J. Membrane Biol. 150, 275–282 (1996)

The Journal of
Membrane
Biology

Inhibition of High-conductance, Calcium-activated Potassium Channels of Rabbit Colon Epithelium by Magnesium

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Received: 25 August 1995/Revised: 16 November 1995

Abstract. High-conductance, Ca^{2+}-activated K^+ channels from the basolateral membrane of rabbit distal colon epithelial cells were reconstituted into planar phospholipid bilayers to examine the effect of Mg^{2+} on the single-channel properties. Mg^{2+} decreases channel current and conductance in a concentration-dependent manner from both the cytoplasmic and the extracellular side of the channel. In contrast to other K^+ channels, Mg^{2+} does not cause rectification of current through colonic Ca^{2+}-activated K^+ channels. In addition, cytoplasmic Mg^{2+} decreases the reversal potential of the channel. The Mg^{2+}-induced decrease in channel conductance is relieved by high K^+ concentrations, indicating competitive interaction between K^+ and Mg^{2+}. The monovalent organic cation choline also decreases channel conductance and reversal potential, suggesting that the effect is unspecific. The inhibition of channel current by Mg^{2+} and choline most likely is a result of electrostatic screening of negative charges located superficially in the channel entrance. But in addition to charge, other properties appear to be necessary for channel inhibition, as Na^+ and Ba^{2+} are no (or only weak) inhibitors. Mg^{2+} and possibly other cations may play a role in the regulation of current through these channels.

Keywords: Ca^{2+}-activated K^+ channels — Magnesium — Rabbit colon epithelium — Surface charges — Ion channel reconstitution

Introduction

The basolateral membrane of epithelial cells contains K^+ channels which are primarily responsible for the conductance of this cell membrane. The regulation of the basolateral K^+ conductance is of central importance for the function of epithelial cells, but the intracellular signals responsible for K^+ channel regulation are largely unknown [5, 33].

Besides other types of K^+ channels, high-conductance (''maxi'' or ''big'') Ca^{2+}-activated K^+ (BK_{Ca}) channels are found in the basolateral membrane of many epithelial cells [5]. Previous studies using the patch clamp technique or reconstitution of membrane vesicles in planar lipid bilayers also demonstrated the presence of basolateral BK_{Ca} channels[1] in epithelial cells of rabbit distal colon [18, 21, 36]. These channels are highly selective for K^+ over Na^+ and Cl^- and display voltage-gating similar to BK_{Ca} channels of other cell membranes, i.e., depolarization of the cell interior increases channel activity. Removal of Ca^{2+} from the solution bathing the cytoplasmic side of the channel abolishes channel activity, whereas charybdotoxin, a high-affinity inhibitor of BK_{Ca} channels, blocks from the extracellular side.

As part of studies on the regulation of basolateral K^+ channels by putative intracellular mediators, we examined the effect of Mg^{2+} on the activity of the BK_{Ca} channel of rabbit colonocytes. Mg^{2+} is the most abundant inorganic divalent cation in the cytoplasm and has many functions. Aside from being a necessary cofactor for G-protein mediated mechanisms and many enzymes, especially those involved in phosphate transfer, Mg^{2+} has

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[1] *Abbreviations:* BK_{Ca} channels: high-conductance, Ca^{2+}-activated K^+ channels; E_r: zero-current or reversal potential of the channel; G_c: single-channel conductance; I_c: single-channel current; P_o: probability that the channel is in its conductive or open state; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PS: phosphatidylserine; V_m: holding or command voltage, *cis* solution with respect to the *trans* solution.

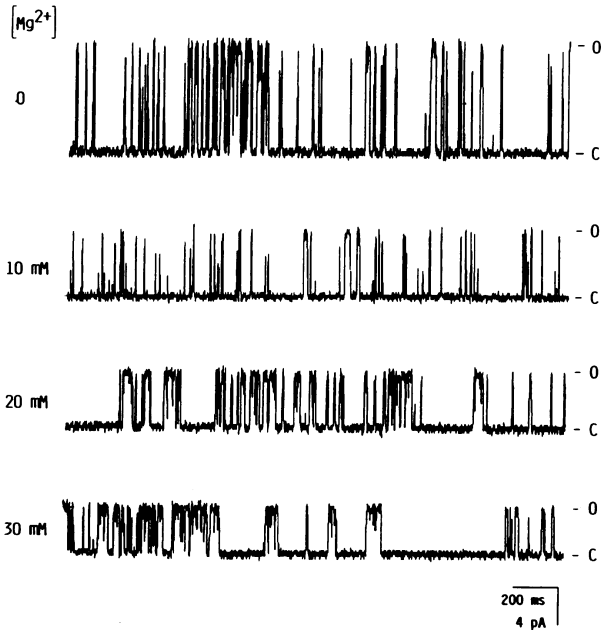

Fig. 1. Example of the effect of Mg^{2+}, added to both the *cis* and the *trans* solution, on current through a BK_{Ca} channel from the basolateral membrane of rabbit colonocytes (V_m = +30 mV) under conditions of asymmetrical KCl solutions (150 mM *cis*, 5 mM *trans*). *−o* and *−c* denote the open (conductive) and closed (nonconductive) state of the channel.
Note that Mg^{2+} causes a concentration-dependent decrease in the amplitude of the channel current.

been reported to regulate co-and counter-transport systems and ion channels [11, 12, 26, 28].

Materials and Methods

Plasma Membrane Preparation

Basolateral membrane vesicles (BLMV) of surface absorptive epithelial cells of rabbit distal colon were prepared from mucosal scrapings as described by Wiener, Turnheim and Van Os [40]. The isolated BLMV were suspended in 250 mM sucrose and 10 mM HEPES-Tris, pH 7.2, frozen in liquid nitrogen, stored at −80°C, and thawed immediately prior to use.

Reconstitution of Ion Channels from BLMV into Planar Lipid Bilayers

Single channel measurements were made by fusing BLMV with planar lipid bilayers consisting of either 25 mg/ml bovine brain phosphatidylethanolamine (PE) and 25 mg/ml phosphatidylcholine (PC) or equal amounts of PE and phosphatidylserine (PS) in decane. The method for production of planar lipid bilayers was essentially that of Schindler [31]. A tiny droplet (~0.5 μl) of the phospholipid solution was taken up into a 5 or 10 μl glass capillary pipette, and the piston of the pipette was moved up and down several times to coat the inside of the glass capillary with the phospholipid. To form a bilayer, an air bubble from the capillary pipette was applied to the aperture (diameter 175 or 250 μm) of a Teflon septum (thickness 12 μm) mounted vertically between two halves of a Teflon chamber. The lipid bilayer separated the *cis* solution (1.5 ml), to which the BLMV were added, from the *trans* solution (1.5 ml). The *cis* solution contained initially 150 mM KCl and the *trans* solution 5 mM KCl, both in 10 mM HEPES-Tris, pH 7.2, and 250 μM $CaCl_2$. Following fusion, i.e., appearance of channel activity, free Ca^{2+} was lowered to approximately 28 μM by addition of a buffered (pH 7.2) K^+-EGTA solution. Free Ca^{2+} was calculated using a Ca^{2+}-EGTA buffer program [27]. The bilayer chamber was shielded in a grounded Faraday cage and placed on a pneumatic antivibration table. All experiments were performed at room temperature (22–24°C).

The solutions in both chamber halves were connected to a patch clamp amplifier (List EPC-7) via 0.5 M KCl-agar bridges in series with Ag/AgCl electrodes. The ''holding'' voltage, V_m, was defined as the electrical potential of the *cis* compartment with respect to the *trans* compartment (ground). Positive current represents the flow of a cation from the *cis* to the *trans* solution. The current across the bilayer was passed through an 8-pole low-pass Bessel filter (Model 902, Frequency Devices) and visualized on a storage oscilloscope. The unfiltered current was recorded via a pulse code modulator (PCM-2 A/D VCR Adaptor, Medical Systems) on a commercial video recorder. For analysis of channel activity with the program pCLAMP (Axon Instruments, Foster City, CA) on a personal computer, the data recorded on the VCR were filtered at 500 Hz and digitized at a frequency of 2,000 Hz (TL-1, Labmaster DMA acquisition system, Axon Instruments). 30–40 sec of channel activity were analyzed at each holding voltage. The channel current, I_c, at a given voltage was obtained from the mean of the Gaussian distribution of the open-state current-amplitude histogram. The probability that the channel is in its conductive or open state, P_o, was defined as the fraction of total time the channel was open. The open-closed discriminator was set halfway between the zero current and full open current levels.

The K^+ concentration in the *cis* and *trans* solution was measured by flame photometry. The K^+ activities were calculated using the activity coefficients published by Robinson and Stokes [30]. At the conclusion of the experiments the K^+ activity in the *cis* solution averaged 111.0 ± 2.0 mM (n = 47) and 10.3 ± 0.5 mM (n = 50) in the *trans* solution, when the initial *cis* and *trans* K^+ concentrations were 150 and 5 mM, respectively.

Materials

Phospholipids were obtained from Avanti Polar Lipids and stored at −20°C for daily use. Stock lipids were kept at −80°C. All other chemicals were analytical grade.

Statistics

Results are given as mean ± SEM. The statistical significance of a difference between means was calculated using the unpaired *t*-test. Linear and nonlinear regression analyses were performed using the least-square method.

Results

Addition of Mg^{2+} to the *cis* and *trans* solution caused a concentration-dependent reduction in current across high-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channels

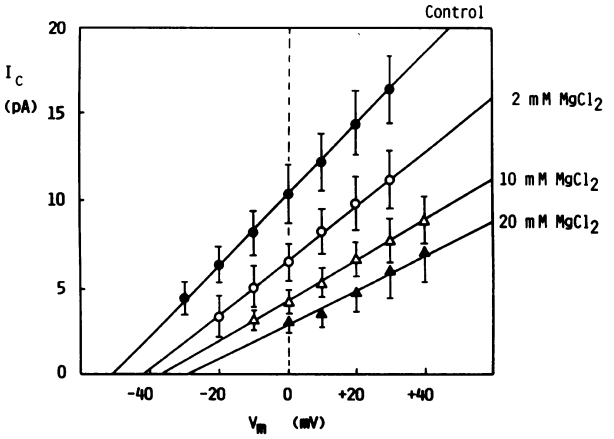

Fig. 2. Effect of Mg^{2+}, added to both the *cis* and *trans* solution, on the relation between I_c and V_m under conditions of asymmetrical KCl solutions (150 mM *cis*, 5 mM *trans*). Means ± SEM of 6 experiments.

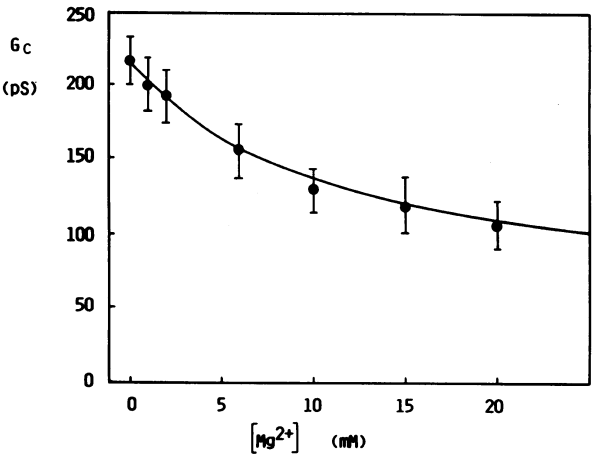

Fig. 3. Concentration-dependence of the effect of Mg^{2+} on channel conductance, G_c. The curve was calculated by approximating the parameters of simple saturation kinetics to the measured values using an iterative curve-fitting procedure.

from the basolateral membrane of rabbit colonocytes (Fig. 1).

The current-voltage (I_c-V_m) relations of the channel exposed to asymmetrical KCl solutions (150 mM *cis*, 5 mM *trans*) in the absence and presence of varying Mg^{2+} concentrations are illustrated in Fig. 2. Mg^{2+} reduced the conductance, G_c, of the BK_{Ca} channel, i.e., the slope of the I_c-V_m relation was decreased. The concentration-dependence of the reduction in G_c by Mg^{2+} conformed to simple saturation kinetics (Fig. 3). From this relation a maximal inhibitory effect of Mg^{2+} on G_c of 160 ± 25 pS can be calculated. This maximum decrease of G_c by Mg^{2+} was not significantly different from the channel conductance without Mg^{2+}, 215 ± pS.

In our reconstitution experiments, we used electroneutral PE/PC or negatively charged PE/PS bilayers. Mg^{2+} inhibited G_c in both types of phospholipid bilayers (Table 1).

In addition to its reducing effect on G_c, Mg^{2+} caused a rightward shift of the reversal potential of the channel, E_r, as is clear from the *x*-axis intersects of the I_c-V_m relations in the absence and presence of varying Mg^{2+} concentrations (Fig. 2).

The voltage-dependence of the probability that the channel is in its open state, P_o, is shown in Fig. 4. Mg^{2+} in concentrations from 2 to 20 mM did not significantly affect P_o.

Further, Fig. 4 illustrates that P_o was high when the transmembrane electrical potential difference was negative (*cis* with respect to *trans*). From this finding and the previously established voltage-dependence of channel gating [36], it follows that the cytoplasmic side of the channels faced the *trans* solution in these experiments. Hence, the orientation of the channels is such that the solution bathing the cytoplasmic side of the channels contained 5 mM KCl, whereas the extracellular side was

Table 1. Effect of Mg^{2+} on the conductance, G_c (pS), of colonic BK_{Ca} channels reconstituted in PE/PC or PE/PS bilayers

$[Mg^{2+}]$ (mM)	0	10	20
PE/PC (n = 6)	172 ± 13	100 ± 6	81 ± 6
PE/PS (n = 5)	216 ± 13	149 ± 18	112 ± 10

The fact that G_c is higher in PE/PS than in PE/PC membranes most likely is due to the negative phospholipid surface charge of the PE/PS bilayers compared to the neutral PE/PC bilayers as discussed in more detail elsewhere [25, 37].

exposed to 150 mM KCl, just the reverse of physiological conditions. We therefore performed additional experiments in which the K^+ concentration in the *trans* solution was increased to 150 mM following channel fusion.[2] The effect of Mg^{2+} on the current-voltage relation of the BK_{Ca} channel exposed to symmetrical KCl concentrations is shown in Fig. 5. The I_c-V_m relations in the absence and presence of Mg^{2+} conformed to a straight line for voltages above and below the reversal potential. In other words, Mg^{2+} equally inhibited channel K^+ currents in the direction extra- to intracellular and intra- to extracellular.

In the experiments described above, Mg^{2+} was added symmetrically to both the *cis* and the *trans* solutions. By addition of Mg^{2+} to only the *cis* or the *trans* solution it can be shown that this divalent cation decreases G_c from both sides of the channel, but E_r is

[2] Initially, the *trans* and *cis* K^+ concentrations were always 5 and 150 mM, respectively. This gradient in osmolarity is necessary to facilitate channel fusion with the bilayer [1].

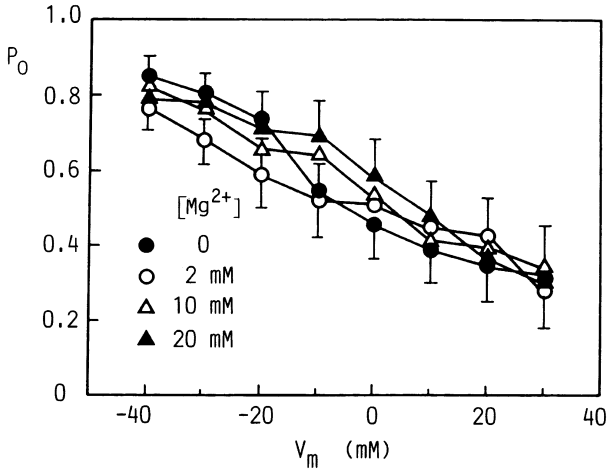

Fig. 4. Effect of Mg^{2+} on the relation between P_o and V_m. Experimental conditions as in Fig. 2.

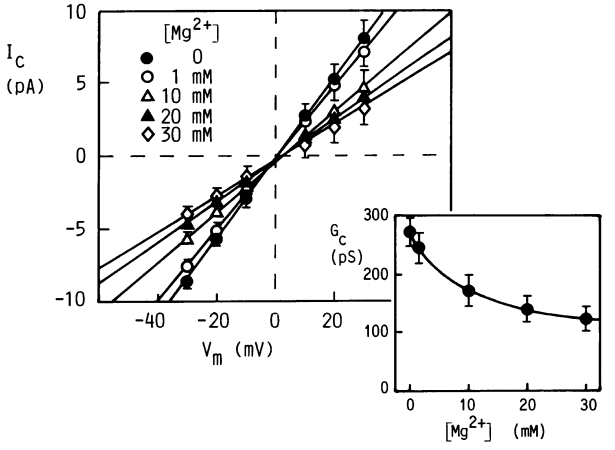

Fig. 5. Effect of Mg^{2+}, added to both the *cis* and *trans* solution, on the I_c-V_m relation under conditions of symmetrical KCl solutions (150 mM *cis* and *trans*). Means ± SEM of 3 experiments. Inset: concentration-dependence of the effect of Mg^{2+} on G_c.

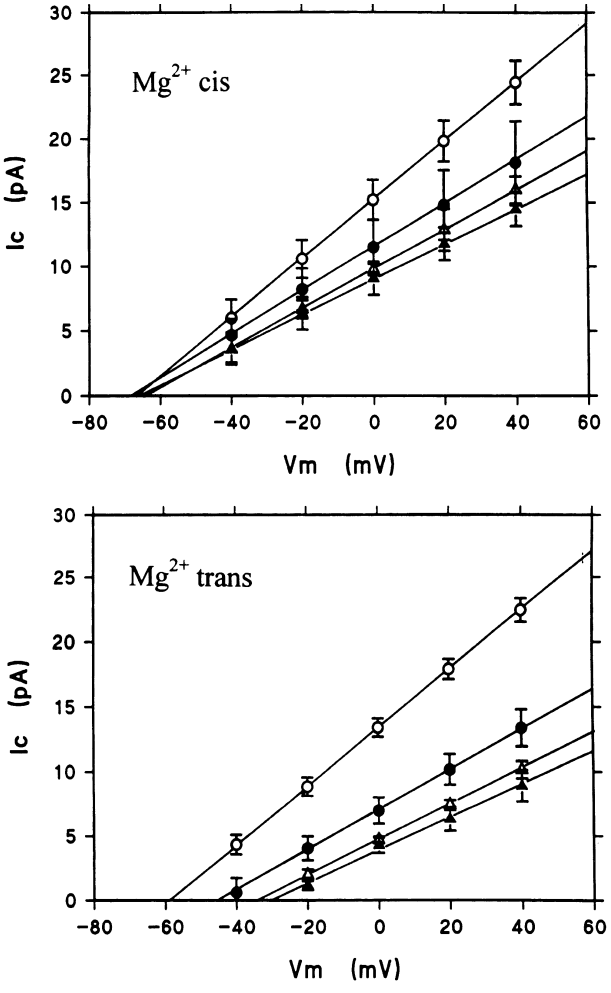

Fig. 6. Effect of Mg^{2+}, added to only the *cis* (top) or the *trans* solution (bottom), on the I_c-V_m relation under conditions of asymmetrical KCl solutions (150 mM *cis*, 5 mM *trans*). Means ± SEM of 3–4 experiments. Mg^{2+} concentrations: 0 (empty circles), 10 mM (full circles), 20 mM (empty triangles), and 30 mM (full triangles).

reduced only from the *trans*, i.e., the cytoplasmic side (Fig. 6).

As illustrated in Figs. 2, 5 and 6, the I_c-V_m relations were linear or ohmic in the presence of Mg^{2+}. This is in contrast to the inhibitory effect of Mg^{2+} on other K^+ channels in which the Mg^{2+}-induced reduction in G_c increases as V_m is made more positive [8, 10, 14, 15, 23]. To further test the voltage-dependence of the Mg^{2+} effect on BK_{Ca} channels of rabbit colonocytes, the apparent dissociation constant of Mg^{2+}, K_D, was determined at different holding voltages. If the Mg^{2+} effect is voltage-dependent, K_D should vary exponentially with V_m according to

$$K_D = K_{D(0)} \exp(\delta z F V_m / RT)$$

[2, 13], where $K_{D(0)}$ is the zero-voltage dissociation constant of the inhibitor and δ the fraction of the transmembrane electrical potential difference acting at the inhibitor site (''electrical distance''). *z*, *F*, *R*, and *T* have their usual meanings. Figure 7 illustrates this analysis for *cis*, *trans* and symmetrical addition of Mg^{2+} under conditions of 150 mM KCl *cis* and 5 mM *trans*. The values of δ obtained are quite low, 0.14 for *trans* Mg^{2+} and 0.06 for *cis* Mg^{2+}. Thus, Mg^{2+} at the inhibitory site on the *trans* side of the channel experiences a somewhat larger fraction of the transmembrane voltage difference than Mg^{2+} on the *cis* side. This sidedness in voltage-sensitivity most likely is responsible for the small voltage dependence of K_D for symmetrical Mg^{2+}, which varied between 2.5 and 4 mM at voltage differences from −20 to +60 mV (Fig. 7). With 150 mM KCl in both the *cis* and

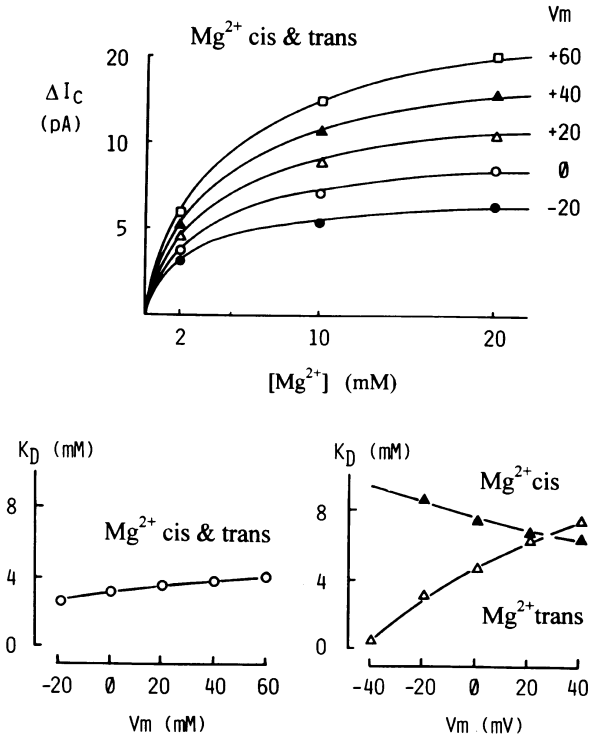

Fig. 7. Top: Voltage- and concentration-dependence of the effect of Mg^{2+} (*cis* and *trans*) on channel current, I_c. ΔI_c is the difference in I_c before and after addition of Mg^{2+}. The curves at each value of V_m were calculated by nonlinear regression analysis, underlying the Michaelis-Menten equation. By this procedure a value for K_D, the apparent dissociation constant of Mg^{2+}, is obtained for each V_m. Bottom: Dependence of K_D on V_m, when Mg^{2+} was added to the *cis* or *trans* solution or to both. Means ± SEM of 3–6 experiments, SEM (*not shown*) ranged between 7 and 22% of the respective means.

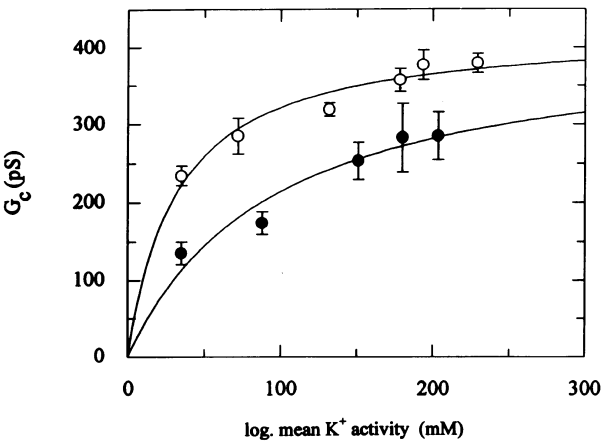

Fig. 8. Dependence of the channel conductance, G_c, on the logarithmic mean K^+ activity, ($\dot{K}$), across the channel under control conditions (empty symbols) or in the presence of 30 mM Mg^{2+} (full symbols). ($\dot{K}$) was increased by adding KCl to the *cis* or *trans* solution in a stepwise manner. Means ± SEM of 3–4 experiments. The curves represent nonlinear regression analyses underlying simple saturation kinetics. Using this procedure an apparent maximum G_c of 422 ± 6 pS was calculated for control conditions (no Mg^{2+}), the corresponding value in the presence of Mg^{2+} was 406 ± 26 pS, which is not significantly different. But the ($\dot{K}$)-value, at which G_c is half maximal, was three times higher in the presence of Mg^{2+} (90 ± 3 mM) than in its absence (31 ± 3 mM).

trans solution, the value of K_D for the inhibitory effect of Mg^{2+} on channel current was increased to 5.5–8 mM (analysis of the data shown in Fig. 5).

Additionally, the interaction between Mg^{2+} and K^+ is illustrated in Fig. 8 which shows the relation between G_c and the logarithmic mean K^+ activity, ($\dot{K}$), given by $[(K)_c\text{-}(K)_t]/\ln[(K)_c/(K)_t]$.[3] G_c depended on ($\dot{K}$) in a hyperbolic manner that conforms to saturation kinetics. Mg^{2+} shifted the G_c-($\dot{K}$) curve to the right, the maximum G_c, however, was not changed by Mg^{2+}. But ($\dot{K}$) at which G_c is half-maximal was increased threefold (Fig. 8). This finding is consistent with competitive inhibition of K^+ current through the channel by Mg^{2+}.

In a final set of experiments, we tested the effects of the monovalent cations choline and Na^+ on the colonic BK_{Ca} channel. Similar to Mg^{2+}, choline caused a marked reduction in G_c and E_r, whereas Na^+ reduced G_c by only a small amount (Table 2).

Mannitol had no effect on G_c or E_r, indicating that the reduction in channel current by the tested cations was not due to changes in osmolarity (Table 2).

Discussion

Mg^{2+} inhibits the current through high-conductance, Ca^{2+}-activated K^+ channels from the basolateral membrane of rabbit distal colon epithelial cells. Reduction of single-channel current by Mg^{2+} has been observed with different K^+ channels, the muscarinic-receptor K^+ channel [14], the inward-rectifier K^+ channel [23, 35], the ATP-sensitive K^+ channel [10, 15], and the Ca^{2+}-activated K^+ channel of skeletal muscle [8]. However, the effect of Mg^{2+} on the BK_{Ca} channel of colon epithelium differs from that of the other K^+ channels twofold:

(1) The I_c-V_m relation of colonic BK_{Ca} channels in the presence of Mg^{2+} is linear (ohmic) in the range −40 to +40 mV, whereas Mg^{2+} confers rectifying properties on the muscarinic receptor, the inward rectifier, the ATP-sensitive K^+ channel and the skeletal BK_{Ca} channel. With other words, in contrast to these channels the voltage dependence of the inhibitory effect of Mg^{2+} on colonic BK_{Ca} channels is small or negligible. Hence, it appears that Mg^{2+} does not sense a major fraction of the

[3] $(K)_c$ and $(K)_t$ are the K^+ activities in the *cis* and *trans* solution, respectively.

Table 2. Effects of choline, Na^+, and mannitol on G_c and E_r of colonic BK_{Ca} channels in comparison to control values before addition of the respective compounds

	G_c (pS)	E_r (mV)
Choline (50 mM *trans*, $n = 3$)	139 ± 9	−22.0 ± 6.8
Control	201 ± 19	−61.9 ± 4.4
Na^+ (50 mM *trans*, $n = 5$)	174 ± 12	−49.5 ± 5.1
Control	188 ± 15	−58.1 ± 1.5
Mannitol (100 mM *trans*, $n = 2$)	225 ± 9	−60.1 ± 4.0
Control	220 ± 11	−60.8 ± 4.2

transmembrane voltage difference at its inhibitory site, i.e., the inhibitory site of Mg^{2+} is located superficially in the channel mouth.

(2) Mg^{2+} reduces the reversal potential, E_r, of the colonic BK_{Ca} channel, whereas no such effect was reported for other K^+ channels. A shift in E_r may suggest that Mg^{2+} permeates the channel, but this is unlikely because infinite Mg^{2+} concentrations seem to block the channel almost completely.[4] The observation that Mg^{2+} changes E_r only when present on the cytoplasmic side of the channel also argues against the possibility that Mg^{2+} permeates through the channel. The ionic radius of unhydrated Mg^{2+} (0.65 Å) is smaller than that of K^+ (1.33 Å), but Mg^{2+} tends to remain hydrated even when interacting with biological macromolecules because of its strongly polarizing nature (effective radius of hydrated Mg^{2+}: 7.0 Å). Mg^{2+} therefore passes through small water-filled channels only with difficulty [11, 41].

A possible explanation for the decrease in E_r by Mg^{2+} is a reduction in the channel selectivity for K^+ over Cl^-. In the absence of Mg^{2+}, the permeability of the channel to K^+, P_K, is approximately 30 times greater than to Cl^-, P_{Cl}. From the decrease in E_r an apparent reduction in P_K/P_{Cl} to 8 can be calculated for 20 mM Mg^{2+}. The notion that Mg^{2+} decreases the K^+/Cl^- selectivity remains to be tested directly.

Mechanism of the Mg^{2+} Inhibition

The reduction of G_c by Mg^{2+} is relieved by increasing K^+ concentrations, indicating competitive (reversible) interaction between Mg^{2+} and K^+. Many chemically unrelated cations besides Mg^{2+} cause a decrease in the conductance of different K^+ channels, for instance Cs^+ [2, 42], monovalent organic cations such as choline [3, *present study*] and tetraethylammonium [19, 42], and polyvalent organic cations such as protamine, poly-L-arginine [6], spermine and spermidine [7, 9, 22, 38]. Hence, the simplest explanation for the inhibitory effect of Mg^{2+} on channel current is an unspecific effect such as screening of negative charges rather than binding to a site specific for Mg^{2+}. Generally, polyvalent cations were shown to be stronger inhibitors, i.e., lower concentrations are required to reduce channel current (in the nM to μM range with spermine, protamine, and poly-L-arginine [6, 7, 9]), than monovalent cations (mM range with Cs^+, TEA, and choline [2, 3]). This relation between cation valence and inhibitory potency is in agreement with surface charge theory [4, 13, 24].

Screening of negative charges in the channel entrance may also be responsible for the finding that G_c tends to saturate with increasing concentrations of K^+. If current across the channel conforms to simple electrodiffusion, a linear relation between G_c and the logarithmic mean K^+ activity, $(\dot{K})$, would be expected that passes through the origin (''independence principle'') [13, 32]. The observed hyperbolic dependence of G_c on $(\dot{K})$ is consistent with multi-ion occupancy of the channel but also with a ''Mg^{2+}-like'' effect of K^+ at high concentrations. At low ionic strength, the negative charges in the channel entrance attract cations into the pore to many times their bulk concentration. In fact, this effect of protein surface charges probably is responsible for the apparently contradictory channel properties of high conductivity and high selectivity [4, 17]. But as the surface potential in the channel mouth is decreased by increasing permeant cation concentrations, this ion concentrating mechanism and the corresponding increase in channel conductance levels off similar to saturation kinetics [4, 17, 24].

The negative charges screened do not appear to be located in the phospholipid bilayer but rather on the channel protein itself, because the inhibitory effect of Mg^{2+} on channel conductance was similar in electroneutral (*PE/PC*) or negatively charged bilayers (*PE/PS*). Thus, the channel mouth does not seem to be formed by the phospholipid molecules of the bilayer.

The nature of the negative charges on the rabbit colon BK_{Ca} channel that appear to be involved in the

[4] If the decrease of E_r in the presence of Mg^{2+} were a result of passage of this divalent cation through the channel, the size of the rightward shift of E_r would indicate a high permeability of the channel for Mg^{2+}, P_{Mg}, compared to that for K^+, P_K. A value for P_K/P_{Mg} cannot be given, because an equation analogous to the Goldman-Hodgkin-Katz (GHK) equation for a mixture of permeant ions of different valence is not available [13]. However, for the monovalent cation choline we can estimate its apparent permeability relative to that for K^+ using the GHK equation. From the shift in E_r caused by choline (Table 2), a $P_K/P_{choline}$ ratio of 1.1 is calculated, indicating that the permeability of choline through the channel would have to be almost as high as that of K^+ to account for the decrease in E_r. But a high permeability of the BK_{Ca} channel for choline is not consistent with the marked reduction in conductance observed in the presence of choline. Passage of choline is also unlikely in view of its size (molecular cross sectional area 23 $Å^2$ compared to 5.5 $Å^2$ for K^+ [3]).

Mg^{2+} effect is not clear. In another K^+ channel, the inward rectifier, the carboxyl group of aspartate has been implicated in mutagenesis studies to be responsible for the Mg^{2+} sensitivity [23, 35]. Replacing aspartate by a cationic amino acid renders the channel Mg^{2+} insensitive, but the I_c-V_m relation now rectifies in the absence of Mg^{2+}. Hence any positive charge appears to cause a decrease in conductance or rectification. In the nicotinic acetylcholine receptor channel, in which Mg^{2+} also reduces conductance, the negative charges responsible for this interaction with Mg^{2+} have been defined by similar mutagenesis techniques [16].

However, simple charge screening should be dependent only on the valence of the screening compound but not on the particular species. Thus, all impermeant monovalent cations should produce identical reductions in channel current or conductance [24]. This was not the case, as choline reduced G_c of colonic BK_{Ca} channels much more effectively than Na^+ (Table 2). The inhibitory potency also varies among divalent cations: in contrast to Mg^{2+}, Ba^{2+} does not significantly affect G_c of colonic BK_{Ca} channel, as reported earlier [36]. Differences in the blocking effects of a number of divalent cations were also observed with the BK_{Ca} channel of rat skeletal muscle [8]. Hence, properties in addition to positive charge appear to determine the potency of a compound to decrease current through BK_{Ca} channels. Some of these properties such as molecular size and shape of the inhibitory species and channel dimensions are discussed by Dani [4].

Decreasing Ca^{2+} reduces P_o but does not change G_c of colonic BK_{Ca} channels [36]. It is therefore improbable that the inhibitory effect of Mg^{2+} on single channel current is a result of a Ca^{2+}-antagonistic effect.

Mg^{2+} is known to facilitate dephosphorylation of proteins [39], hence it is possible that the observed decrease in conductance with Mg^{2+} is a consequence of channel dephosphorylation. We believe that this is an unlikely possibility because increasing K^+ concentrations antagonized the Mg^{2+} effect. In the absence of ATP, it is inconceivable how K^+ would cause rephosphorylation of the channel.

Physiological Significance

Total intracellular Mg^{2+} concentration ranges between 15–17 mM of which approximately 0.5–1.3 mM are free. Under hypoxic conditions or other states of decreased metabolic energy supply, intracellular free Mg^{2+} is doubled or tripled as a result of release of Mg^{2+} bound to ATP [11, 20, 26, 34]. The Mg^{2+} concentration at which the inhibitory effect on current through the colonic BK_{Ca} channel is half-maximal was 2.5–4 mM at K^+ concentrations of 150 mM *cis* and 5 mM *trans* and 5.5–8 mM at a K^+ concentration of 150 mM *cis* and *trans*, corresponding to Mg^{2+} activities (free Mg^{2+} concentrations) of 1.3–2 mM and 2.8–4 mM, respectively.[5] Hence it is possible that Mg^{2+} participates in the physiological regulation of colonic BK_{Ca} channels at least under conditions of decreased energy supply. The increase in cell Mg^{2+} during states of ATP depletion may serve to prevent an inordinate loss of cell K^+ by limiting the outflow through the BK_{Ca} channels. In agreement with such a scenario, decreasing cell ATP in frog proximal tubules with cyanide was found to result in a reduction of basolateral membrane K^+ conductance [29]. It should be noted that intracellular Ca^{2+} also rises when the supply of metabolic energy is impaired. The resulting stimulation of BK_{Ca} channels may be attenuated by the simultaneous rise in intracellular Mg^{2+}.

This study was supported in part by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (Austrian Research Council), project P6487M. Christoph Wachter was supported by a fellowship of the Medizinisch-Wissenschaftlicher Fonds of the Mayor of the City of Vienna, Austria. The chamber used for the reconstitution experiments was kindly provided by Dr. H. Schindler, University of Linz, Austria.

References

1. Cohen, F. 1986. Fusion of liposomes to planar bilayers. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 131–139. Plenum, New York
2. Coronado, R., Miller, C. 1979. Voltage-dependent caesium block of a cation channel from fragmented sarcoplasmic reticulum. *Nature* **280:**807–810
3. Coronado, R., Miller, C. 1982. Conduction and block by organic cations in a K^+ selective channel from sarcoplasmic reticulum incorporated into planar phospholipid bilayers. *J. Gen. Physiol.* **79:**529–547
4. Dani, J.A. 1986. Ion-channel entrances influence permeation. Net charge, size, shape, and binding considerations. *Biophys. J.* **49:**607–618
5. Dawson, D.C., Richards, W. 1990. Basolateral K conductance: role in regulation of NaCl absorption and secretion. *Am. J. Physiol.* **259:**C181–C195
6. Deutsch, N., Matsuoka, S., Weiss, J.N. 1994. Surface charge and properties of cardiac ATP-sensitive K^+ channels. *J. Gen. Physiol.* **104:**773–800
7. Fakler, B., Brändle, U., Glowazki, E., Weidemann, S., Zenner, H.-P., Ruppersberg, J.P. 1995. Strong voltage-dependent inward rectification of inward rectifier K^+ channels is caused by intracellular spermine. *Cell* **80:**149–154
8. Ferguson, W.B. 1991. Competitive Mg^{2+} block of a large-conductance, Ca^{2+}-activated K^+ channel in rat skeletal muscle. *J. Gen. Physiol.* **98:**163–181
9. Ficker, E., Taglialatela, M., Wible, B.A., Henley, C.M., Brown, A.M. 1994. Spermine and spermidine as gating molecules for inward rectifier K^+ channels. *Science* **266:**1068–1072
10. Findlay, I. 1987. The effect of magnesium upon adenosine triphos-

[5] The Mg^{2+} activity was calculated using an activity coefficient of 0.5, assuming a total ionic strength of 100–150 mM [30].

phate-sensitive potassium channels in a rat insulin-secreting cell line. *J. Physiol.* **391:**611–629
11. Flatman, P.W. 1984. Magnesium transport across cell membranes. *J. Membrane Biol.* **80:**1–14
12. Grubbs, R.D., Maguire, M.E. 1987. Magnesium as a regulatory cation: criteria and evaluation. *Magnesium* **6:**113–127
13. Hille, B. 1992. Ionic Channels in Excitable Membranes, 2nd ed. Sinauer, Sunderland, MA
14. Horie, M., Irisawa, H. 1987. Rectification of muscarinic K^+ current by magnesium ion in guinea pig atrial cells. *Am. J. Physiol.* **253:**H210–H214
15. Horie, M., Irisawa, H., Noma, A. 1987. Voltage-dependent magnesium block of adenosine-triphosphate-sensitive potassium channel in guinea-pig ventricular cells. *J. Physiol.* **387:**251–272
16. Imoto, K., Busch, C., Sakmann, B., Mishina, M., Konno, T., Nakai, J., Bujo, H., Mori, Y., Fukuda, K., Numa, S. 1988. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature* **335:**645–648
17. Jordan, P.C. 1987. How pore mouth charge distributions alter the permeability of transmembrane ionic channels. *Biophys. J.* **51:**297–311
18. Klaerke, D.A., Wiener, H., Zeuthen, T., Jørgensen, P.L. 1993. Ca^{2+} activation and pH dependence of a maxi K^+ channel from rabbit distal colon epithelium. *J. Membrane Biol.* **136:**9–21
19. Lerche, H., Fahlke, C., Iaizzo, P.A., Lehmann-Horn, F. 1995. Characterization of the high-conductance Ca^{2+}-activated K^+ channel in adult human skeletal muscle. *Pfluegers Arch.* **429:**738–747
20. Li, H.-Y., Dai, L.-J., Quamme, G.A. 1993. Effect of chemical hypoxia on intracellular ATP and cytosolic Mg^{2+} levels. *J. Lab. Clin. Med.* **122:**266–272
21. Loo, D.D.F., Kaunitz, J.D. 1989. Ca^{2+} and cAMP activate K^+ channels in the basolateral membrane of crypt cells isolated from rabbit distal colon. *J. Membrane Biol.* **110:**19–28
22. Lopatin, A.N., Makhina, E.N., Nichols, C.G. 1994. Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. *Nature* **372:**366–369
23. Lu, Z., MacKinnon, R. 1994. Electrostatic tuning of Mg^{2+} affinity in an inward-rectifier K^+ channel. *Nature* **371:**243–246
24. MacKinnon, R., Latorre, R., Miller, C. 1989. Role of surface electrostatics in the operation of a high-conductance Ca^{2+}-activated K^+ channel. *Biochemistry* **28:**8092–8099
25. Moczydlowski, E., Alvarez, O., Vergara, C., Latorre, R. 1985. Effect of phospholipid surface charge on the conductance and gating of a Ca^{2+}-activated K^+ channel in planar lipid bilayers. *J. Membrane Biol.* **83:**273–282
26. Murphy, E., Freudenrich, C.C., Lieberman, M. 1991. Cellular magnesium and Na/H exchange in heart cells. *Annu. Rev. Physiol.* **53:**273–287
27. Oiki, S., Yamamoto, T., Okada, Y. 1994. Apparent stability constants and purity of Ca-chelating agents evaluated using Ca-selective electrodes by the double-log optimization method. *Cell Calcium* **15:**209–216
28. Preston, R.R., Kung, C. 1994. Inhibition of Mg^{2+} current by single-gene mutation on *Paramecium*. *J. Membrane Biol.* **139:**203–212
29. Rehwald, W., Lang, F. 1986. The effect of cyanide on apparent potassium conductance across the peritubular cell membrane of frog proximal tubules. *Pflueger Arch.* **407:**607–610
30. Robinson, R.A., Stokes, R.H. 1959. Electrolyte Solutions, 2nd ed. Academic Press, New York
31. Schindler, H. 1989. Planar lipid-protein membranes: Strategies of formation and of detecting dependencies of ion transport functions on membrane conditions. *Methods in Enzymology* **171:**225–253
32. Schultz, S.G. 1980. Basic Principles of Membrane Transport. Cambridge University Press, New York
33. Schultz, S.G., Hudson, R.C. 1991. Biology of sodium absorbing cells: dawning of a new era. *In:* Handbook of Physiology, Sect. 6: The Gastrointestinal System, Vol. IV: Intestinal Absorption and Secretion. Field, M., Frizzell, R.A., volume editors. pp. 45–81. American Physiological Society, Bethesda, MD
34. Silverman, H.S., Lisa, F. Di, Hui, R.C., Miyata, H., Sollott, S.J., Hansford, R.G., Lakatta, E.G., Stern, M.D. 1994. Regulation of intracellular free Mg^{2+} and contraction in single adult mammalian cardiac myocytes. *Am. J. Physiol.* **266:**C222–C233
35. Stanfield, P.R., Davies, N.W., Shelton, P.A., Sutcliffe, M.J., Khan, I.A., Brammar, W.J., Conley, E.C. 1994. A single aspartate residue is involved in both intrinsic gating and blockade by Mg^{2+} of the inward rectifier, IRK1. *J. Physiol.* **478:**1–6
36. Turnheim, K., Costantin, J., Chan, S., Schultz, S.G. 1989. Reconstitution of a calcium-activated potassium channel in basolateral membranes of rabbit colonocytes into planar lipid bilayers. *J. Membrane Biol.* **112:**247–254
37. Wachter, C., Turnheim, K. 1995. Surface changes influence the properties of a Ca^{2+}-activated K^+ channel in planar lipid bilayers. *Ital. J. Gastroenterol.* **27:**171 (*Abstr.*)
38. Weiger, T., Hermann, A. 1994. Polyamines block Ca^{2+}-activated K^+ channels in pituitary tumor cells (GH3). *J. Membrane Biol.* **140:**133–142
39. Wen, Y., Famulski, K.S., Carafoli, E. 1984. Ca^{2+}-dependent K^+ permeability of heart sarcolemmal vesicles. Modulation by cAMP-dependent protein kinase activity and by calmodulin. *Biochem. Biophys. Res. Comm.* **122:**237–243
40. Wiener, H., Turnheim, K., Os, C.H. van. 1989. Rabbit distal colon epithelium: I. Isolation and characterization of basolateral plasma membrane vesicles from surface and crypt cells. *J. Membrane Biol.* **94:**147–162
41. Williams, R.J.P. 1970. The biochemistry of sodium, potassium, magnesium, and calcium. *Quart. Rev.* (*Chemical Soc. London*) **24:**331–36
42. Yellen, G. 1984. Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffine cells. *J. Gen. Physiol.* **84:**157–186