CAFFEINE REGULATION OF CARDIAC CONTRACTILITY IN *SQUALUS ACANTHIAS*

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Caffeine in millimolar concentrations exerts a strong effect on excitation-contraction coupling mainly by triggering or enhancing the Ca^{2+}-induced Ca^{2+}-release mechanism in the mammalian heart (Usachev Y., Verkhratsky A., *Cell Calcium* 17:197-200, 1995). It was intriguing, therefore, to find in initial experiments (Fig. 1A) that caffeine also had a strong potentiating effect on the contraction of shark myocardium, which is known to lack releasable Ca^{2+} stores (Maylie et al. *MDIBL Bulletin* 19:84-87,1979). Alternative explanations as to why caffeine enhances contractility in the shark heart include: (1) augmentation of Ca^{2+} influx via the Ca^{2+} channel and/or via Na^{+}-Ca^{2+} exchanger, (2) suppression of adenosine receptors (Choi et al., *Life Sci.* 43:387-398,1988), (3) elevation of cyclic AMP levels through inhibition of phosphodiesterase (Choi et al., *Life Sci.* 43:387-398,1988), and (4) increased Ca^{2+} sensitivity of myofilaments (Palmer S. et al., *J. Mol. Cell. Cardiol.* 28: 797-805, 1996). To examine these possibilities in the dogfish heart, we measured the force of contraction in atrial strips and Ca^{2+} currents in voltage-clamped, freshly isolated ventricular myocytes.

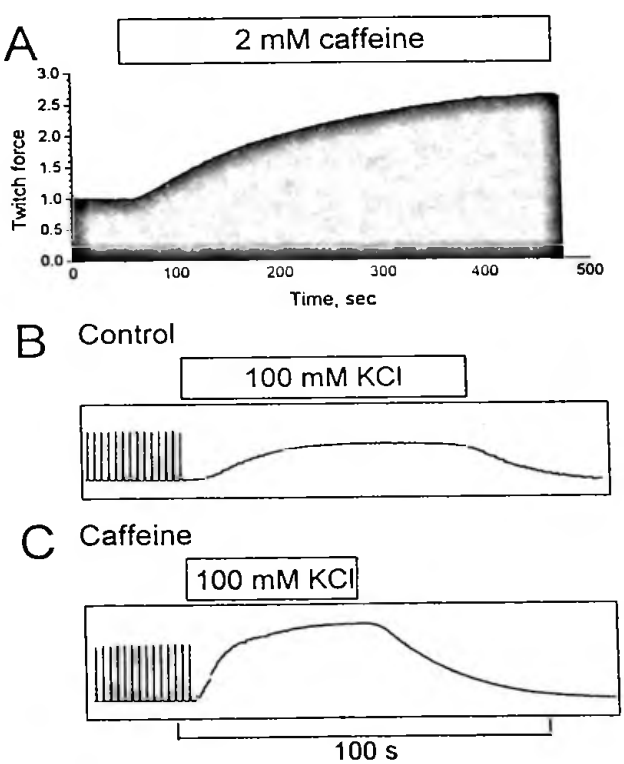

Figure 1. Effect of caffeine on twitch force (panel A) and KCl contractures (panels B and C). Panel A: Gradual increase in twitch force after addition of 5 mM of caffeine. Panel B: twitch and contracture force under control conditions. Panel C: twitch and contracture force in the presence of 2 mM caffeine. Shark atrial strip, 0.3 mm diameter, stimulation at 0.5 Hz, 5mM $[Ca^{2+}]_o$, room temperature.

Twitch- and contracture-force were measured from thin atrial strips (0.3-0.5 mm in diameter) by tightly holding one end of the muscle (10-15% of the muscle length) under a perspex block containing a stimulating Ag/AgCl electrode in the experimental chamber. The other end of the atrial strip was attached to an isometric tension transducer. Small stimulating voltages (0.1-2 V), applied between the electrode in the perspex block and the ground electrode in the bath, produced reproducible and stable twitches for well over 1-2 hours.

The single ventricular myocytes used in voltage clamp experiments were isolated by enzymatic dissociation as described previously (Dorfman et al. *MDIBL Bulletin*, 37:15-16, 1998). Briefly, the coronary arteries of the heart were canulated and perfused with Ca^{2+} free elasmobranch physiological solution (270 mM NaCl, 4 mM KCl, 10 mM $MgCl_2$ 0.5 mM KH_2PO_4, 0.5 mM Na_2SO_4, 350 mM urea, 10 mM glucose, and 10 mM HEPES titrated to a pH of 6.7) for 20 minutes. The heart was then perfused with 25 ml of the same solution containing 20

mg collagenase (type B, Boehringer Manheim) and 6 mg protease (type XIV, Sigma) for another 20 minutes. Next the enzyme containing solution was washed out using Ca^{2+} free physiological solution for 10 minutes. The heart was then removed from the Langendorff apparatus and was cut in number of smaller pieces which were gently shaken to release the individual isolated myocytes. Finally the myocytes were transferred into physiological solution containing 2 mM Ca^{2+} titrated to the pH of 7.2 and were voltage clamped using borosilicate patch pipettes filled with a dialyzing solution containing in mM: 20 NaCl, 1 $MgCl_2$, 300 urea, 240 CsCl, 5 MgATP, 0.2 EGTA, and 20 HEPES adjusted to pH 7.2 with CsOH.

In strips stimulated at constant frequency, caffeine (2-5 mM) caused a reversible increase of twitch force up to 300% (Fig. 1A). This occurred without a change in time-to-peak of contraction. Since the time-to-peak of contraction corresponds directly to the duration of action potential in shark heart, this finding suggests that the action potential duration was unchanged. Steady state depolarization induced by rapid application of 100 mM KCl (Fig. 1B) produced contractures, which were enhanced by about 100% in the presence of 5 mM caffeine (Fig.1C). The potentiation of force observed during a prolonged (≅ 60 s) depolarization by KCl, where the Ca^{2+} current is likely to be fully inactivated, suggests that Ca^{2+} entry through Ca^{2+} channels is not likely to be a reason for the positive inotropic effect of caffeine. However, the possibility of a direct effect of caffeine on the Na^{+}-Ca^{2+} exchanger could not be ruled out by this experiment. Figure 2 examines the tension potentiating effect of caffeine on an atrial strip in the presence of 2mM of Ni^{2+}, known to suppress Na^{+}-Ca^{2+} exchanger (Fan et al., *PNAS* 93:5527-5532, 1996). At this concentration, Ni^{2+} not only fully blocks Ca^{2+} channels, but also significantly suppresses the exchanger (note the slow rate of relaxation, right lower traces in the Fig. 2). In the presence of Ni^{2+}, even though twitch tension was significantly suppressed (Fig. 2, middle panel), addition of 5mM caffeine enhanced contractions by more than 3 folds (Fig. 2, lower pannel). This finding suggests that the caffeine effect does not directly depend on activation of the exchanger.

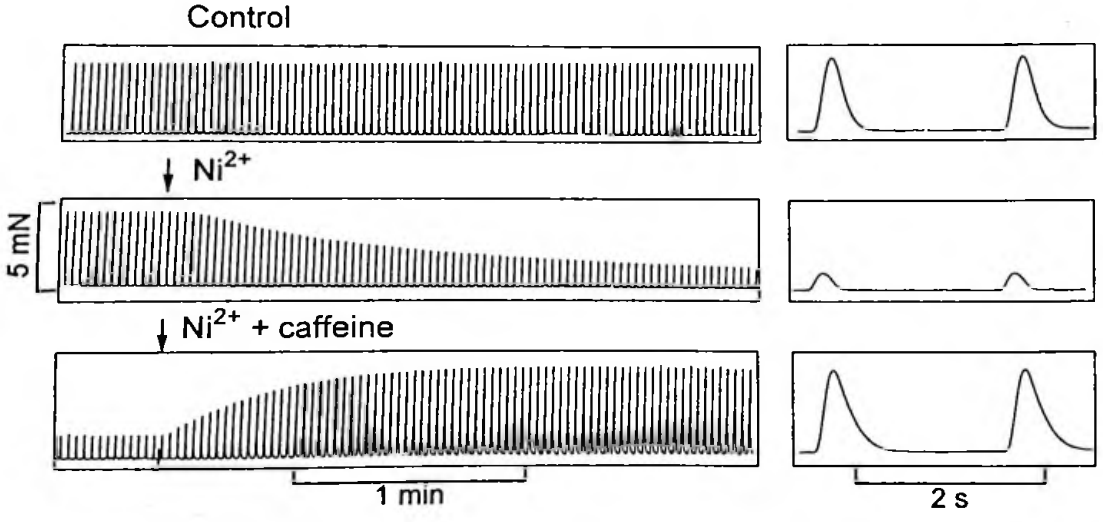

Figure 2. Effect of caffeine on the contractility of atrial strips. Upper panel: the magnitude of contraction in the 5 mM Ca^{2+} containing control solution. Middle panel: 2 mM Ni^{2+} suppressed twitch tension. Lower panel: in the presence of 2mM Ni^{2+}, 5 mM caffeine strongly enhances twitch tension. The time course of twitches, occurring at 2 s intervals, under the 3 experimental conditions is depicted in the right panels. Stimulation frequency 0.5 Hz, $[Ca^{2+}]$ = 5 mM, room temperature.

To directly evaluate the effect of caffeine on Ca^{2+} channels, we measured Ca^{2+} current in whole-cell clamped ventricular myocytes. Figure 3 shows that caffeine (2mM) had little or no effect on the time course or the magnitude of Ca^{2+} current measured at potentials between -30 and +60 mV (n=3 cells). In fact, the slight decrease in the Ca^{2+} current observed in Fig. 2C, was most likely due to the run-down of the current, since subsequent removal of caffeine was also accompanied by a similar slight decline of the current. Moreover, absence of significant effect of caffeine on Ca^{2+} current (Fig.3) suggests that pathways involved in modulation of intracellular concentrations of cAMP via inhibition of phosphodiesterase enzyme receptor are not likely to play significant role in the caffeine induced enhancement of contraction.

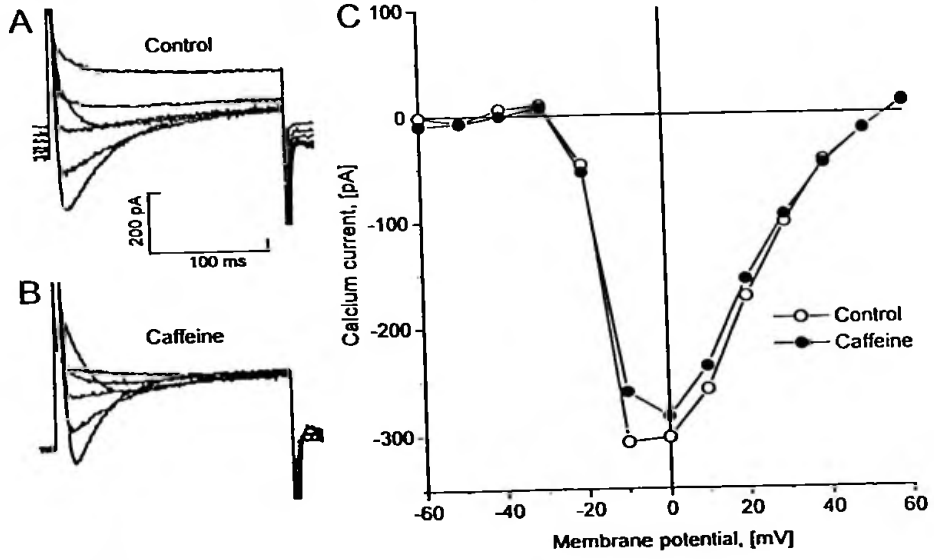

Figure 3. Caffeine does not change the magnitude of Ca^{2+} current in shark ventricular myocytes. Ca2+ currents measured in the absence (Panel A) and presence (Panel B) of 2 mM caffeine were plotted versus clamped membrane potential (Panel C). Shown sample traces (Panels A and B) were measured at 20mV intervals. Plotted Ca2+ current was measured relative to the current in the end of the voltage clamp. Holding potential: -60 mV.

The absence of functional Ca^{2+} release stores activated by caffeine is supported the findings that rapid application of 20 mM caffeine had no effect on base line tension, and that the caffeine induced enhancement of twitch tension in Ni^{2+} containing solution could be completely suppressed in the absence of extracellular Ca^{2+}. Thus, it is unlikely that mobilization of intracellular Ca^{2+} stores plays a significant role in caffeine induced potentiation of tension.

Our findings suggest that enhancement of twitch force in shark heart by caffeine is mediated neither by increased Ca^{2+} influx through the Ca^{2+} channels and Na^{+}-Ca^{2+} exchanger, nor by mobilization of dormant Ca^{2+} stores. Since caffeine is known to enhance the Ca^{2+} sensitivity of myofilaments in the mammalian heart, it is possible that a similar mechanism may be mediating the tension enhancing effect of caffeine in the shark heart. In the absence of the direct experimental evidence for this possibility such an eventuality must remain a possibility in the shark heart. Simultaneous measurements of intracellular Ca^{2+} signals and contraction (or cell shortening) might serve to assess this possibility directly.

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