The Effect of Isoproterenol and Nifedipine on Electrically Stimulated Ca_i-transients in Ventricular Myocytes of *Squalus acanthias*

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Mammalian cardiac myocytes contract via a process called Ca^{2+}-induced Ca^{2+} release (CICR), in which I_{Ca} through voltage gated Ca^{2+}channels causes the opening of Ca^{2+} release channels (RyR) on the sarcoplasmic reticulum [5]. There is little functional or ultrastructural evidence, however, for such Ca^{2+} release stores in shark heart [3]. Instead, Ca^{2+} influx through L-type Ca^{2+} channels and Na^+/Ca^{2+} exchanger (NCX) directly causes contraction, while the removal of Ca^{2+} from the cell on NCX results in relaxation. It has been shown that β-adrenergic agonists augment the force of contraction by increasing I_{Ca} (secondary to phosphorylation of Ca^{2+} channels) and enhance relaxation through phosphorylation of phospholamban and decreased myofilament sensitivity [4]. Here, we have investigated the effects of isoproterenol and nifedipine on Ca_i-transients in single, intact ventricular myocytes from dogfish sharks (*Squalus acanthias*).

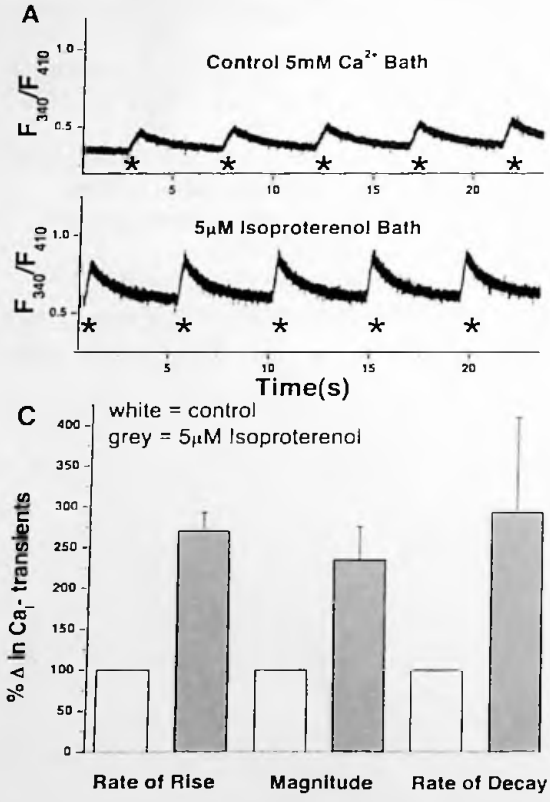

Figure 1. Comparison of electrically stimulated (*) Ca_i-transients in control vs. 5μM isoproterenol (Panel A). Magnified trace from panel A showing differential kinetics of control and isoproterenol Ca_i-transients (Panel B). Quantification of enhancement of rate of rise (ΔF/rise time), magnitude (ΔF), and rate of decay (ΔF/relax half time) with isoproterenol treatment in paired cells (n =5).

Ventricular myocytes were isolated from hearts of dogfish. Following complete spinal pithing, hearts were excised and placed in Ringer solution containing (in mM) 270 NaCl, 6 K^+-glutamate, 5 $CaCl_2$, 10 glucose, 10 $MgCl_2$, 10 HEPES, 350 Urea, 0.5 KH_2PO_4, 0.5 Na_2SO_4, at pH 7.4 and refrigerated at 2-8 °C until ready for use. For cell isolation, the heart was mounted on a Langendorff apparatus and the aorta and two major coronary vessels were perfused with Ringer solution containing zero $CaCl_2$ for 15 min, with zero $CaCl_2$ Ringer plus 1 mg/ml Collagenase A (Roche) and 0.2 mg/ml Protease type XIV (Sigma) for 15 min, and finally with 0.2 mM $CaCl_2$ Ringer solution for 10 min to washout the enzyme. The ventricle was cut off and gently agitated to dissociate ventricular myocytes. Cells were then plated on glass coverslips and loaded with ratiometric Ca^{2+} fluorescent dye Fura-2 AM (Molecular Probes). Ca_i-transients were measured using alternating 340

and 410 nm excitation waves at 1.2 kHz to monitor the fluorescence of Ca^{2+}-bound and Ca^{2+}-free Fura-2 respectively [2].

To confirm the absence of intracellular Ca^{2+} release pools, an electronically controlled microbarrel "puffer" system was used to apply a solution of 10 mM caffeine upon well-attached ventricular myocytes. In agreement with the previously published data [3], we found no evidence for intracellular Ca^{2+} release upon caffeine treatment (n=10). Interestingly, the puffing of bathing solution onto these myocytes failed to elicit the shear stress-activated Ca_i-transients so prevalent in rat atrial myocytes [1].

To probe possible effects of isoproterenol on the shark NCX, we also investigated the effect of isoproterenol on electrically stimulated Ca_i-transients. Figure 1 (upper trace, Panel A) clearly shows that depolarization causes a rapid $[Ca^{2+}]_i$ rise followed by a slower relaxation as Ca^{2+} is removed from the cell. After 2 minutes of exposure to isoproterenol (5 μM), the magnitude as well as rate of rise and relaxation of Ca_i-transients were significantly increased (Panel C). This finding is consistent with the idea that Ca^{2+} channel and NCX phosphorylation may be critically involved in enhancement of Ca_i-transients and acceleration of its relaxation respectively.

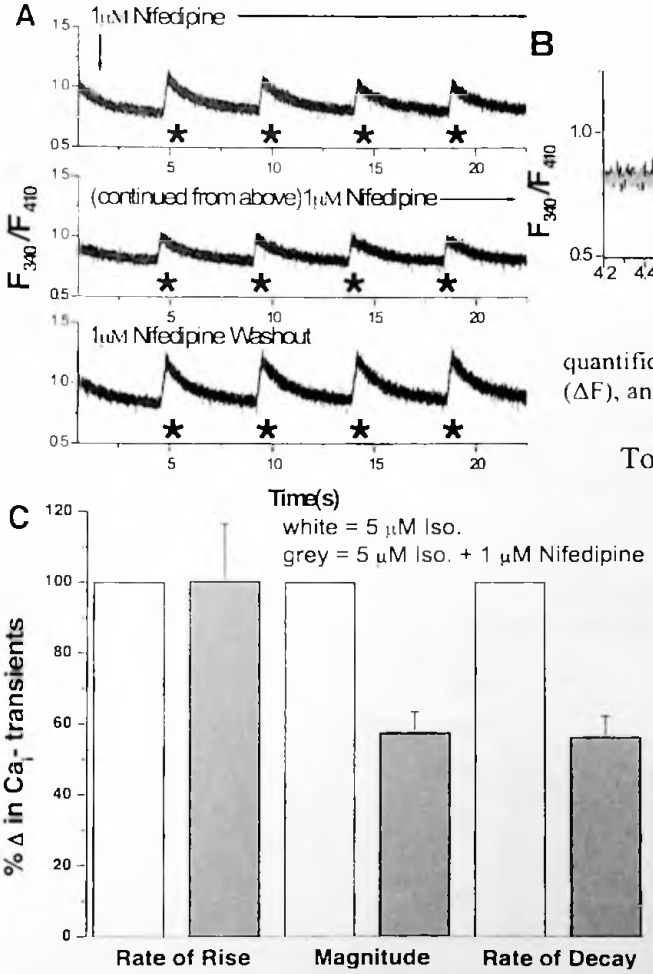

Figure 2. Nifedipine reversibly reduces the magnitude of electrically stimulated (*) Ca_i-transients compared to 5 μM isoproterenol (Panel A). Panel B is a magnified trace from panel A showing reduced magnitude and relaxation rate in 1 μM nifedipine. Panel C shows quantification of nifedipine effect on rate of rise (ΔF/rise time), magnitude (ΔF), and rate of decay (ΔF/relax half time) in paired cells (n =3).

To probe whether Ca^{2+} channel blocker nifedipine could reverse the effects of isoproterenol, we exposed the myocytes to 1 μM nifedipine. Figure 2, panels A and B show that nifedipine reversibly reduces the magnitude of Ca_i-transients while also reducing the rate of relaxation (Panel C). The rate of rise, however, is not significantly altered by nifedipine (n=3).

These findings provide support for the lack of functional intracellular Ca^{2+} stores in shark myocytes and secondarily suggest that the presence of functional Ca^{2+} release stores is critical for activation of shear-stress responses in rat atrium. Even though isoproterenol enhanced the rates of rise and relaxation of Ca_i-transients, the selective suppression of isoproterenol-induced rate of decay by nifedipine, but not the rate of rise of Ca_i-transients was unexpected and requires further experimentation. It has been suggested that isoproterenol enhances the magnitude and the rate of relaxation of Ca_i-transients via phosphorylation of putative PKA site on the NCX [6], although our data show that suppression of peak Ca_i-transients also plays a critical role in regulating relaxation rate of Ca_i-transients.

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