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SPECIAL REPORT

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Caffeine-induced decreases in the inward rectifier potassium and the inward calcium currents in rat ventricular myocytes

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The effects of high (20 mM) concentrations of caffeine were studied on the transmembrane voltage and currents in rat single ventricular myocytes by the whole cell configuration of the patch clamp technique. Rapid application of caffeine released Ca^{2+} from the sarcoplasmic reticulum and induced a Ni^{2+}-sensitive transient inward current with concomitant change of the transmembrane voltage from -72.6 ± 0.4 to -68.0 ± 0.6 mV ($n = 4$). Maintained application of caffeine lengthened the action potential duration (APD_{90}) from 66.7 ± 16.9 to 135.1 ± 34.1 ms ($n = 4$) and depressed the amplitude of both the inward rectifier potassium and the inward calcium currents. It is concluded that these effects of caffeine should be recognized when it is used as a tool to study electromechnical coupling.

Keywords: Caffeine; heart; voltage clamp; calcium current; potassium current

Introduction Caffeine in high concentrations (5–20 mM) is widely used as a pharmacological tool in muscular tissues to study excitation-contraction coupling. Caffeine enhances Ca^{2+} permeability of the sarcoplasmic reticulum (SR) membrane by increasing the frequency and the duration of open events of the SR Ca-release channels (Rousseau & Meissner, 1989). The change of the intracellular Ca^{2+} concentration may affect the properties of various transmembrane ionic currents in the heart. Also, there are reports that caffeine may directly influence ionic currents (Goto *et al.*, 1979; Eisner *et al.*, 1979; Yatani *et al.*, 1984; Habuchi *et al.*, 1991). The effect of caffeine on the L-type Ca current is controversial. Some studies suggest an increase of I_{Ca} (Goto *et al.*, 1979; Yatani *et al.*, 1984) probably due to the suppression of phosphodiesterase activity (Beavo *et al.*, 1970). Other studies indicate an inhibitory action of caffeine on the I_{Ca} (Eisner *et al.*, 1979; Hughes *et al.*, 1990). Recently, it has also been reported that caffeine decreased the fast inward sodium current in guinea-pig ventricular myocytes (Habuchi *et al.*, 1991). However, there is still little information available about the effect of caffeine on the ionic currents in the heart. Therefore, in this study we investigated the effects of high (20 mM) concentrations of caffeine on the action potential and ionic currents in rat ventricular myocytes.

Methods Single myocytes isolated from the rat ventricle by a technique modified from that of Powell *et al.* (1980) were voltage clamped using the whole cell configuration of the patch clamp technique. Cell shortening was measured with a video edge-detector. Electrodes of 3–6 MΩ resistance were filled with the following solution (mM): potassium glutamate 140, KCl 10, NaCl 7, $MgCl_2$ 5, HEPES 10, K_2EGTA 0.1, K_2ATP 5, pH 7.2. The cells were bathed in the following solution (mM): NaCl 133.5, KCl 4, NaH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 10, glucose 11, $CaCl_2$ 1, pH = 7.4. All experiments were performed at room temperature (20–24°C).

Results Rapid application of 20 mM caffeine released Ca^{2+} from the SR, and induced an inward current (-45.4 ± 13.9 pA, $n = 4$, Figure 1b) at -80 mV. This current was completely abolished by 5 mM $NiCl_2$ (not shown) and therefore, was attributed to the Na-Ca exchange (Clusin, 1983). This transient caffeine-induced inward current changed the transmembrane potential from -72.6 ± 0.4 to -68.0 ± 0.6 mV ($n = 4$; Figure 1a). Following these transient effects, maintained application of caffeine lengthened the action potential duration at 50 and 90% repolarization from 13.9 ± 2.8 and 66.7 ± 16.9 ms to 51.5 ± 20.0 and 135.1 ± 34.1 ms, respectively ($n = 4$, $P < 0.05$, Figure 1c).

Maintained application of 20 mM caffeine decreased the amplitude of the steady state current voltage relation at the end of the 400 ms voltage pulse from -40 mV holding potential (Figure 2a). This effect of caffeine was completely reversed upon washout. A similar result was obtained in five other cells. When the inward rectifier potassium current was greatly reduced with 100 μM $BaCl_2$, caffeine did not change the steady-state current voltage relation (Figure 2a). The effects of caffeine on the inward calcium current were studied in the presence of 5 mM 4-aminopyridine to abolish the transient outward current which may interfere with I_{Ca} measurements. Cells were held at -40 mV and 400 ms voltage pulses were applied with a pulse frequency of 0.33 Hz. As Figure 2b shows, maintained application of 20 mM caffeine reversibly decreased the amplitude of the peak inward calcium current without shifting its voltage-dependence. Similar results were obtained in four other rat ventricular cells. Caffeine, however, not only decreased the amplitude of the inward calcium current but significantly slowed its inactivation kinetics (t_{inac} increased from 29.3 ± 5.2 to 42.7 ± 5.2 ms, $n = 5$, $P < 0.05$).

Discussion Caffeine has various effects on the myocardium. The nickel-sensitive transient effects of caffeine on both currents and transmembrane voltage can be satisfactorily explained by the Na-Ca exchanger (Clusin, 1983). The effect of caffeine on the action potential repolarization is not entirely clear. The lack of calcium release during the action potential greatly diminishes the depolarizing Na-Ca exchanger current, therefore, it should shorten the action potential duration in rat ventricular myocardium as it was reported with ryanodine (Mitchell *et al.*, 1984). However, the significant slowing of the inactivation kinetics of I_{Ca} and inhibition of I_{k1} have the opposite effect, i.e. may lengthen the repolarization. Also, an additional effect of caffeine on other currents such as the fast and slow component of the transient outward potassium current (Apkon & Nerbonne, 1991) cannot be ruled out. The depressant effect of caffeine on the steady state current-voltage relation is probably due to its inhibition of I_{k1}, because this effect was not seen in conditions where I_{k1} was greatly inhibited, i.e. in the presence of $BaCl_2$.

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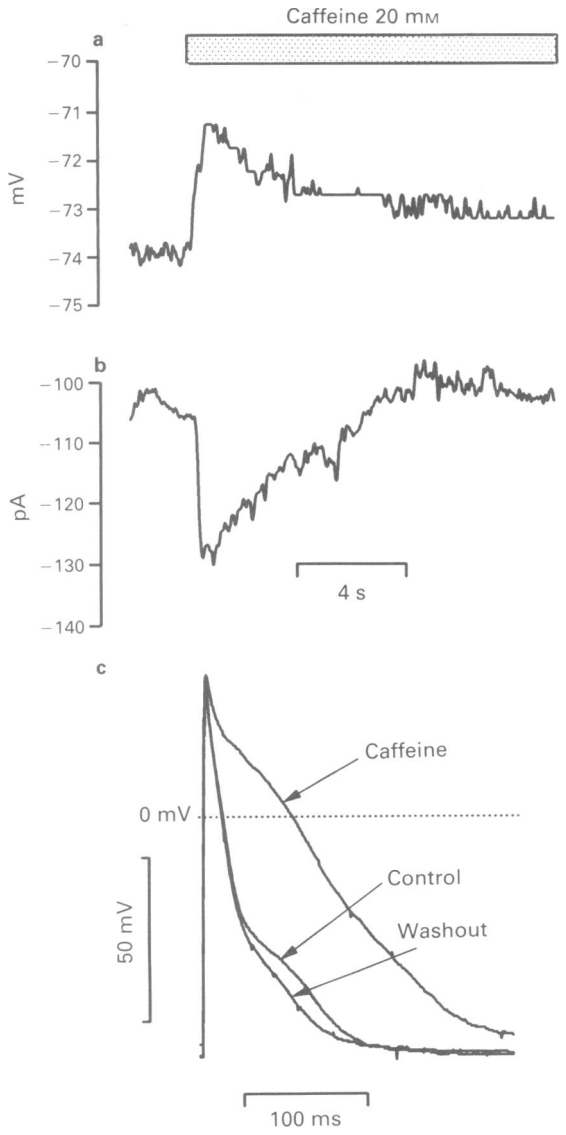

Figure 1 The effect of rapid application of 20 mM caffeine on transmembrane voltage (a) and transmembrane ionic currents (b). Cell was held at − 80 mV. (c) Shows the effect of maintained application of 20 mM caffeine on the action potential.

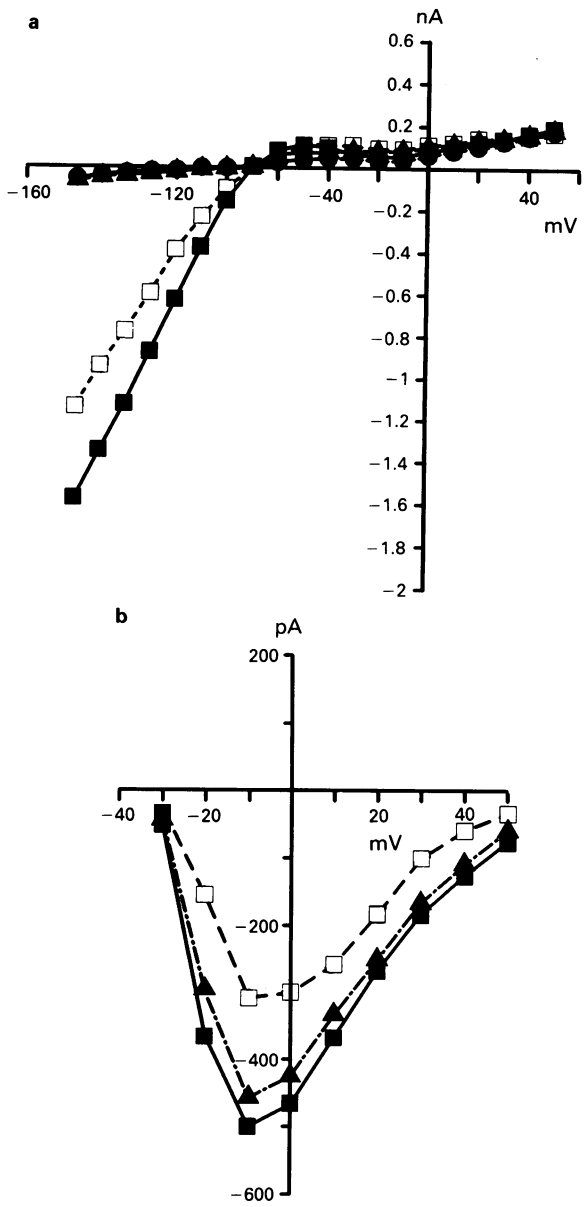

Figure 2 (a) The effect of maintained application of 20 mM caffeine on 'steady state' current voltage relation in the absence and presence of 100 μM $BaCl_2$: (■) control; (□) caffeine; (●) control + Ba; (▲) caffeine + Ba. (b) The effect of maintained application of 20 mM caffeine on peak inward calcium current: (■) control; (□) caffeine; (▲) washout. Calcium current was measured as the difference between the peak inward and the steady state current. Holding potential = − 40 mV, pulse duration = 400 ms, pulse frequency = 0.33 Hz. External solution contained 5 mM 4-aminopyridine to block the transient outward current.

The reported data with caffeine on the inward calcium current are conflicting. Yatani *et al.* (1984) found that caffeine increased the inward calcium current in rat single ventricular myocytes, but Eisner *et al.* (1979) observed the opposite effect with caffeine on the inward calcium current in sheep cardiac Purkinje fibres. Our data agree with the results of the latter study, but the differences between our present and previous studies is not understood. Although, the above effect of caffeine is not relevant therapeutically, we conclude that it should be recognized when the drug is used as a tool to study electromechanical coupling.

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