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Rapid Report

The conductance of single cardiac sodium channels from guinea pig depends on the intracellular sodium concentration

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Currents through DPI 201–106 modified single sodium channels have been measured in cell-free inside-out patches from guinea-pig ventricular myocytes. Single-channel conductance and reversal potential of the sodium channel have been calculated at different intracellular sodium concentrations ($[Na^+]_i$) from microscopic *I–V* curves, which were obtained by application of linear voltage ramps. The relation between the reversal potential and $[Na^+]_i$ could be fitted with a modified Goldman-Hodgkin-Katz equation with a relative permeability for K^+ over Na^+ ions of 0.054. The zero-current conductance of the Na channel as a function of $[Na^+]_i$ shows a plateau value at low Na concentrations, and increases in a sigmoidal manner at higher concentrations. It is concluded that the Na channel can carry outward currents and that its conductance depends on $[Na^+]_i$.

Cardiac sodium channels are responsible for the fast depolarizing phase of the action potential. Recently, it has been suggested that these channels might be involved in the initial repolarization or at least in limiting the depolarization [1]. Outward currents through sodium channels during the early repolarization become imaginable when sodium accumulates near the internal side of the membrane as a result of stimulation and a large influx through the sodium channels themselves. A 6-fold higher sodium concentration was measured near the inner side of the membrane compared to the central cytosolic concentration when cardiomyocytes were stimulated repetitively and shock-frozen in diastole [2]. Until now it has not been known whether a change in $[Na^+]_i$, beside its effect on the driving force for Na^+ ions, can modify currents through Na channels. In this paper we present data showing that $[Na^+]_i$ modulates the conductance of single cardiac Na channels.

Single cardiomyocytes from guinea-pig ventricular tissue were dissociated as described previously [3]. All experiments were carried out on cell-free patches in the inside-out configuration of the patch-clamp technique. The solution in the patch pipette contained in mmol/l: 140 Na^+, 4 K^+, 1 Ca^{2+}, 1 Mg^{2+}, 147 Cl^-, 5 Hepes (pH 7.4). The sodium concentration in the bath solution was changed between 1 and 140 mmol/l. Osmolarity was kept constant by adding complementary amounts of potassium ($[Na^+] + [K^+] = 140$ mmol/l). In some experiments a 500 mmol/l NaCl solution was used. All solutions contained additionally 5 Hepes and 5 EGTA (pH 7.2). 5 μM of the *S*-enantiomer of the piperazinylindole compound DPI 201–106 (Sandoz Ltd., Basel) were added to the bath solution in order to remove the fast inactivation of the voltage-dependent sodium channel [4,5]. All experiments were carried out at room temperature (21 ± 1°C).

In order to demonstrate an Na-outward current through Na channels, we have used a double-step voltage protocol. A 20 ms step from a holding potential of −140 mV to −60 mV was applied to activate the Na channels, and was followed by a second step to a potential of +60 mV to induce outward currents. Fig.1A shows typical single-channel recordings from the DPI-modified Na channel activated by a rectangular voltage step from a holding potential of −140 mV to a test potential of −60 mV in a bath solution containing 140 mmol/l K^+ but no Na^+. No inactivation could be observed during the 20 ms lasting step. During the consecutive step to +60 mV no openings of either the Na channel or any other channel (e.g. ATP-dependent potassium channel) could be recorded. During the same voltage protocol in a bath solution with 140 mmol/l Na^+ instead of K^+ long-lasting outward

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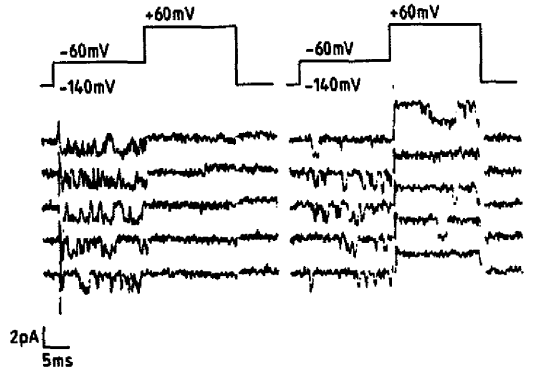

Fig. 1. (A) Unitary currents through DPI-modified Na channels were evoked by a voltage step of 20 ms duration from a holding potential of −140 mV to −60 mV, which was followed by an additional step to +60 mV (see the voltage protocol at the top of the figure). The patch pipette contained 140 mmol/l Na^+ as the main cation, whereas the bath solution contained (in mmol/l) 140 K^+, 5 Hepes, and 5 EGTA. Typical inward currents could be measured during the first step, but channel activity was absent during the second one. (B) Outward currents through the sodium channel at +60 mV in the same patch as in A after replacing K^+ ions in the bath solution by 140 mM Na^+ (at least two channels in the patch).

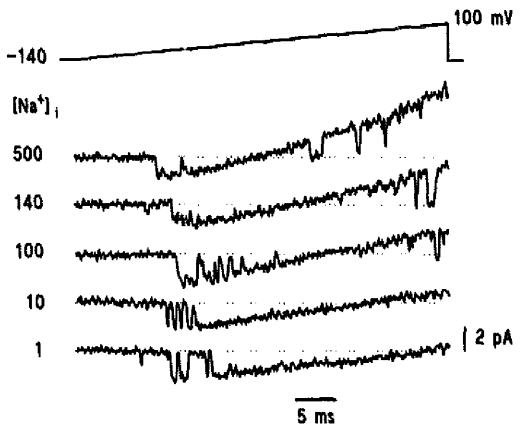

Fig. 2. Single-channel currents through DPI-modified sodium channels during voltage ramps from −140 to 100 mV recorded in inside-out cell-free patches at different intracellular Na concentrations (140 mmol/l Na in the pipette solution). Note the shift in reversal potential and the steeper slope of the current trace at higher Na concentrations. Records have been corrected for capacitive transients and leakage current by subtracting an ensemble averaged current from empty sweeps measured under the same conditions.

currents, representing outward Na currents through sodium channels, appeared during the second step to +60 mV (Fig. 1B).

Permeation and selectivity properties of single cardiac sodium channels were studied in cell-free inside-out patches by analyzing current–voltage relationships (*I–V* curves) at different intracellular sodium concentrations ($[Na^+]_i$). For non-inactivating ion channels, such *I–V* curves can be easily obtained by applying linear voltage ramps. When such ramps of 50 ms duration from a holding potential of −140 mV up to +100 mV were applied to excised patches the first openings of the sodium channel appeared from a potential of about −80 mV on. For our analysis we have used records during which a single channel remained open for almost the complete duration of the ramp and which were not disturbed by openings of an additional channel. Representative traces at each $[Na^+]_i$ are shown in Fig. 2, from which it can be observed that the reversal potential of the Na current shifts with $[Na^+]_i$ and that the slope of the current traces becomes steeper at higher $[Na^+]_i$. Because of the linear change in voltage with time, these traces can be easily converted to *I–V* curves. The non-linearity of the current traces and therefore of the *I–V* relations in the range of inward currents is due to a block of sodium channels by extracellular Ca^{2+} [6]. The zero-current conductance (g_{Na}) and reversal potential (E_{rev}) were obtained from polynomal fits through the data points, using the equation

$$i = g_{Na} \cdot (V - E_{rev}) + a \cdot (V - E_{rev})^2 + b \cdot (V - E_{rev})^3 \qquad (1)$$

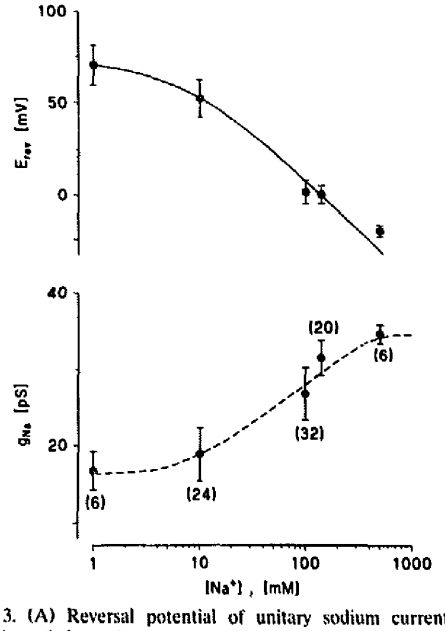

Fig. 3. (A) Reversal potential of unitary sodium currents as a function of the sodium concentration at the inner surface of the sodium channel. The smooth line shows the fit of these data to a modified Goldman-Hodgkin-Katz equation (Eqn. 2), with a permeability ratio for $Na^+ : K^+$ of 1:0.054. (B) Dependence of the single-channel Na conductance on $[Na^+]_i$. The dashed line has been drawn by eye. Data points correspond to mean values ± standard deviation, the number of patches from which these data have been obtained is given between brackets in the lower panel.

The quadratic and cubic terms in this equation were used to describe the deviation from linearity, but the coefficients a and b are not further used. The dependence of g_{Na} and E_{rev} on $[Na^+]_i$ is represented in Fig. 3. It is obvious that both g_{Na} and E_{rev} change monotonically with $[Na^+]_i$. In Fig. 3A the mean reversal potentials were plotted as a function of the sodium concentration at the internal surface of the patch membrane. The values for the reversal potential obtained at constant $[Na^+]_i$ + $[K^+]_i$ were fitted to a modified Goldman-Hodgkin-Katz equation

$$E_{rev} = \frac{RT}{F} \cdot \ln \frac{[Na]_0 + P_{rel} \cdot [K]_0}{[Na]_i + P_{rel} \cdot (140 - [Na]_i)} \quad (2)$$

It was assumed that sodium and potassium are the main charge carriers in the solutions on both sides of the membrane, and it was taken into account that $[K^+]_i = 140 - [Na^+]_i$. In this equation R, T and F have their usual meaning, and P_{rel} represents the relative permeability of K^+ over Na^+. From this fit a value of 0.054 for P_{rel} was obtained.

Fig. 3B shows the dependence of the single-channel conductance of the Na channel on $[Na^+]_i$. At low $[Na^+]_i$ it is independent of the Na concentration with a value of about 17 pS, in the range about 100–140 mmol/l it increases with $[Na^+]_i$ and reaches a maximum level of about 35 pS at still higher Na concentrations. The monotonic increase of g_{Na} with $[Na^+]_i$ and the monotonic increase of the outward current with increasing $[Na^+]_i$ at all potentials are consistent with a single-ion pore [7]. However, the lack of an anomalous mole fraction behavior does not exclude a multi-ion pore mechanism, which behaves as a single ion pore. This could be due to saturation of the multi-ion pore with permeant cations under our experimental conditions. Such a behavior is also expected if the binding depths for Na^+ and K^+ ions are not very dissimilar energetically. The values of the reversal potential showed a large scatter, especially at the lower Na concentrations, as can be judged from the large error bars. This dispersion also occurred in consecutive sweeps from the same patch during the same experimental protocol. A possible explanation could be a variable accumulation of Na^+ ions in an unstirred layer near the inner side of the excised Ω-shaped patch membrane. In this case, the shifts in reversal potential should be correlated with the channel activity in the preceding record. However, in four patches that we have analyzed, no such correlation could be observed. Changes in selectivity are an alternative explanation for the observed heterogeneous values for the reversal potential. Such inherent fluctuations in selectivity, which would lead to prominent changes in reversal potential, especially at low Na, high K concentrations, have been reported previously [8].

References

1 Wellis, D.P., DeFelice, L.J. and Mazzanti, M (1990) Biophys. J. 57, 41–48.
2 Isenberg, G. and Wendt-Gallitelli, M.F. (1990) J. Physiol. 420, 86P.
3 Mitra, R. and Morad, M. (1985) Am. J. Physiol. 249, H1056-H1060.
4 Kohlhardt, M., Fröbe, U. and Herzig, J.W. (1987) Proc. R. Soc. Lond. B 232, 85–100.
5 Nilius, B., Vereecke, J. and Carmeliet, E. (1989) Pflügers Arch. 413, 234–241.
6 Nilius, B. (1988) J. Physiol. 399, 537–558.
7 Hille, B. (1984) Ionic channels of Excitable Membranes, Sinauer, Sunderland, MA.
8 Albitz, R., Droogmans, G. and Nilius, B. (1991) Gen. Physiol. Biophys. 10, 3–9.