# REGULATION OF CONTRACTION BY $Ca^{2+}$ CHANNEL AND $[Na^+]_i$ IN ISOLATED DOGFISH (SQUALUS ACANTHIUS) CARDIAC MYOCYTES

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The development of tension in heart muscle appears to be regulated by a number of mechanisms which include the $Ca^{2+}$ channel, sarcoplasmic reticulum, and the $Na^+$-$Ca^{2+}$ exchange system (Morad and Cleemann, J. Mol. Cell. Cardiol. 19: 527, 1987). The differential contribution of each system varies greatly in different species and appears to be related, in part, to the development of sarcoplasmic release pools. In this report, we examine the role of the $Ca^{2+}$ channel and $[Na^+]_i$ on the development of tension in single isolated ventricular myocytes of dogfish heart. Ventricular myocytes were enzymatically isolated (Mitra and Morad, Am. J. physiol. 249, H1056-H1060, 1985; Sorbera et al., this issue) and internally dialyzed with (in mM) 200 K-gluconate, 10 MgATP, 300 Urea, 10 HEPES, 0.1 BAPTA (bis-[O-aminophenoxy]-ethane-N,N,N',N'-tetraacetic acid) at pH = 7.2 and 0 or 60 $Na^+$, and superfused with a solution containing 270 NaCl, 4 KCl or 10 CsCl, 3 MgCl, 0.5 $KH_2PO_4$, 250 Urea, 10 HEPES and 1 $Ca^{2+}$ at pH = 7.2. In some experiments, $i_{Na}$ esd blocked by TTX (5 uM). Cells were depolarized at a frequency of 1/5 s from a holding potential of -80 mV. Myocyte shortening was measured using a photodiode array technique.

The threshold potential for cell shortening was -30 mV, coinciding with the activation of the high-threshold $Ca^{2+}$-channel. In cells dialyzed with 0 $Na^+$, the voltage-shortening relation was bell-shaped, reflecting the voltage-dependence of $i_{Ca}$ (Fig. 1A). On prolongation of the depolarizing pulses (to 800 ms), cell shortening peaked after about 400 ms (at 0 mV), followed by almost complete relaxation during the clamp pulse. Thus, little or no "tonic" shortening was measured. In myocytes dialyzed with 0 $Na^+$, nifedipine (5 uM) completely suppressed $i_{Ca}$ and cell shortening (200 ms clamp pulses).

In myocytes dialyzed with a solution containing 60 mM $Na^+$, the voltage-shortening relation was sigmoid. Thus, in contrast to cells dialyzed with 0 $Na^+$, contractions were observed at positive potentials > +50 mV (Fig. 1B). During long depolarizations, cell shortening increased for up to 1000 ms (at 0 mV) and did not relax unless the cell membrane was repolarized. At higher positive potentials (>+50 mV), prolongation of depolarization beyond 300 ms frequently caused an irreversible contracture of the cell. Block of $i_{Ca}$ by nifedipine reduced cell shortening only below +50 mV but appeared to be unaffected when depolarizations were to more positive potentials (Fig. 1C). Prolongation of the clamp duration between 50 and 800 ms ($[Na^+]_i$ = 60 mM) led to an increase in duration and size of the slow tail currents in parallel with the amplitude of cell shortening (not shown). These slow inward tail currents may therefore be generated by the $Na^+$-$Ca^{2+}$ exchanger (Hume, Am. J. Physiol. 252, H666-H670, 1987).

In conclusion, contraction in single ventricular myocytes of dogfish appears to be activated by $Ca^{2+}$-influx through the high threshold (L-type) $Ca^{2+}$ channel. The intracellular $Na^+$ concentration, in addition, has a powerful modulating influence on contraction, which appears to be mediated by the $Na^+$-$Ca^{2+}$ exchanger. Thus, during the action potential, the $Na^+$-$Ca^{2+}$ exchanger might contribute to $Ca^{2+}$ influx and $Ca^{2+}$ extrusion, depending on the intracellular $Na^+$ concentration.

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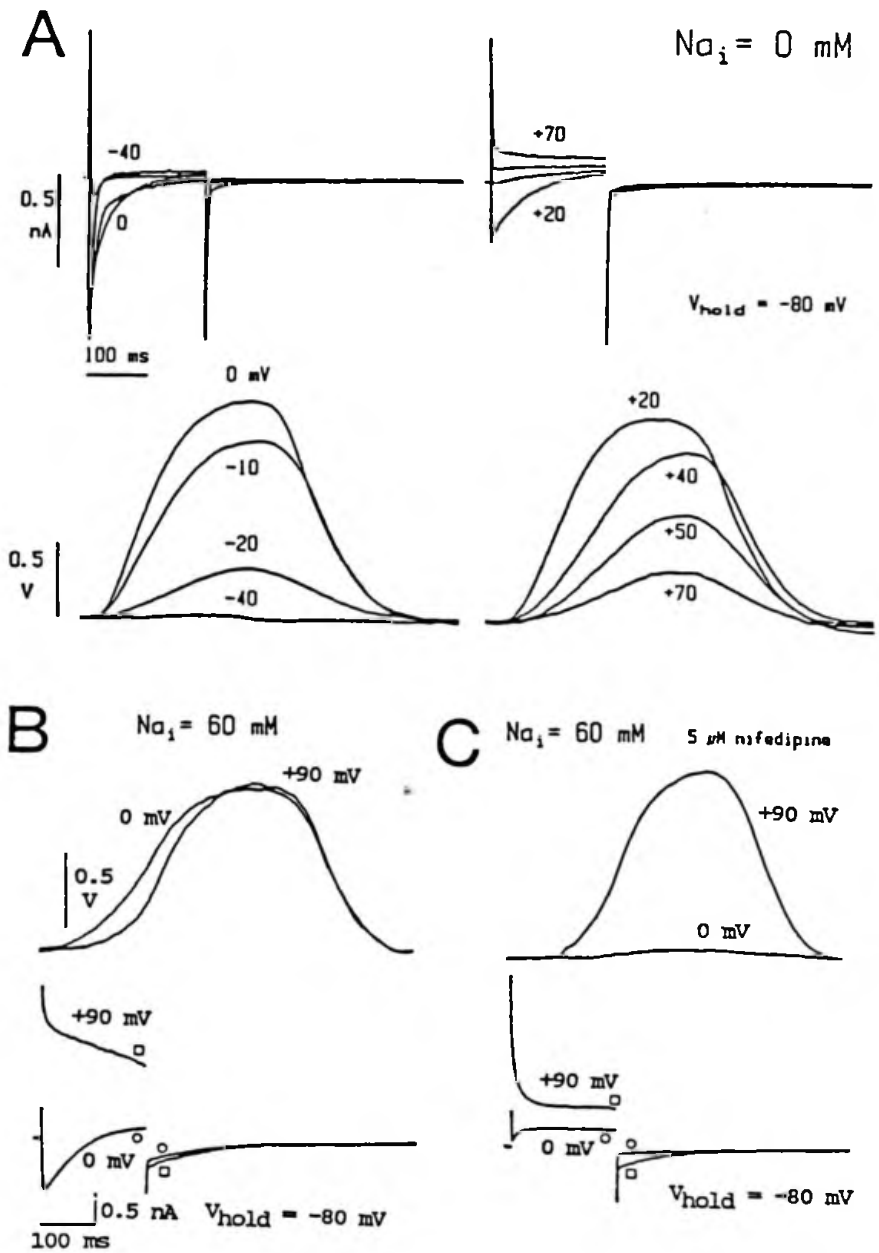


Figure 1. A: Voltage-dependence of $i_{Ca}$ in cells dialyzed with 0 $Na^+$.
B: Contractions at positive potentials (>+50 mV) in myocytes dialyzed with solution containing 60 nM $Na^+$.
C: Block of $i_{Ca}$ by nifedipine: effects on cell shortening below +50 mV and at higher (positive) step potentials.