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Block of single cardiac sodium channels by intracellular magnesium

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Abstract. Currents through single cardiac sodium channels have been measured in inside-out patches from guinea pig ventricular cells. To abolish the fast inactivation, Na channels were modified by DPI 201-106. In symmetrical Na solutions, a diminution of outward sodium currents can be observed that depends on the intracellular magnesium concentration and the membrane potential. Inward currents were not altered by the concentrations of magnesium used (between 0 and 22.5 mmol/l). In Mg free solutions a linear current-voltage relation can also be measured in the range of outward Na currents. At +60 mV (symmetrical Na solutions, single channel conductance 24 pS) a half maximal block of cardiac Na channels by intracellular magnesium was found at 2.1 mmol/l. From the analysis of single channel current-voltage relationships the concentration and voltage-dependent block by intracellular magnesium of cardiac sodium channels could be described as binding of Mg at one site with a K_d value of 5.1 mmol/l at 0 mV. The site is located at an electrical distance of 0.18 from the inside.

Key words: Cardiac sodium channels – Patch clamp – Block – Intracellular magnesium

Introduction

A number of ion channels are blocked by intracellular magnesium (L-type Ca channels: White and Hartzell (1988); Agnus et al. (1989); ATP-sensitive K channels: Horie et al. (1987); muscarinic K channels: Horie and Irisawa (1987); NMDA-activated channels: Mayer et al. (1984)). At the level of macroscopic currents, a block of outward sodium currents has been described (Pusch et al. 1989). Here we report on the block of single cardiac sodium channels by intracellular magnesium. It is shown that magnesium causes a fast block of sodium channels at physiological intracellular concentrations.

Methods

All experiments were carried out with single ventricular cells from guinea-pig hearts. Cells were dissociated by a procedure similar to that used by Mitra and Morad (1985). The dissociated cells were stored in an Eagle's minimum essential medium. For single channel recordings, cells were incubated in a solution that contained in mmol/l: 140 NaCl, 5 EGTA, 10 Hepes, pH 7.4 with NaOH. The free magnesium concentration in the bath solution was calculated to be between 0 and 22.5 mmol/l by considering two binding constants for Mg to EGTA ($pK_1 = 5.21$, $pK_2 = 3.37$). In the pipette solution the same concentrations were used except that the Ca concentration was 1.8 mmol/l (without EGTA) and 5.4 mmol/l KCl was added.

To prolong the open times of single Na channels, the bath solution was changed by the addition of 5 μM of the S-enantiomer of the piperazinylindole compound DPI 201-106 (Sandoz Ltd., Basel, kindly provided by Professor G. Scholtysik, Bern). DPI 201-106 causes removal of inactivation thereby inducing a prolonged mean open time and long-lasting bursts of openings with no changes in channel selectivity (for detailed properties of the DPI 201-106 modified channel see Nilius et al. (1989)). Owing to the prolonged open times, voltage ramps could be used to evaluate single channel current-voltage relationships.

All experiments were carried out at room temperature (20 ± 1 °C).

The patch clamp device used was standard (Hamill et al. 1981). The currents were digitized at 10 kHz, and filtered with 2 kHz by a 4-pole low-pass Bessel filter. To evaluate current-voltage relationships, we used 50 ms voltage ramps from −140 to +100 mV or double voltage steps from a holding potential of −130 to −60 and to +60 mV. All measurements were performed on inside-out cell free patches. The channels, therefore, faced sym-

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metrical sodium solutions. The sizes of single channel currents were obtained from amplitude histograms. Numerical analysis and modelling were performed by use of a Levenberg-Marquardt non-linear fitting routine. This fitting routine has been adapted for the 4-barrier-3-well model of Na channels as described in detail by Nilius 1988). Pooled data are presented as means ± SEM.

Results

1. Block of sodium channels by intracellular magnesium

Figure 1 shows a typical experiment for the response of cardiac Na channels in excised patches and symmetrical Na solutions. From a holding potential of −130 mV test potentials to −60 and +60 mV were applied symmetrical to the reversal potential under the conditions used. If the internal concentration of Mg was increased from 0 to 22.5 mmol/l in the same patch, a decrease in the amplitude of the outward single channel currents can be observed. In contrast, the inward currents were not altered.

Outward currents showed the same steady state inactivation as inward Na currents and completely disappeared when Na free solutions were applied from the inner side of the membrane (not shown). In asymmetrical solutions (140 mmol/l Na and 1.8 mmol/l Ca outside, 140 mmol/l K inside, 0 Mg), a single channel conductance of 18 ± 2.7 pS was measured from inward currents. In symmetrical Na and Mg-free solutions, the single channel conductance as measured from inward currents was 24 ± 2.6 pS, whereas the conductance measured from outward currents was 37 ± 6.1 pS. This difference nicely fits the block by Ca ions of inward Na currents (Nilius 1988).

From this type of experiment the Mg concentration for half maximal block was measured. Figure 2 shows a synopsis of all data obtained from the same protocol as that shown in Fig. 1. Amplitudes of inward single channel currents were not affected by internal Mg. In contrast, if the concentration of intracellular Mg is increased, the amplitude of outward single channel currents is dramatically reduced. From best fits to

$$i = i_{max}/(1 + [Mg_i]/K_d) \quad (1)$$

(i: single channel current in pA, $[Mg_i]$: Mg concentration at the inner side of the membrane, K_d: concentration for half maximal block, i_{max}: maximal Na outward current), a concentration for half maximal block of 2.1 mmol/l could be obtained at +60 mV.

2. Concentration and voltage dependence of the Mg_i block

To study the mechanism of Mg block in more detail, current-voltage relationships (iV curves) which were obtained from linear voltage ramps were analysed. Figure 3 shows a set of iV curves at different intracellular Mg concentrations. With no Mg at the inner side of the membrane, nearly linear iV curves were obtained. No rectification is seen at positive potentials. If the concentration of Mg at the inner side is increased, a strong reduction of outward currents appears. Inward currents are not affected. From these measurements, iV curves were constructed (see Fig. 5). If the currents at positive potentials were normalized to the unblocked current in 0 mmol/l Mg_i, concentration-response curves can be approximated. Figure 4 (left hand panel) shows concentration-response curves at 5 potentials. The curves were fitted by

$$i_{norm} = 1/(1 + [Mg]_i/K_d) \quad (2)$$

where K_d is the concentration of the half maximal block and depends on the membrane potential. K_d values obtained from these fits were plotted against the respective membrane potential and fitted by

$$K_d(V) = K_d(0) \cdot \exp(-2dF \cdot V/RT) \quad (3)$$

where $K_d(0)$ is the concentration of the half maximal block at 0 mV, d the electrical distance of a binding site for intracellular magnesium, V the membrane potential. F, R, and T have the usual meaning. The fit over all

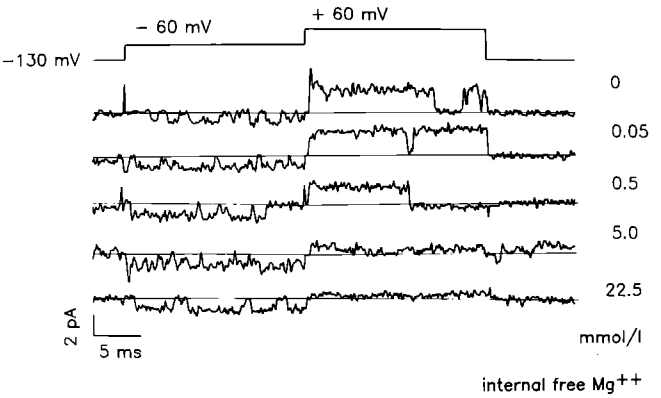

Fig. 1. Single Na channel currents measured from the same inside-out patch by a two-step voltage protocol. From a holding potential of −130 mV, a step to −60 and +60 mV is applied. The concentrations at the right hand side of the panel mark the free Mg concentrations at the inner side of the excised patch. The outward but not the inward currents are reduced due to increased Mg concentrations

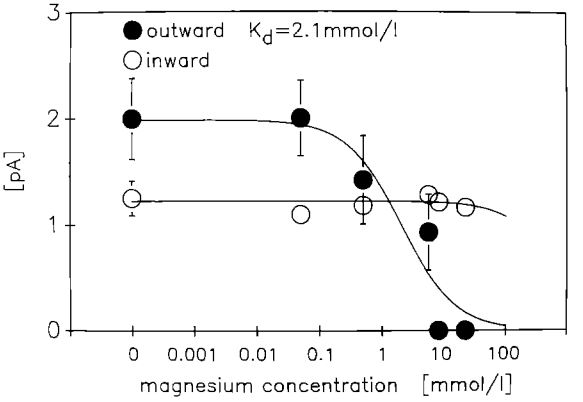

Fig. 2. Dependence of single Na channel currents on the intracellular Mg concentration in inside-out patches. Currents at −60 and +60 mV were measured by use of the pulse protocol shown in Fig. 1. Data from 4 cells were pooled. Whereas inward currents are not changed by intracellular magnesium, outward currents are reduced due to an increase in the intracellular Mg concentration. Smooth curves were obtained from best fits to (1). The concentration for half maximal block was 2.1 mmol/l

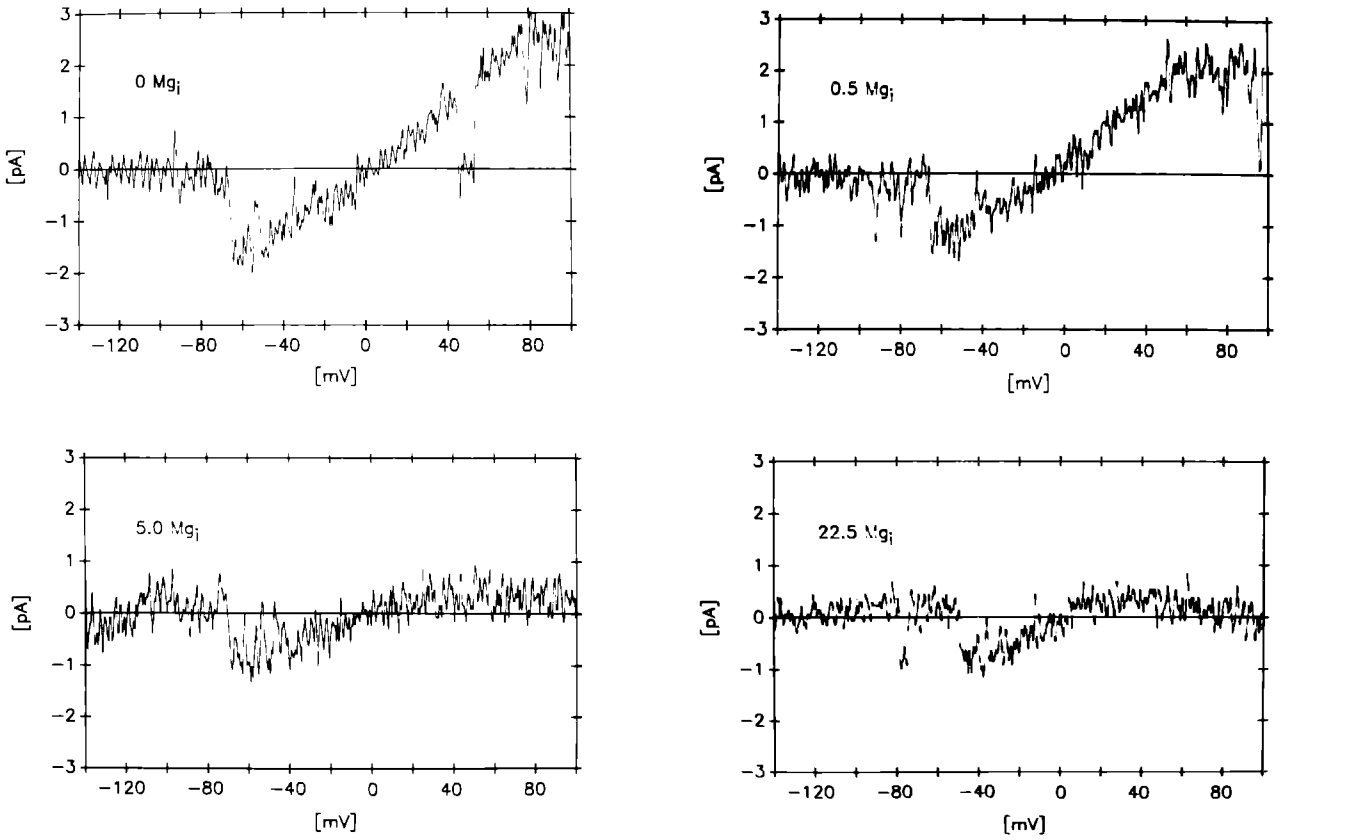

Fig. 3. Instantaneous current-voltage relationships obtained from linear voltage ramps at different intracellular Mg (Mg_i) concentrations (inside-out patches). Shown are individual iV curves from single channel measurements. Mg concentrations are indicated in each panel

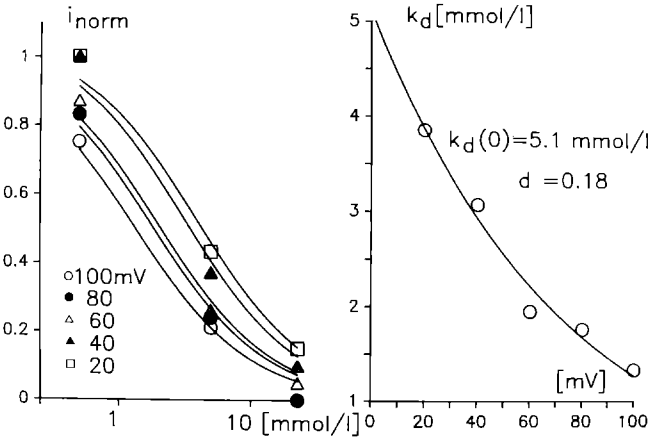

Fig. 4. Quantitative analysis of the block by intracellular magnesium of Na channels. *Left:* from the measurements shown in Fig. 3 (from a total of seven cells) concentration-response curves for intracellular Mg were constructed. The concentration-response curves at different membrane potentials were fitted by (2) to obtain K_d values at different potentials. *Right:* plot of the K_d values obtained from measurements as shown in the left hand panel against the respective membrane potentials. Data were fitted by (3). From best fits the two parameters $K_d(0)=5.1$ mmol/l, and $d=0.18$ were obtained (for details see text)

obtained data points is shown in Fig. 4 (right hand panel). From best fits the following data were obtained: $K_d(0)=5.1$ mmol/l, electrical distance of a Mg binding site $d=0.18$ from the inside. These data were used to evaluate a quantitative model of Na channel block by intracellular magnesium. The left hand panel of Fig. 5 shows current-voltage curves pooled from ramp experiments on 4 patches. At positive potentials a voltage and concentration dependent block by magnesium can be observed. Smooth curves were fitted with energy barrier models shown in the right hand panel. The energy profile in the absence of magnesium at the inner side of the membrane was used for permeation of Na ions. The same profile was applied for all calculations in the presence of Mg. For fitting, initial values of data published previously were used (Nilius 1988). The electrical distances were fixed according to Nilius (1988): $d_1=0.36$, $d_2=0.6$, $d_3=0.8$, barriers are assumed to be symmetrical. The first well is fixed according to the K_d value for sodium to -1 RT units (Nilius 1988), the second and the third well are assumed to be equal. All other parameters were left free and were obtained from best fits. With this profile for Na, the effect of Mg as the second ion was fitted. The approximation was performed as simply as possible: we assume an outer binding site for extracellular magnesium at an electrical distance of 0.36 from the outside (see Nilius (1988) and also Yamamoto et al. (1984)). The affinity of this binding site was estimated to be -2 RT units (first well, see Nilius (1988)). The first barrier for Mg from the outside was assumed to be equal to that for Ca and was set to 10.7 RT units. The second well was set to 0.5 RT. From the measurements shown, the inner well was identified with the inner binding site for Mg at 0.18 from the inside. The Mg affinity is -5.28 RT units (see

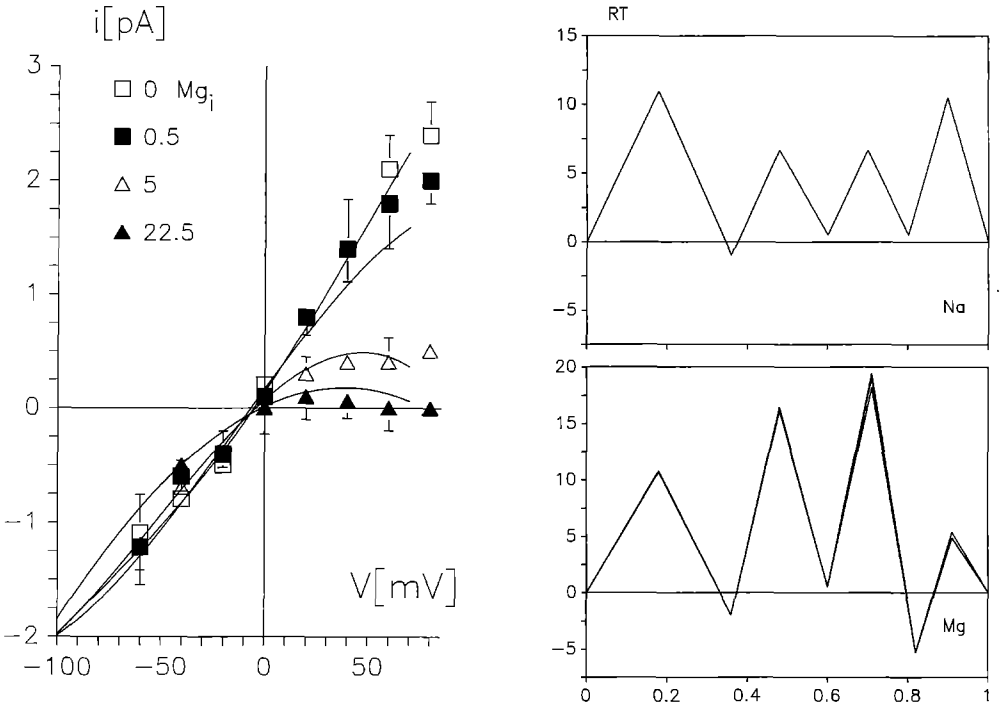

Fig. 5. Modelling of the block by intracellular Mg of cardiac Na channels. *Left:* current-voltage relationships measured at eight selected potentials from protocols as shown in Fig. 3. Data were obtained from four cells (mean ± SEM). The smooth curves were calculated from best fits according to a four-barrier three-well model of the Na channel for two ions (Na and Mg, for details see Hille (1975), Nilius (1988). *Right:* energy barriers obtained from best fits of the means of the currents measured at the selected potentials as shown by iV curves in the left hand panel. *Top:* in the absence of intracellular Mg the following data were obtained (G_{12} – first, G_{23} – second, G_{34} – third, G_{45} – fourth barrier; G_1, G_2, G_3 first to third well; d_1, d_2, d_3 location of the wells from outside; the barriers are assumed to be symmetrical; Na and Mg are for sodium and magnesium): G_{12}Na = 10.9 RT, G_2Na = −1 RT*, G_{23}Na = 6.3 RT, G_3Na = 0.5 RT*, G_{34}Na = 6.7 RT, G_4Na = 0.5 RT*, G_{45}Na = 10.5 RT, d_1 = 0.36*, d_2 = 0.6*, d_3 = 0.8*. *Bottom:* G_{12}Mg = 10.7* RT (0.5 Mg), G_{23}Mg = 16.1, G_{34}Mg = 19.1, G_{45}Mg = 5.1, G_{12}Mg = 10.7* RT (5 Mg), G_{23}Mg = 16.5, G_{34}Mg = 18.2, G_{45}Mg = 4.9, G_{12}Mg = 10.7* RT (22.5 Mg), G_{23}Mg = 16.4, G_{34}Mg = 19.4, G_{34}Mg = 5.4.
* from Nilius (1988). G_2Mg = −2 RT units and d_1 = 0.36, d_2 = 0.6 (all values from Nilius 1988); G_3Mg = 0.5 RT, G_4Mg = −5.28 (Fig. 4), d_3 = 0.82 (Fig. 4) were fixed

Fig. 4). Therefore, fits were performed only by variation of G_{23}, G_{34}, and G_{45} (second, third, fourth barrier from outside) as the free parameters. The graph at the bottom (right hand panel) gives the individual barriers for all fits at 0.5, 5, and 22.5 mmol/l internal Mg. All iV curves were fitted with only minor deviations in the barriers (see legend to Fig. 5).

Discussion

A variety of ion channels are blocked by intracellular magnesium (L-type Ca channels: White and Hartzell (1988), Agnus et al. (1989); ATP-sensitive potassium channels: Horie et al. (1987); muscarinic potassium channels: Horie and Irisawa (1987)). All channels are blocked in the mmol/l range of intracellular Mg concentrations by reducing the amplitudes of single channel currents (fast block). This block seems to be a quite general feature of ion channels. Here we report on a similar block of single cardiac Na channels by intracellular magnesium.

Outward currents through sodium channels have not yet been analysed in detail in cardiac muscle and have even been neglected in many electrophysiological measurements (e.g. Bodewei et al. 1982; Follmer et al. 1987). A frist report on a block by intracellular magnesium of outward currents through sodium channels has been given from type II rat brain sodium channels expressed in oocytes (Pusch et al. 1989). From a tail current analysis a half blocking concentration of magnesium at 0 mV between 3 and 4 mmol/l was determined. The voltage dependence was e-fold per 49 mV. In the present paper a similar block in cardiac muscle is analysed from single channel data. The main finding was a voltage and concentration dependent decrease of the amplitude of the single channel currents. Only outward currents were affected without a change of inwardly directed currents. These findings refer to a fast block of Na channels by magnesium. In the present paper we used the approach of Hille (1975) to describe this block. In the same way as Nilius (1988), we used a four-barrier three well model to describe ion currents through single sodium channels. The fast block can be explained by binding of internal magnesium within the channel to a site that is located at an electrical distance of 0.18 from the inside and shows an affinity of 5.1 mmol/l (−5.28 RT units) at 0 mV. Binding at this low affinity site can explain the diminution of the amplitude of the single channel current. An outward current of Mg is prevented by the high energy barrier of 18.9 ± 0.5 (seven measurements at three different Mg concentrations). This binding site is separated from the intracellular space by a small energy barrier of only 5.1 ± 0.1 RT units (seven

measurements at three different Mg concentrations). From this analysis we propose a fast block of the Na channel pore by binding of Mg at one inner side of the channel.

We found an e-fold decrease of the amplitude of the single channel currents per 69.4 mV. This voltage dependence is in the same range as the voltage dependence of the Mg block obtained from macroscopic currents (e-fold per 49 mV, Pusch et al. (1989)). Also, the K_d values for the block as obtained from two different methods (2.1 mmol/l at +60 mV, see Fig. 2, 5.1 mmol/l at 0 mV from the Hille analysis, see Fig. 4) nicely agree with $\approx$4 mmol/l at 0 mV from Pusch et al. (1989). These values are in the range of physiological intracellular concentrations of magnesium ($\approx$1.7 mmol/l for resting skeletal muscle, Alvarez-Leefmans et al. (1986)).

These data are consistent with data obtained from block by Mg of calcium channels, ATP-sensitive potassium channels, and muscarinic potassium channels. Sodium channels possess at least two binding sites for Mg that blocks the channels from both outside and inside. Both mechanisms are now included in the 4-barrier-3-well scheme as shown in Fig. 5. The block by magnesium from the outside seems to play a minor role whereas the block from the inside would occur at physiological concentrations. It is argued that block of ion channels by intracellular magnesium is a general mechanism.

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