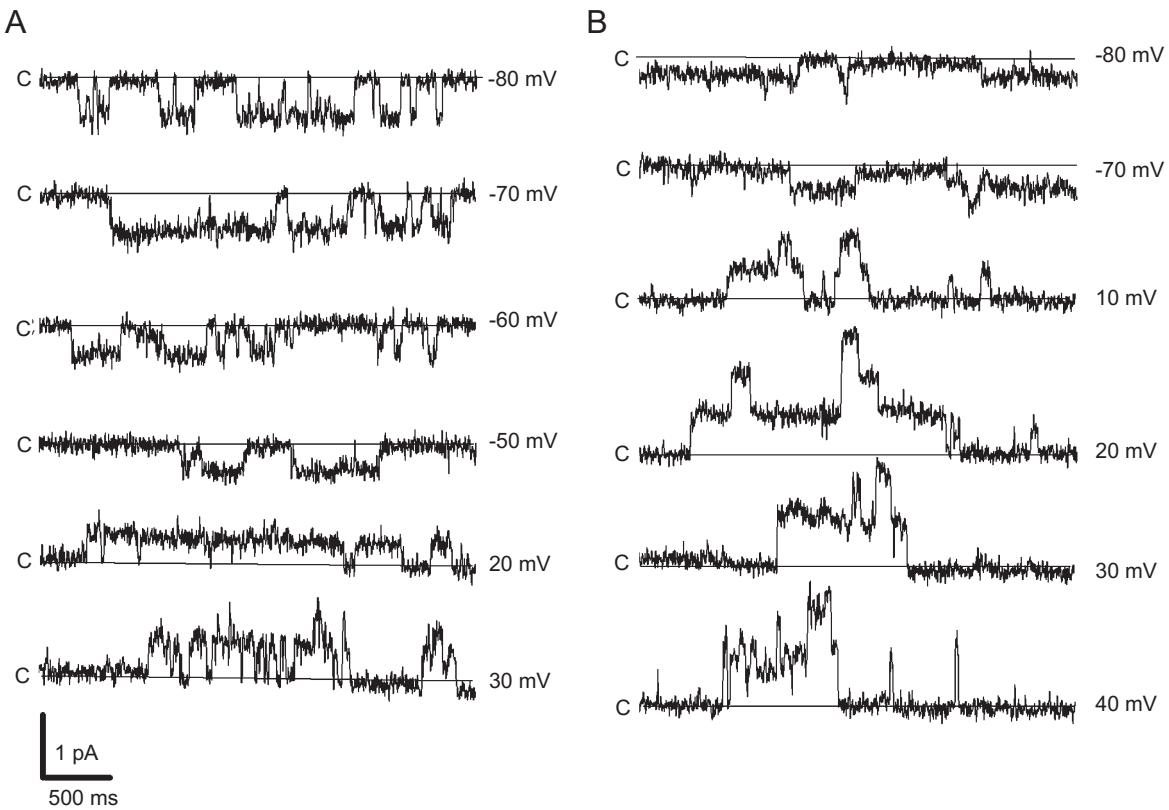

Figure 2 MS channel activity recorded from cell-attached patches at applied negative pressure when Mg^{2+} was the only available carrier of the inward current. Pipette solution contained: 90 mM $MgCl_2$, 10 mM HEPES/Tris **(A)** or 2 mM $MgCl_2$, 137mM NMDGCl, 10 mM HEPES/Tris **(B)**. Holding membrane potentials are indicated near traces; c – closed states.

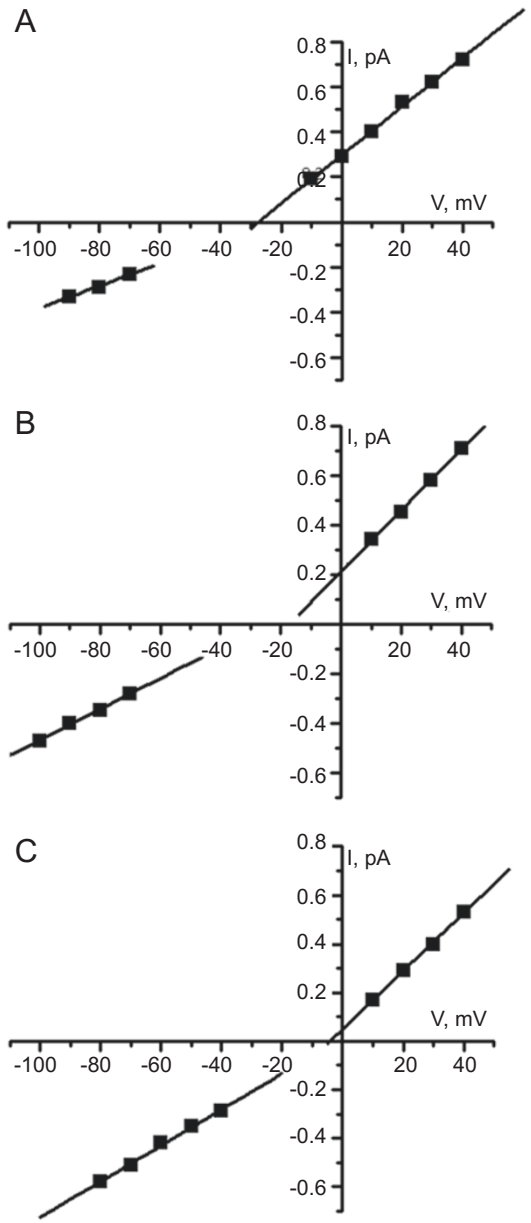

Figure 3 Permeation of MS channels for magnesium ions. Current-voltage relationships measured in cell-attached experiments with various Mg^{2+} concentrations in the pipette solution: **(A)** 2 mM $MgCl_2$, **(B)** 20 mM $MgCl_2$ and **(C)** 90 mM $MgCl_2$. $NMDG^+$ was used for cationic substitution. Inward currents represent magnesium influx through MS channels.

solution in the pipette) and in the presence of 20 mM Mg^{2+} supplemented with $NMDG^+$ in the pipette. In the control experiments, MS channels were found in 55% (n=479) of cell-attached patches. Mechanogated currents were also observed in 53% (n=173) of stable cell-attached patches in the presence of Mg^{2+} as the only permeable cation in the pipette solution. At all applied concentrations of $MgCl_2$, single openings representing MS channel activity were observed over a wide range of membrane potentials (Figures 1 and 2). Outward or inward unitary currents activated by negative pressure application could be measured (Figure 2). Importantly, inward single-channel currents carried by Mg^{2+} cations were detected. These data show that MS channels are permeable to Mg^{2+} at physiological concentration range (1~2 mM) as well as at higher level of Mg^{2+} (20 and 90 mM) in the extracellular (pipette) solution (Figures 1–3). When 0.1-0.2 mM EGTA/Tris-OH was added to the pipette solution to avoid potential impurities, very similar currents and conductance were measured.

Mechanogated currents of outward direction appeared to represent intracellular monovalent (presumably, potassium) ion efflux (cell-attached recordings, Figure 2). Corresponding single-channel conductance measured at positive potentials (12.1 ± 0.6 pS, n=22) did not change when varying the Mg^{2+} concentration in the extracellular solution. Inward currents carried by Mg^{2+} corresponded to the lower values of unitary conductance; current-voltage relationships displayed slight outward rectification (Figure 3). In the presence of 2 mM Mg^{2+} in the pipette, the current-voltage relationship, measured for MS channel activity of inward direction, showed a unitary conductance value of 5 pS. In the presence of 20 and 90 mM $MgCl_2$ in the pipette, mean conductance values for inward currents carried by Mg^{2+} were similar, being equal to 6.8 ± 0.5 (n=13) and 6.4 ± 0.5 (n=8) pS, respectively. Thus, single-channel currents and conductance values show that Mg^{2+} permeation through MS channels is characterized by strong saturation effect.

The reversal potential values could be obtained from current-voltage relationships by extrapolation of linear sections at positive and at negative membrane voltage (Figure 3). Mean values were equal to -27.4 ± 2.7 (n=5), -16.0 ± 1.9 (n=5) and -5.2 ± 1.1 (n=9) mV at 2, 20 and 90 mM $MgCl_2$ in the pipette, respectively. Increasing of Mg^{2+} from 1 to 90 mM resulted in a shift of the reversal potential value to the positive direction providing additional evidence that inward currents objectively represented Mg^{2+} influx through MS channels. The relative permeability of the channel was estimated from reversal potential data according to modified Goldman-Hodgkin-Katz equation [18]. Intracellular K^+ concentration was assumed to be equal to 140 mM. Intracellular Mg^{2+} was assumed to be 0 or 0.5 mM and similar values of permeability ratio have been obtained in both cases. The activity coefficients for cations were calculated according to the extended Debye-Hückel equation: g_K=0.73 and g_{Mg}=0.35. Taking into account the activity coefficients, the relative permeability value (P_{Mg}/P_K) estimated from reversal potential value of –27 mV (at 2 mM extracellular Mg^{2+}) was equal to 18. This

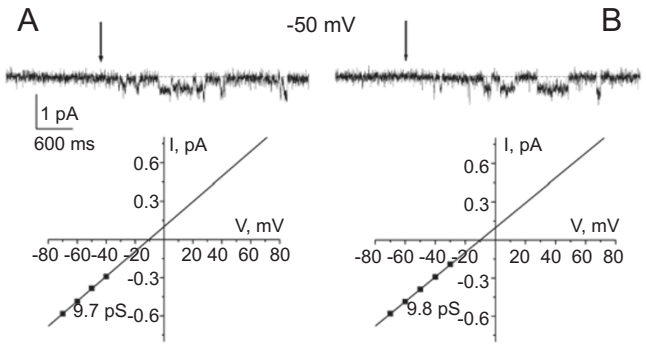

Figure 4 Inside-out recordings of MS channel activity and corresponding current-voltage relationships obtained with anion replacement in the cytosol-like solution: 140 mM KAsp **(A)** or KCl (B). Pipette solution contained 20 mM $MgCl_2$, 110 mM NMDGCl and 10 mM HEPES/Tris. The application of suction is indicated by the arrows.

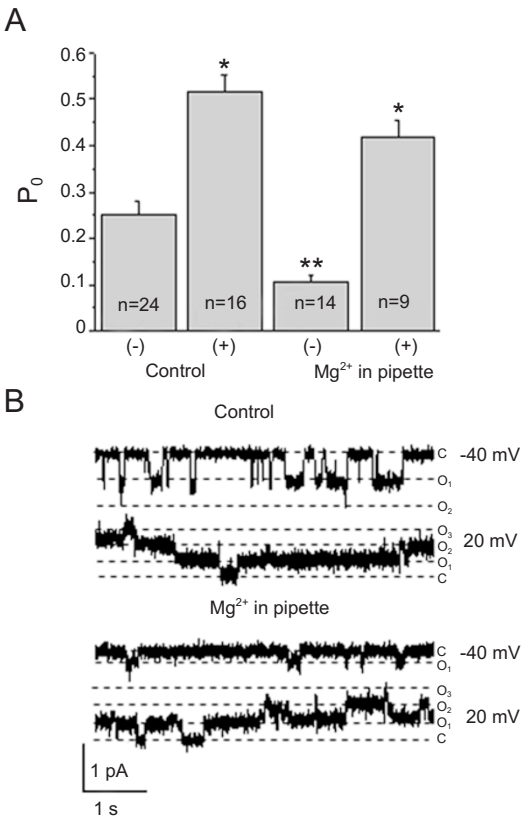

Figure 5 Effect of membrane voltage on channel activity in cell-attached patches in presence of Na^+ (145 mM, control) or Mg^{2+} (90 mM) as a main cation in the pipette solution. **(A)** Mean open probability (P_o) values represent different levels of MS channel activity for inward (-) and outward (+) currents. **(B)** Typical current records obtained at the membrane potential –40 mV (inward currents) and +20 mV (outward currents) in control or at high concentration of Mg^{2+} in the pipette.

implies Mg^{2+} selectivity of the MS channel. However, this estimation should be considered as rough approximation since unitary currents and conductance displayed saturating Mg^{2+} influx when Mg^{2+} concentration was raised above physiological level. Therefore, it would be quite incorrect to estimate channel selectivity from reversal potential measurements at higher Mg^{2+} concentrations in the pipette solution. Moreover, it is reasonable to conceive that deviations from independence for Mg^{2+} fluxes may occur even at low concentrations. Calculations according constant field equation for 20 and 90 mM Mg^{2+} give P_{Mg}/P_K values of about 3 and 1, respectively. There are very inconsistent values that have been calculated from reversal potential data at various bivalent concentrations. Thus, the detection of single currents carried by Mg^{2+} is the primary source to characterize permeation properties of the channels.

In our previous studies, high cation/anion selectivity of MS channels in leukaemia cells has been indicated using several series of ion-substitution experiments [15]. Nevertheless, to address the possibility that Cl- flux might be involved in the inward currents recorded from cell-attached patches, additional experiments on excised fragments were performed. MS channel activity was recorded from inside-out patches on K562 cells in the presence of aspartate as a main anion in the bath of cytosol-like solution (Figure 4A); Mg^{2+} (20 mM) was the only available carrier for inward currents in the pipette solution. Single-channel conductance for inward currents activated by suction was equal to 8.5 ± 0.6 pS. Additional evidence to identify the inward currents as Mg^{2+} influx has been obtained in inside-out experiments with anionic substitution. Figure 4 shows mechanogated currents recorded from inside-out patch in the presence of aspartate or chloride in the bath solution and Mg^{2+} as the only permeant cation in the pipette. Inside-out recordings demonstrate that substitution of anions in the cytosol-like solution caused no changes in the amplitude of single-channel openings.

It is known that macroscopic ionic currents underlying membrane transport considerably depend on kinetic properties of single channels and on the level of channel activity. MS channels in K562 cells were shown to be active throughout the range of holding membrane potentials [15, 19]. Figure 5 shows the effect of membrane voltage on channel activity in cell-attached patches in the presence of Na^+ (145 mM, control) or Mg^{2+} (90 mM) as a main cation in the pipette solution. In the presence of Na^+, MS channel activity estimated as open probability (P_o) displayed obvious voltage sensitivity (Figure 5A, on the left): P_o at positive voltages was higher than that at negative voltages, in agreement with our previous data for control patches on

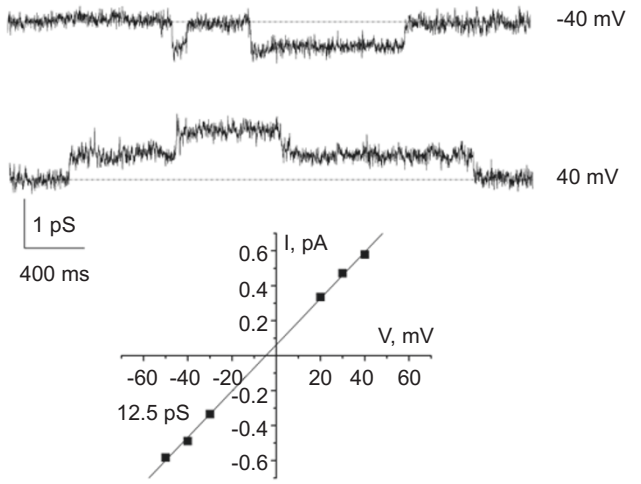

Figure 6 Mechanogated currents and corresponding current-voltage relationship obtained on an inside-out patch when Mg^{2+} (20 mM $MgCl_2$) was added to Na-containing (115 mM NaCl) pipette solution. Bath solution contained 115 mM NaCl and 30 mM NMDGCl.

K562 cells [19]. Figure 5A also shows evident differences of open probability values for inward and outward currents in the presence of 90 mM $MgCl_2$ in pipette solution: mean P_o at negative potentials (inward currents carried by Mg^{2+}) was considerably lower than at positive potentials (outward currents). Representative current records (Figure 5B) additionally demonstrated that at high external Mg^{2+} concentrations, channel activity strongly depended on membrane voltage or on current direction. A comparison of mean open probabilities obtained for negative voltage ranges revealed that P_o value is lower for Mg^{2+}-transported currents than correspondent P_o for monovalent currents through MS channels (Figure 5A). It is reasonable to suggest that on an integral current level, this effect may also contribute to the saturation or even inhibition of Mg^{2+} influx.

Single-current records clearly demonstrate permeation of magnesium when the divalent is the only charge carrier. Direct measurements of unitary currents carried by Mg^{2+} ions together with the estimation of permeability ratio allow one to assume that the channel activity may contribute to Mg^{2+} entry at physiological levels of extracellular Mg^{2+}. However, it remains unclear whether the possible contribution of MS channels in Mg^{2+} transport really occurs under physiological ionic conditions in the presence of competition between extracellular Mg^{2+} and Na^+. We tried to test this possibility using current recording from inside-out patches bathed in Na-containing solutions when Mg^{2+} ions were added only from the extracellular membrane side. It should be noted that MS channel activity in inside-out patches was characterized by less stability as compared to cell-attached patches. Figure 6 shows the results of the experiment in which outward and inward currents recorded from the same inside-out patch could be compared. Inward and outward currents corresponded to very similar conductance values (12.5 pS), thus providing no evidence that the channel carries Mg^{2+} in the presence of sodium.

Discussion

Our data document Mg^{2+} permeation through MS channels in the plasma membrane of K562 leukaemia cells. The estimation of selectivity in terms of permeability ratios using the constant field equation gave rather high values of P_{Mg}/P_K, implying the channel is Mg^{2+}-selective. However, it is reasonable to consider that the application of constant field equation is very limited because of strong saturation of single-channel Mg^{2+} currents and conductance. Therefore, the detection of inward single currents at various Mg^{2+} concentrations is stronger evidence for Mg^{2+} permeation via MS channels when magnesium is the only charge carrier.

Numerous works described inhibitory or modulatory effects of extracellular and intracellular Mg^{2+} on various MS cationic channels, whereas their permeability for Mg^{2+} remained largely unknown. Particularly, stretch-activated K^+ channels in *Lymnaea stagnalis* and TREK-1, an MS K^+ channel, have been found to be blocked by external Mg^{2+} [20, 21]. Wu *et al*. [22] suggested that Mg^{2+} permeated and blocked MS channels and this effect partly contributed to the inward rectification. To address Ca^{2+} or Mg^{2+} permeation, single currents were recorded routinely at extremely high concentrations of bivalent cations in the extracellular solutions (pipette solution in the case of cell-attached). However, it was shown that channel properties strongly depended on the permeant ion concentration [23]. Recently, we have also demonstrated that permeation and blocking effects of Ca^{2+} on MS channels were critically dependent on the external Ca^{2+} level [15]. These data emphasize that concentration range is of primary importance for the correct estimation of channel permeability properties. Notably, in the present work, we managed to record inward single-channel currents carried by Mg^{2+} in the wide range of external concentration including rather low level (1-2 mM) that is similar to physiological values.

A number of observations indicate electrodiffusive transfer of Mg^{2+} consistent with transport through a channel in specialized renal epithelia [7]. In a variety of cells of different specialization, Mg^{2+} entry via non-specific cation channels has been suggested. Mg^{2+} influx through Ca^{2+} channels was observed in frog motor nerve terminals [24]. ADP-induced integral currents carried by different cations, including Ca^{2+}, Ba^{2+} and Mg^{2+} (at 110 mM), have been found in megakaryocytes [25]. The ability of Mg^{2+}

to pass through and to carry substantial currents has been shown for the RyR channel [26] and for the IP_3-gated channel from the cerebellum [27]. The authors suggest that Mg^{2+} ions are able to keep their inner shell of water molecules when they pass through the selectivity filters of both intracellular Ca^{2+} channels. The non-selective voltage-activated cation channel in human red blood cells was reported to be permeable for Ca^{2+} and Ba^{2+}, and even Mg^{2+}; currents were also recorded at extremely high extracellular divalent concentrations [28]. Measurements of macroscopic Mg^{2+} currents in *Paramecium* gave a rare example of a membrane conductance that was strongly Mg^{2+}-specific [5]. The physiological contribution of Mg^{2+} current to normal membrane excitability and behaviour in this ciliate has been proposed.

Although data suggesting the existence of Mg^{2+} channels have been observed in *Paramecium*, functional identification of membrane transport mechanisms for Mg^{2+} entry in other eukaryotic cells was considered to be an unsolved question. Recently, the members of TRP family, namely TRPM subfamily, have been described as Ca^{2+}- and Mg^{2+}-permeable channels and their implication in cellular magnesium homeostasis has been assumed [12, 13]. Specifically, electrophysiological analysis of HEK-293 cells overexpressing recombinant LTRPC7 (TRPM7) showed large currents regulated by millimolar levels of Mg-ATP and Mg-GTP, with the permeation properties of a voltage-independent divalent cation pathway [11, 29]. Mg^{2+} permeation and block of the TRPM6-induced channels were characterized using different approaches, particularly significant inward whole-cell currents were measured in the presence of 20 Mg^{2+} and 120 $NMDG^+$ in the external solution [14]. The genetic analysis together with the expression studies and functional channel characteristics highlight a crucial role of TRPM6 for epithelial Mg^{2+} transport [13]. Alternatively, a participation of different members of the TRP family in the formation of physiologically active Mg^{2+} channel is hypothesized. Various functions of TRP channels have been proposed that are correlated with their diverse activation properties [30]. It is of interest that the activation mechanism of TRPV4 channel is linked to hypotonic cell swelling and its expression pattern is assumed to be in agreement with a possible role as an osmo- or volume sensor [30, 31]. Our single-current data allow us to speculate that MS stretch-activated channels could participate in passive cation transport and Mg^{2+} regulation in mammalian cells. Convincing evidence has shown that MS channels are ubiquitously present in various cell types [32, 33]. Stretch-activated channels described in specialized mammalian cells are thought to be involved in mechanical signal transduction. However, in a large number of non-specialized cells, the functional significance as well as molecular identification of cation-permeable MS channels is poorly understood. Two roles are usually proposed: to pass cations (mainly Na^+) leading to membrane depolarization, and to pass Ca^{2+} to increase the free cytosolic Ca^{2+} concentration either directly or indirectly (by triggering Ca^{2+} release from stores in muscle cells). Evidence for Ca^{2+} permeability has been obtained using fluorescence measurements and single-current recordings at physiological Ca^{2+} levels [15, 34]. Measurements of Mg^{2+} currents and permeability for MS channels imply that additional functional roles could be considered, particularly in cells of blood origin. An essential point that needs to be addressed is to obtain direct evidence for Mg^{2+} influx under physiological conditions. To demonstrate Mg^{2+} entry into the cytosol via the channels, other approaches would be required in addition to single-current identification.

Our patch-clamp recordings show strong saturation effect of inward currents carried by Mg^{2+}: the amplitude of single-channel currents did not significantly increase with the rise of the Mg^{2+} concentration in the solution from the extracellular membrane side. These data correlate with the recent observations, indicating that large variations in magnesemia (an elevation of circulating Mg^{2+} level) are well tolerated *in vivo* [3]. It is worth noting that the absence of apparent physiological effects despite the magnitude of the increase in serum Mg^{2+} level is a striking difference with a comparable increase in serum Ca^{2+}, which induces muscle weakness and arrhythmia.

The saturation of currents and conductance may be due to characteristics of hydratation-dehydratation reactions of the Mg^{2+} ion. In terms of barrier models, the ion-transporting pore appears to include highly saturable binding site for Mg^{2+} that is consistent with known slow dehydratation for this cation. Mg^{2+} currents corresponded to the lower unitary conductance compared to K^+ or Na^+ currents (Figure 2). Accordingly, there is evident difference in the description of channel selectivity in terms of conductance ratios or permeability ratios calculated from reversal potentials. This observation somewhat resembles a dramatic deviation from independence described earlier in classical studies on proton permeability of sodium channels [35, 18]. In conclusion, our single-current measurements provide evidence for Mg^{2+} permeation through MS cation channels and allow one to assume their possible involvement in membrane transport mechanisms. Notably, inside-out current recordings have indicated that MS channel activity in leukaemia cells was not inhibited by internal Mg^{2+} [15] in distinction from Mg^{2+}-inhibited TRP (TRPM7) channels or so-called MIC channels [11, 36]. Mg^{2+} effects on MS channels can be exploited as a fingerprint to search for molecular correlates of stretch-activated channels in mammalian cells [37].

Acknowledgments

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