NEGATIVE SHIFT OF CARDIAC Ca^{2+} CHANNEL INACTIVATION BY REDUCING EXTERNAL $[Na^+]$

Geert Callewaert[1], Adam Orkand[2], Ajay Chawla[2] and Martin Morad[2]
[1]Laboratory of Physiology, University of Leuven, 3000 Leuven, Belgium
[2]Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104

For accurate measurement of the Ca^{2+} current in cardiac cells, it is necessary to eliminate the interference from the fast Na^+ current and K^+ currents. K^+ currents are readily eliminated by replacing internal and external K^+ ions by the impermeant ion Cs^+. The fast Na^+ current is eliminated either by using the specific blocker tetrodotoxin (TTX) or preferably by substituting external Na^+ with an impermeable ion. Here we report that eliminating or reducing external Na^+ by substitution with Cs^+ or Li^+ markedly affects the Ca^{2+} current.

Single rat and guinea-pig ventricular cells were enzymatically dissociated using the method described by Mitra and Morad (Am.J.Physiol. 249:H1056-H1060, 1985). Experiments were performed at room temperature (20 to 25 °C) using the whole-cell patch-clamp technique (Hamill et al., Pflügers Arch. 391:85-100, 1981). Patch pipettes had a resistance of 2 to 5 MOhm when filled with a solution containing (in mM): 45 NaCl, 80 CsCl, 10 EGTA, 5 Na_4BAPTA (glycine, N,N'-(1,2-ethanediylbis(oxy-2,1-phenylene))bis(N-carboxymethyl)-, sodium salt), 0.01 to 0.1 cAMP, 0 to 0.1 GTP-γ-S (guanosine 5-O-(γ-thio) triphosphate), 2 MgATP, 25 HEPES, adjusted with CsOH to pH 7.4. The standard external solution contained (in mM): 140 NaCl, 2 $CaCl_2$, 0 to 2 KCl, 2 CsCl, 1 $MgCl_2$, 10 HEPES, 0.002 TTX, adjusted to pH 7.4 with NaOH. The low Na^+ external solution contained (in mM): 5 NaCl, 135 CsCl, 2 $CaCl_2$, 0 to 2 KCl, 1 $MgCl_2$, 10 HEPES, 0.002 TTX, adjusted to pH 7.4 with CsOH. External Ca^{2+} was replaced by Ba^{2+} in some experiments, but there was no apparent difference with regard to the observed effects. External solutions were exchanged rapidly using a multi-barrel pipette with a common opening placed approximately 100 μm from the cell under investigation.

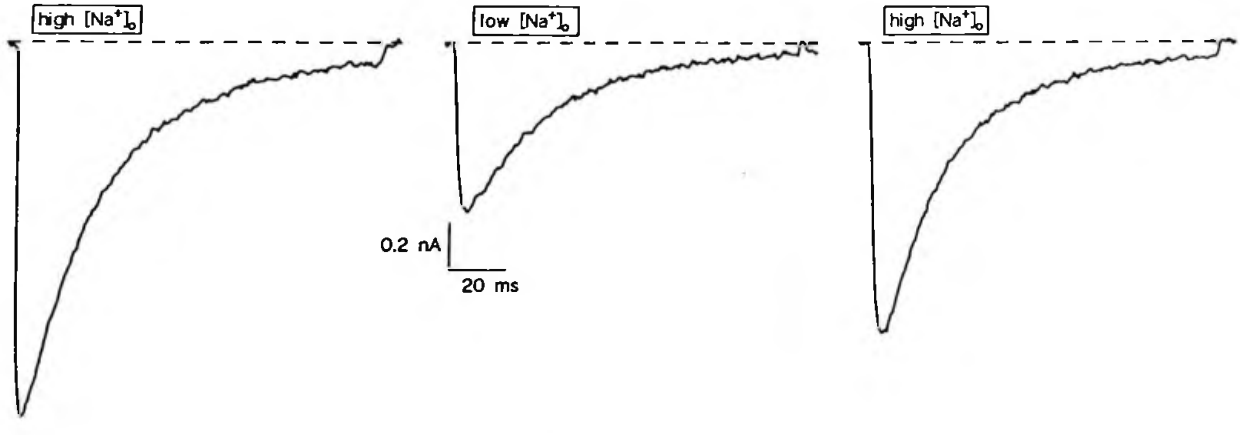

Figure 1. Effect of low external $[Na^+]$ on the amplitude and time course of the Ca^{2+} current in a rat ventricular myocyte. The Ca^{2+} current was elicited by step depolarizations from -40 to 0 mV at 0.2 Hz. In low external $[Na^+]$ the Ca^{2+} current is reduced by more than 50% (middle trace) and this effect is reversible upon returning to control medium (right trace).

Figure 1 illustrates the effect of reducing external [Na^+] on the Ca^{2+} current in a rat ventricular myocyte. In control conditions i.e. in the presence of 150 mM external Na^+, depolarizations from a holding potential of -40 to 0 mV elicited a Ca^{2+} current with an amplitude of 1.7 nA. When the cell was perfused with the low [Na^+] medium, the Ca^{2+} current at 0 mV declined to 0.8 nA. Time-to-peak current and the decay phase of the Ca^{2+} current did not appear to be affected. When the decay phase of the Ca^{2+} current was fitted by a single exponential, the time constant was 25 ms in control conditions as compared to 30 ms in low [Na^+] medium. Upon return to control medium the Ca^{2+} current was restored to 1.3 nA. The Ca^{2+} current in guinea-pig ventricular myocytes showed a similar reduction upon exposure to a low [Na^+] medium.

Figure 2 (left panel) shows peak Ca^{2+} current-voltage relations at a holding potential of -35 mV in control and low external [Na^+]. Perfusing the cell with low [Na^+] medium reduces the Ca^{2+} current at all voltages. This reduction was not related to a change in driving force since the reversal potential of the Ca^{2+} current was near +50 mV in both relations. In the range -35 to +95 mV currents were simply scaled down by perfusing with low [Na^+] medium with little apparent change in voltage-dependence.

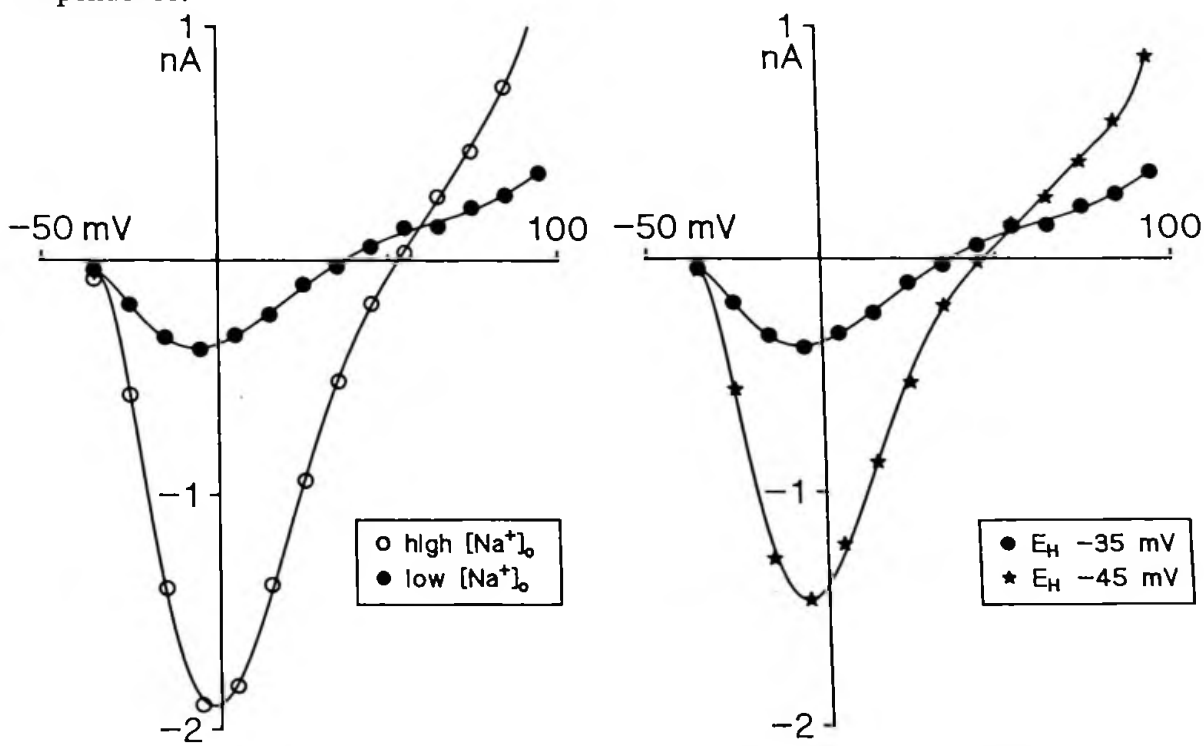

Figure 2. Peak Ca^{2+} current-voltage relations in a rat ventricular myocyte. Left panel: Current-voltage relations determined by pulsing from a holding potential of -35 mV in control and low external [Na^+] medium. Peak Ca^{2+} current is plotted as a function of test potential. Right panel: Current-voltage relations in low external [Na^+] medium determined by pulsing from a holding potential of -35 or -45 mV. Peak Ca^{2+} current is plotted as a function of test potential.

The reduction of the Ca^{2+} current by reducing external [Na^+] may be the result of a voltage shift of Ca^{2+} channel kinetics. Figure 2 (right panel) shows peak Ca^{2+} current-voltage relations in low external [Na^+] medium at a holding potential of either -35 or -45 mV. Shifting the holding potential to a more negative level enhanced the Ca^{2+} current at all voltages, restoring about 75% of the Ca^{2+} current obtained in

control conditions at a holding potential of -35 mV (figure 2, left panel). This finding thus indicates that the reduction of the Ca^{2+} current by lowering external $[Na^+]$ is mainly caused by a negative shift of the steady-state inactivation curve.

To confirm the negative shift of the inactivation curve, the Ca^{2+} current at a test potential of 0 mV was measured while varying the holding potential. The steady-state inactivation curves thus obtained are shown in figure 3 (left panel). The midpoint of inactivation was -34 mV in control conditions and shifted to -42 mV in low $[Na^+]$ medium.

By contrast with the effect on channel inactivation, reducing external $[Na^+]$ had only minimal effects on the voltage dependence of activation. Steady-state activation curves were obtained either by measuring maximum tail currents on return to the holding potential of -45 mV from various depolarizations (figure 3, right panel) or estimated from the maximum conductance of the Ca^{2+} current according to Isenberg and Klöckner (Pflügers Arch. 395:525-544, 1982). It is obvious that reducing external $[Na^+]$ does not affect Ca^{2+} channel activation : the midpoint of activation in both conditions is -17 mV (figure 3, right panel).

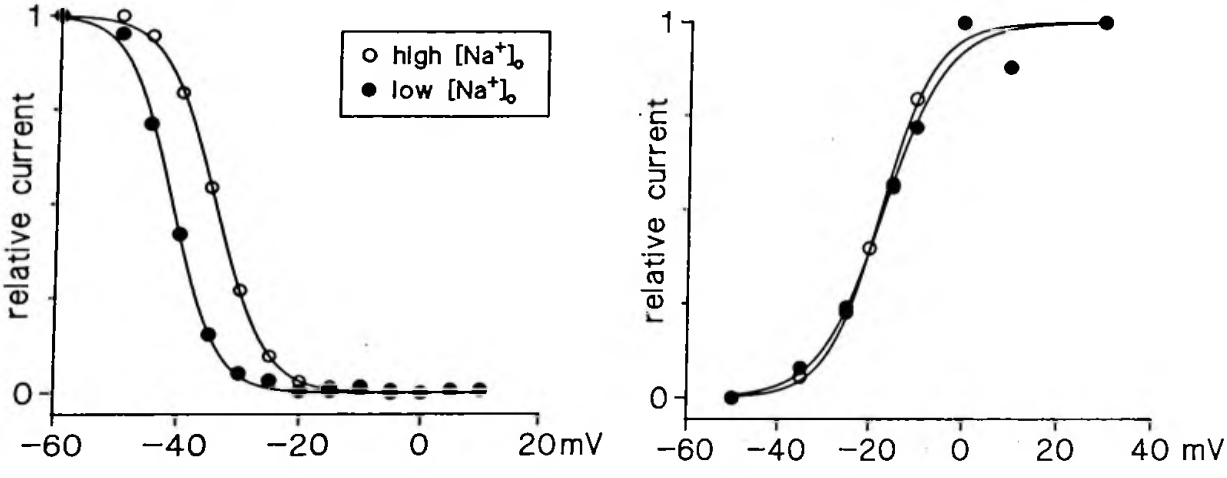

Figure 3. Effect of low external $[Na^+]$ on steady-state inactivation and activation. Left panel: Negative shift of the voltage dependence of Ca^{2+} channel inactivation by reducing external $[Na^+]$. Midpoints of inactivation are -34 and -42 mV for control and low external $[Na^+]$, respectively. Right panel: Ca^{2+} channnel activation is not modified by reducing external $[Na^+]$. Midpoint of activation is -17 mV for both conditions. Curves are nonlinear least-squares fits of Boltzmann functions to the data.

The conclusion from these experiments is that the reduction of the Ca^{2+} current by reducing external $[Na^+]$ is due to a negative shift of the voltage dependence of channel inactivation. The negative shift was also observed when Ba^{2+} was used as charge carrier or when external Na^+ was replaced with Li^+. Based on the results, it is difficult to assume a change of the fixed charges on the external surface of the membrane. We therefore suggest a direct interaction of Na^+ ions with the gating of the Ca^{2+} channel or the existence of a Na^+-specific binding site on the Ca^{2+} channel.

[Supported by a Lucille P. Markey Fellowship to G.C.]