CAFFEINE-INDUCED Ca^{2+} RELEASE STIMULATES Ca^{2+} EFFLUX VIA THE Na^{+}-Ca^{2+} EXCHANGER IN SINGLE MAMMALIAN CARDIAC MYOCYTES.

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Brief exposures to caffeine in mM concentration induce a small transient inward current in atrial 'cardioballs' and embryonic heart cells (S. Mechmann and L. Pott, Nature 319:597-599, 1986; W.T. Clusin, R. Fischmeister, G. DeHaan, Am. J. Physiol. 245:H528-H532, 1983). This current was thought to be generated by the Na^{+}-Ca^{2+} exchanger as Ca^{2+} released from the sarcoplasmic reticulum was sequestered from the sarcoplasm through the surface membrane in exchange for Na^{+}. To test this hypothesis we carried out experiments where the inward current was compared directly to the intracellular Ca^{2+} transient measured with the fluorescent metallochromic dye fura-2.

Single rat and guinea pig ventricular myocytes were prepared by enzymatic dissociation. The cells were voltage clamped using the whole cell technique. The patch pipette had a resistance of 1 to 4 Mohm and was filled with a solution containing 120 mM CsCl, 20 mM TEACl, 5 mM MgATP, 0.1 mM cAMP, 10 mM Hepes (pH 7.2) and 0.4 mM K_5 fura-2. Fura-2 distributed itself uniformly within the cell and was used with dual wavelength excitation (335 nm and 410 nm) to measure intracellular Ca^{2+} activity. The standard extracellular solution contained 140 mM NaCl, 5.4 mM KCl, 3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM Hepes (pH 7.4) and 10 mM glucose and could be exchanged rapidly using a multibarrel pipette with a common outlet (200 um) placed directly over the cell under investigation.

Figure 1 compares the Ca^{2+} transients generated in a rat ventricular myocyte either by a depolarizing voltage clamp pulse from -70 to 0 mV (Panel A) or by fast application of 5 mM caffeine at a fixed holding potential of -70 mV (Panel B). The Ca^{2+} transient elicited by depolarization (lower trace) rose with a halftime of about 15 msec and was gated by the Ca^{2+} current, i.e. like the Ca^{2+} current it activated around -50 mV, reached a maximum at 0 mV and decreased to a very low value with further depolarization to +80 mV. The extracellular application of caffeine evoked a larger Ca^{2+} transient which had a much slower rise time (halftime 150 ms) and relaxed spontaneously with a halftime of 2.5 s. This signal was accompanied by a transient inward current which is barely noticeable when seen on the same scale as the Ca^{2+} current (cf. panels A and B). When expanded it is clear, however (Panel C), that this current (upper trace) develops with nearly the same time course as the Ca^{2+} transient (lower traces).

Experiments were also carried out to test if interventions which block Na^{+}-Ca^{2+} exchange also blocked the transient inward current. One such experiment is shown in Panel D. Ni^{2+} was added in a concentration, 5 mM, which is known to markedly inhibit Na^{+}-Ca^{2+} exchange (J. Kimura, S. Miyamae, A. Noma, J. Physiol. 384: 199-222, 1987). This reduced the transient inward current by 60% (upper traces) but had little effect on the caffeine induced intracellular Ca^{2+} transient. Notice also that the Ca^{2+} transient signal relaxes much more slowly in the presence of Ni^{2+}. This confirms the notion that removal of intracellular Ca^{2+} via the Na^{+}-Ca^{2+} exchanger is blocked. In another series of experiments the extracellular Na^{+} was replaced by TEA^{+} in order to block the outward movement of Ca^{2+} via the Na^{+}-Ca^{2+} exchanger. As anticipated this had little effect on the Ca^{2+} transients but did eliminate the transient current.

The magnitude of the transient inward current was about 100 pA and lasted about 2 s, thus carrying 200 pC or 2 * 10^{-15} equivalents across the membrane. With 3 Na^+ ions being exchanged for each Ca^{2+} ion, we therefore estimate that 2 fmole of Ca^{2+} is being removed from the cell. The cell volume is about 10 um * 20 um * 100 um or 20 pliter and the cell therefore contains 0.4 mM * 20 pliter = 8 fmole of fura-2. This suggests that a noticeable fraction of the Ca^{2+} bound to fura-2 may be removed by the Na^+-Ca^{2+} exchanger and that the measured inward current transients are of the expected order of magnitude.

These results show that the transient inward current (1) has a time course similar to that of the intracellular Ca^{2+} transients, (2) is blocked by interventions which block Na^+-Ca^{2+} exchange without affecting the intracellular Ca^{2+} transients, and (3) has a magnitude consistent with the estimated Ca^{2+} movements. Our results are consistent with the idea that the caffeine induced inward current transients are generated by the Na^+-Ca^{2+} exchanger.

Supported by NIH Grants HL-33720 and HL-16152.

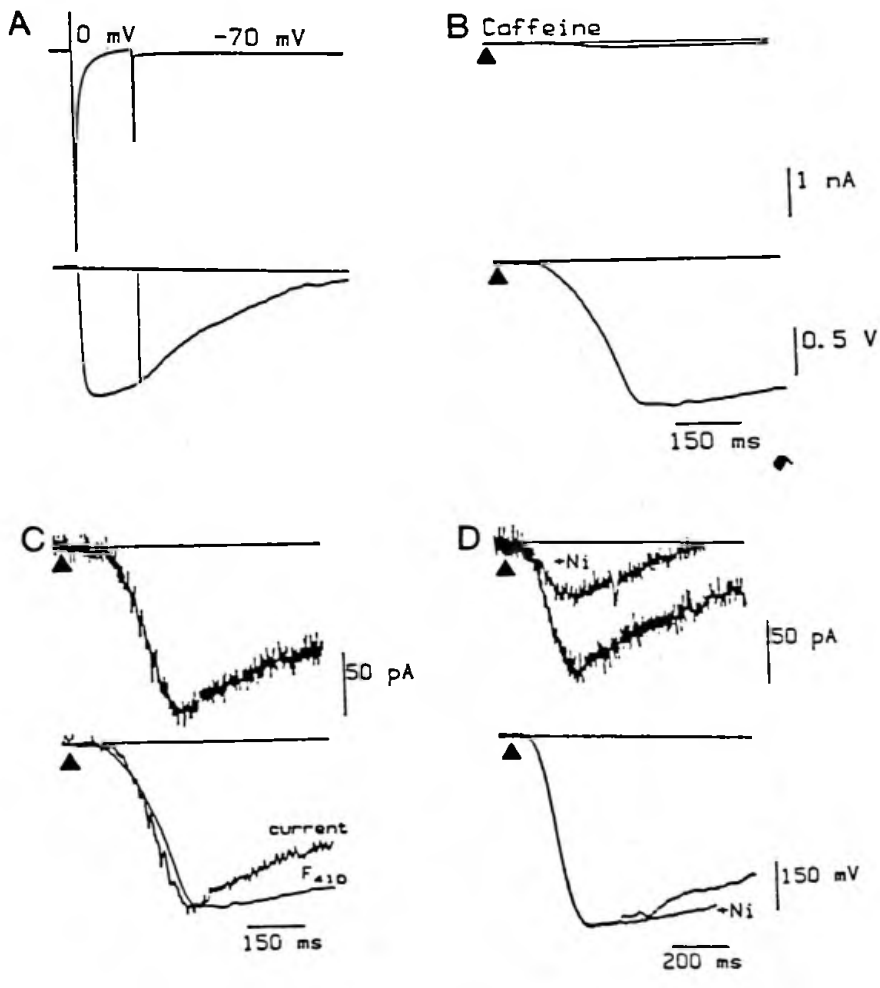

Figure 1. Membrane current and intracellular Ca^{2+} transients in a rat ventricular myocyte exposed to voltage clamp depolarization (Panel A) or rapid perfusion with 5 mM caffeine (Panels B, C and D). Each panel shows the membrane current at the top and the fura-2 signal measured with 410 nm excitation at the bottom. At this wavelength addition of Ca^{2+} gives decreasing fluorescence so that a downward deflection corresponds to increasing Ca^{2+} activity. Ratio measurements based on simultaneous measurements with excitation at 335 nm and 410 nm indicate that the depolarization-induced release in panel A increased the Ca^{2+} activity from 50 nM to 250 nM while the caffeine-induced release in panel B resulted in a peak Ca^{2+} activity of 1100 nM. Panel C compares the timecourse of the caffeine-evoked transient inward current to that of the intracellular Ca^{2+} transient. Panel D shows that 5 mM Ni^{2+} decreases the Na^+-Ca^{2+} exchange but has little effect on the caffeine-induced Ca^{2+} release.