Effects of Caffeine, Tetracaine, and Ryanodine on Calcium-dependent Oscillations in Sheep Cardiac Purkinje Fibers

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ABSTRACT Membrane current and tension were measured in voltage-clamped sheep cardiac Purkinje fibers. Elevating the intracellular calcium concentration ($[Ca^{2+}]_i$) results in oscillations of membrane current and tension both at rest and during stimulation. During stimulation, an oscillatory transient inward current and an aftercontraction follow repolarization. We have examined the effects on the oscillations of changing the extracellular calcium concentration ($[Ca^{2+}]_o$) and of adding various drugs. In agreement with previous work, high concentrations of drugs that affect the sarcoplasmic reticulum, namely caffeine (10–20 mM), tetracaine (1 mM), and ryanodine (10 μM), abolish the oscillations. However, at lower concentrations, these three drugs have different effects on the oscillations. Caffeine (1–2 mM) decreases the oscillation amplitude but increases the frequency. Tetracaine (100–500 μM) has little effect on the magnitude of the oscillations but decreases their frequency. Ryanodine, at all concentrations used (0.1–10 μM), eventually abolishes the oscillations but, in doing so, decreases the magnitude, leaving the frequency unaffected. When $[Ca^{2+}]_o$ was changed in order to vary $[Ca^{2+}]_i$, both the frequency and the magnitude of the oscillations always changed in the same direction. This suggests that these three drugs have effects in addition to just changing $[Ca^{2+}]_i$.

INTRODUCTION

Elevating the intracellular calcium concentration ($[Ca^{2+}]_i$) in mammalian cardiac muscle produces oscillations of $[Ca^{2+}]_i$ (Orchard et al., 1983; Wier et al., 1983). These Ca oscillations produce oscillations of membrane current (Lederer and Tsien, 1976) and tension (Kass et al., 1978; Eisner and Lederer, 1979; Kass and Tsien, 1982). Although the oscillations can be seen in an unstimulated preparation (Kass and Tsien, 1982; Matsuda et al., 1982), they become larger on stimulation. After an action potential or a depolarizing voltage-clamp pulse, repolarization produces an oscillatory aftercontraction and an accompanying oscillatory transient inward current (Lederer and Tsien, 1976). The stimulated oscillations of either $[Ca^{2+}]_i$ itself (Allen et al., 1984) or membrane current (Kass

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and Tsien, 1982) have a frequency similar to those of the spontaneous oscillations, and it has been suggested that the larger stimulated oscillations may result from a synchronization of the spontaneous oscillations. The oscillations of $[Ca^{2+}]_i$ are important for the following reasons. (*a*) The oscillatory transient inward current has been shown to be the cause of certain ventricular arrhythmias produced by digitalis intoxication (Rosen et al., 1973; Lederer and Tsien, 1976). (*b*) The tension oscillations and underlying oscillations of $[Ca^{2+}]_i$ may interfere with normal excitation-contraction coupling (Kort and Lakatta, 1984; Allen et al., 1985; Valdeolmillos and Eisner, 1985).

The oscillations of $[Ca^{2+}]_i$ are thought to be produced by oscillatory cycling of Ca ions between the cytoplasm and the sarcoplasmic reticulum (SR). The evidence for this comes from various observations. (*a*) Oscillations of tension can be seen in mechanically skinned preparations, thus excluding a role for the sarcolemma (Fabiato, 1983). (*b*) Spontaneous $[Ca^{2+}]_i$ oscillations can be seen in preparations of isolated SR (Palade et al., 1983). (*c*) Finally, drugs that interfere with the SR, such as caffeine (Eisner and Lederer, 1982; Karagueuzian and Katzung, 1982), tetracaine (Tsien et al., 1978), and ryanodine (Sutko and Kenyon, 1983), abolish the oscillations of current and tension in intact cardiac preparations. These pharmacological studies have, however, only shown that the drugs abolish the oscillations and have given no insight into the mechanism of the effects. Of particular interest is the fact that, whereas tetracaine and ryanodine principally act to decrease Ca release from the SR, caffeine is thought to promote the release. One might therefore expect to see this reflected in a differential effect of these drugs on the properties of the oscillations.

We have therefore investigated the mechanism of action of these drugs on the oscillations by looking at the effects of concentrations of the drugs that do not completely abolish the oscillations. We have compared the effects of the drugs on the magnitude and frequency of the oscillations with the changes produced by altering the extracellular calcium concentration ($[Ca^{2+}]_o$). The results show that although the various drugs all abolish the oscillations if applied in sufficient concentration, they modify the amplitude and frequency of the oscillations in different ways. A preliminary report of some of these results has appeared (Eisner and Nieman, 1984).

METHODS

Hearts were removed from freshly killed sheep, obtained from the local slaughterhouse, and transported to the laboratory in a phosphate-buffered solution at 4°C. The composition of the solution was (mM): 127 NaCl, 16 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 5 Na_2HPO_4/NaH_2PO_4, pH 7.5. The high $[K^+]_o$ was used to arrest the heart. Free-running Purkinje fibers were dissected from both ventricles of the hearts and placed in a dish of modified Tyrode's solution, gassed with 100% O_2 at room temperature. The composition of the modified Tyrode's solution was (mM): 145 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 glucose, 5 U/liter insulin. This was buffered with 5 mM Tris-Hepes to give a pH of 7.3 at 36°C.

Purkinje fibers, of 0.3–0.5 mm diam, were shortened to ~2.0 mm and placed in an experimental bath. One end was tied with a fine silver wire hook and attached to a tension transducer, and the other end was secured with a pin. The standard solution was the same modified Tyrode's solution that was used to store the dissected Purkinje fibers. This, as

well as the other solutions used, was gassed with 100% O_2, heated to 36 ± 0.5°C, and continuously perfused through the bath with a nonpulsatile pump.

Preparations were voltage-clamped with a conventional two-microelectrode technique (Deck et al., 1964). The microelectrodes were filled with 3 M KCl and had resistances between 5 and 8 MΩ. In most of the experiments, $[Ca^{2+}]_i$ was raised by inhibiting the Na-K pump either by removal of K_o^+ (standard solution without KCl) or by addition of 10 μM strophanthidin (Sigma Chemical Co., Poole, England). Caffeine and tetracaine (both from Sigma Chemical Co.) were added to Tyrode's solution as solids. Ryanodine (a kind gift from Dr. J. Kenyon, obtained from Penick Corp.) was stored in a frozen aqueous solution (10 mM).

Results were stored on FM tape for subsequent analysis. Some results were averaged with a DL 4000E averager (Datalab, Mitcham, Surrey, England). In some cases, an HP3582A computer (Hewlett-Packard Co., Palo Alto, CA) was used to obtain Fourier amplitude spectra. The spectra are shown in the range 0–10 Hz and were calculated from 256 equally spaced points.

RESULTS

Effects of Increasing $[Ca^{2+}]_i$ on Current and Tension Oscillations

Fig. 1 shows the effects of changing $[Ca^{2+}]_o$ on a Purkinje fiber that had been exposed to a K-free solution in order to inhibit the Na-K pump. In agreement with previous work (e.g., Eisner and Lederer, 1979), inhibition of the Na-K pump produced a component of tonic tension during the depolarizing pulse, and an aftercontraction (AC) and a transient inward current (TI) on repolarization. Fig. 1 shows that increasing $[Ca^{2+}]_o$ increases both the tonic tension and the AC. Furthermore, the time to peak of the AC and TI is decreased. This is shown more clearly in Fig. 1*C*, where the records have been expanded and only the repolarization phase is shown for tension. It can be seen that as the $[Ca^{2+}]_o$ increases, so the time taken for the AC to peak decreases and the amplitude increases. This is shown graphically in Fig. 1*B*; the top graph shows that although the AC magnitude increases with increasing $[Ca^{2+}]_o$, it starts to level off at ~5 mM, as does the increase in frequency (or the decrease in time to peak). In fact, there is an inverse relationship between the latency of the AC and its magnitude over the whole range of $[Ca^{2+}]$ tested, and this relationship can be seen in the lower graph of Fig. 1*B*. This inverse relationship was seen in all preparations examined. However, the linearity was not always seen and may be fortuitous (cf. Fig. 7). As will be shown later, this simple relationship between magnitude and frequency contrasts with the effects of caffeine, tetracaine, and ryanodine.

Effects of High Concentrations of Caffeine, Tetracaine, and Ryanodine

It has previously been reported that high concentrations of certain drugs that affect the SR inhibit the oscillations. This is confirmed by the results of Fig. 2. In this experiment, K_o^+ was absent throughout, and the first panel shows that a TI and an AC were present. The application of 1 mM tetracaine (third panel) reduced the twitch by ~10-fold, and abolished the TI, the AC, and the oscillations seen during the depolarizing pulse. After allowing 10 min for recovery (which was complete within 5 min), 20 mM caffeine (second panel) was added and this abolished the twitch, the AC, and the TI, as well as the current and tension

oscillations during depolarization, but it greatly enhanced the tonic tension. Finally, the fourth panel shows that 10 μM ryanodine abolished the twitch, as well as the AC and the TI. The effects of caffeine and tetracaine were reversible, but the effect of ryanodine was not.

Effects of Low Concentrations of Caffeine

The previous section has demonstrated that, in sufficient concentration, caffeine abolished the oscillations. In subsequent experiments, we investigated the effects

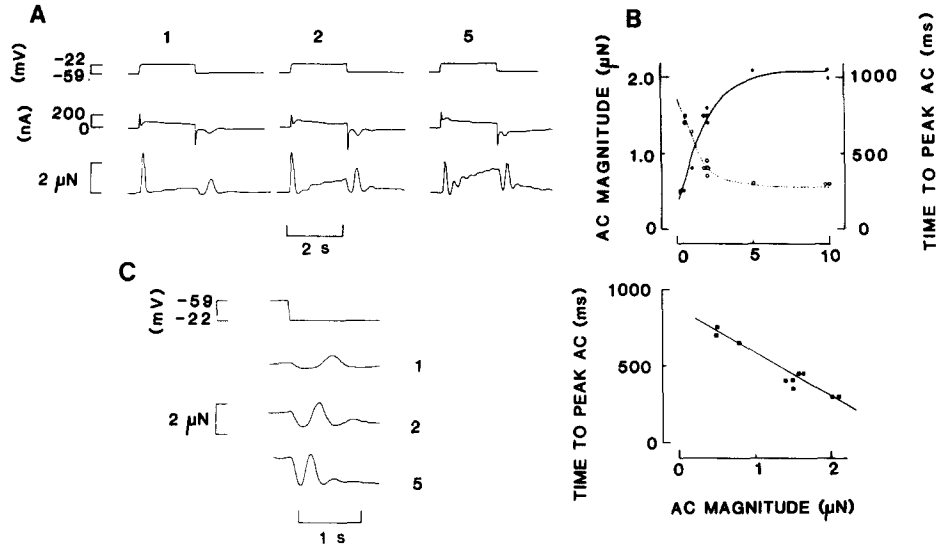

FIGURE 1. Effect of changing $[Ca^{2+}]_o$ on the magnitude and frequency of oscillations. (*A*) Original records. Top: membrane potential; middle: current; bottom: tension. In all panels, a 2-s-long voltage-clamp pulse was applied from −59 to −22 mV at 0.1 Hz. The solutions were K-free throughout and $[Ca^{2+}]_o$ was 1, 2, and 5 mM, as indicated. The records were obtained in the order 2, 5, 1. All traces are the average of four records. (*B*) Graphs from the same experiment. The top graph shows the relationship between $[Ca^{2+}]_o$ and the aftercontraction (AC) magnitude (●) and latency (○). The graph below shows the relationship between AC magnitude and AC latency at different values of $[Ca^{2+}]_o$. Some of the points have been displaced horizontally to avoid overlap. (*C*) Expanded records of repolarization. The top trace is membrane potential and the three lower traces are from the same tension traces as those in *A*.

of lower concentrations. Fig. 3 shows the effect of 2 mM caffeine on a voltage-clamped Purkinje fiber, which had been exposed to 10 μM strophanthidin. It is clear that 2 mM caffeine did not abolish the oscillations. In fact, although the magnitudes of the TI, the AC, and the oscillations during the depolarizing step decreased, the frequency of all these oscillations increased. The effects of caffeine were very fast in onset, i.e., within the first minute of application of the drug, and were also readily reversible. We also investigated the effects of low concen-

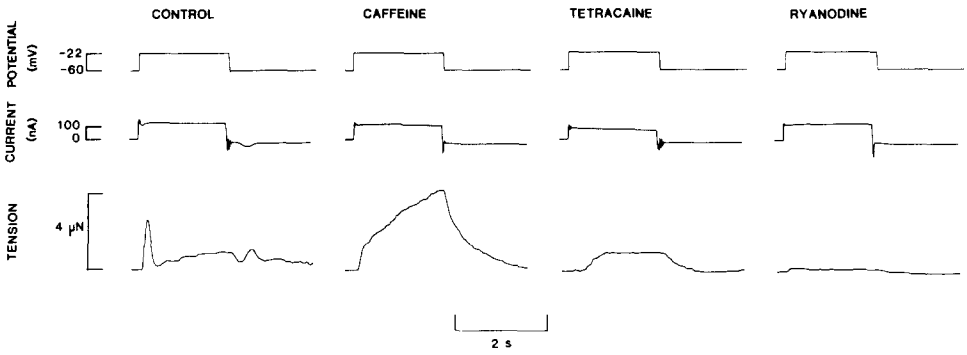

FIGURE 2. Effect of high concentrations of caffeine, tetracaine, and ryanodine. Top: membrane potential; middle: current; bottom: tension. In each panel, a 2-s-long voltage-clamp pulse was applied from −60 to −22 mV at 0.1 Hz. All traces are the average of four records. All solutions were K-free. Panels show (left to right): control, after 1.5 min exposure to 20 mM caffeine, after 1.5 min exposure to 1 mM tetracaine, and after 3 min exposure to 10 μM ryanodine.

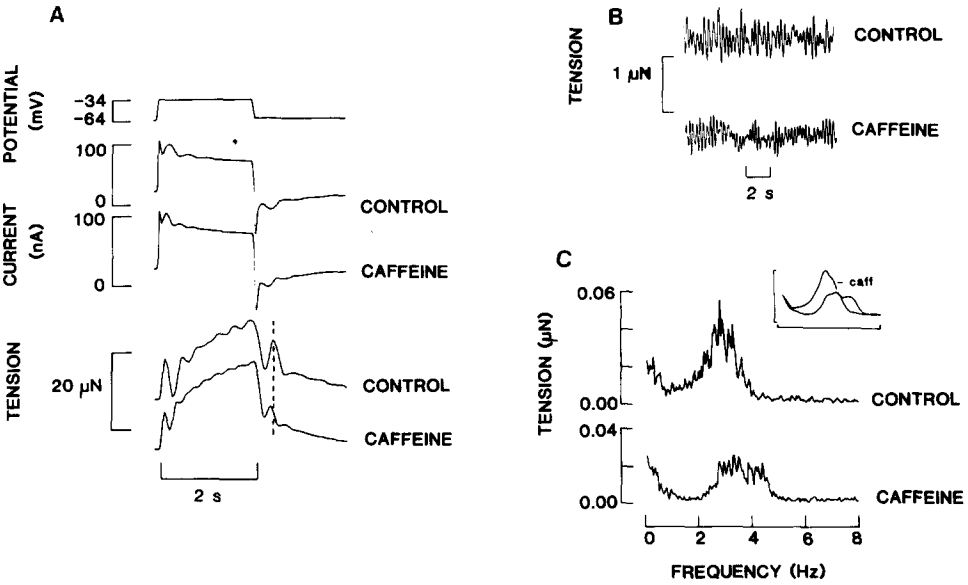

FIGURE 3. Effect of 2 mM caffeine on stimulated and spontaneous oscillations. (*A*) Top: membrane potential; middle: current; bottom: tension. In each panel, a 2-s-long voltage-clamp pulse was applied from −64 to −34 mV at 0.1 Hz. The upper current and tension traces are the controls; the lower current and tension traces are in the presence of 2 mM caffeine. The dotted line is through the peak of the control AC. All traces are the average of four records. (*B*) Original records. The traces show tension records from a fiber held at −34 mV. Top: control; bottom: with caffeine (2 mM). (*C*) Fourier spectra of original records in *B*. Top: control; bottom: with caffeine (2 mM). Spectra have been smoothed and superimposed in the inset. 10 μM strophanthidin was present throughout.

trations of caffeine on the spontaneous oscillations produced at a constant membrane potential. Fig. 3*B* shows that caffeine (2 mM) again decreased the amplitude but increased the frequency of the tension oscillations. To give a more quantitative estimate of this effect, a Fourier amplitude spectrum is shown in Fig. 3*C*. The control spectrum shows that the oscillation has a broad peak in the range 2.5–3.5 Hz. In the presence of caffeine, the frequency shifted to the right, to 3.0–4.5 Hz, and the height of the peak decreased. The results are clearer in the inset, where they have been smoothed and superimposed. This effect of increased frequency with decreased magnitude contrasts with the relationship produced by simply changing $[Ca^{2+}]_o$, where frequency and magnitude changed in parallel.

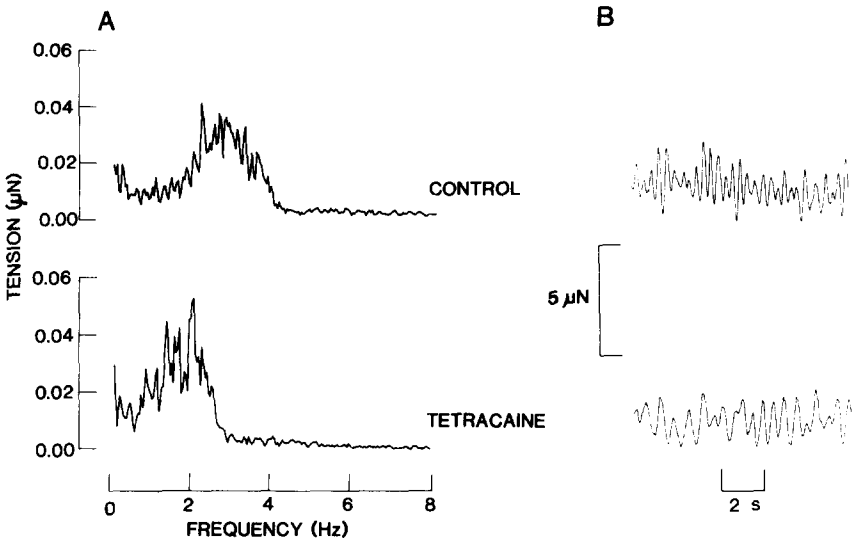

FIGURE 4. Effect of tetracaine on spontaneous tension oscillations. (*A*) Fourier spectra of tension from a fiber held at −32 mV. (*B*) Original records. For *A* and *B*: top: control; bottom: tetracaine (100 μM). All solutions were K-free throughout.

Effect of Low Concentrations of Tetracaine

Fig. 4 shows that 100 μM tetracaine decreased the frequency of spontaneous tension oscillations while having little effect on their magnitude. This is confirmed by the Fourier spectra. The control spectrum had a peak in the range 2.0–4.0 Hz, but, in the presence of 100 μM tetracaine, the peak was shifted to the left, to 1.0–3.0 Hz. The effects of tetracaine, like caffeine, were rapid in onset and were also readily reversible. The same result of decreased frequency with no change in magnitude was seen for TI and AC in stimulated oscillations.

We used tetracaine because it is a drug that is known to interfere with the SR. However, it is important to exclude an alternative explanation for its effects. Tetracaine is a local anesthetic that blocks Na channels and thereby decreases

the passive influx of Na into the cell and thence $[Na^+]_i$. Such a decrease of $[Na^+]_i$ would (via Na-Ca exchange) decrease $[Ca^{2+}]_i$, and this could explain the reduction of frequency of the oscillations. Such an explanation has been proposed to explain the reduction of Ca-dependent oscillations by lidocaine (Eisner et al., 1983). In order to test this hypothesis, we examined the effects of tetracaine on the oscillations produced by the removal of extracellular Na^+. If tetracaine acts

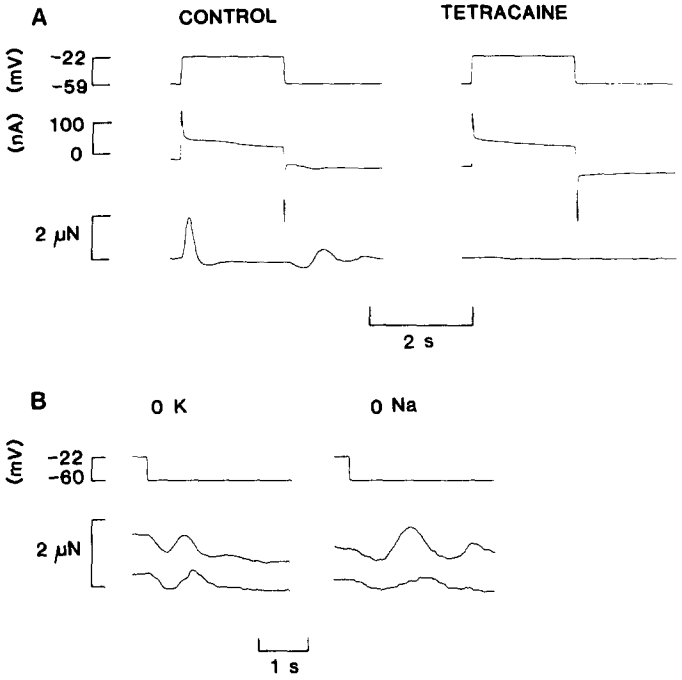

FIGURE 5. Effect of tetracaine in the presence of an Na-free solution. (*A*) High concentrations of tetracaine. Top: membrane potential; middle: current; bottom: tension. In each panel, a 2-s-long voltage-clamp pulse was applied from −59 to −22 mV at 0.1 Hz. The records were obtained ~3 min after replacing all external Na with Li. Left: removal of external Na^+; right: in the presence of 1 mM tetracaine during Na^+ removal. All traces are the average of eight records. (*B*) Low concentration of tetracaine. Top: membrane potential; middle: tension for the control; bottom: tension in the presence of 100 μM tetracaine. In each panel, a 2-s-long voltage-clamp pulse was applied from −60 to −22 mV at 0.1 Hz. Left: in K^+-free solution; right: in Na^+-free solution. Only the repolarization phase is shown. All traces are the average of four records.

solely as a local anesthetic, it should have no effect on the oscillations produced by Na^+ removal.

Fig. 5*A* (left) shows current and tension records obtained 2 min after replacing all external Na^+ with Li^+. In Fig. 5*A* (right), the addition of 1 mM tetracaine at the time of Na^+ removal abolished all oscillations. Furthermore, lower tetracaine concentrations had qualitatively the same effect in 0 Na_o^+ as they did in 0 K_o^+. Fig.

5*B* shows that 100 μM tetracaine slowed the frequency of the oscillations produced by either inhibiting the Na-K pump (K^+-free) or by removing external Na^+. Therefore, in Na-free solutions, the effects of tetracaine are similar to those seen in Na-containing solutions. This suggests that tetracaine is not acting as a local anesthetic.

Effect of Ryanodine

In Fig. 2, it was shown that 10 μM ryanodine abolished the oscillations of current and tension. All concentrations tested down to 0.1 μM abolished the oscillations, and, as expected for an irreversible drug, the lower the concentration, the longer the time to produce the effect. 0.1 μM took ~16 min, whereas 2 μM took ~4

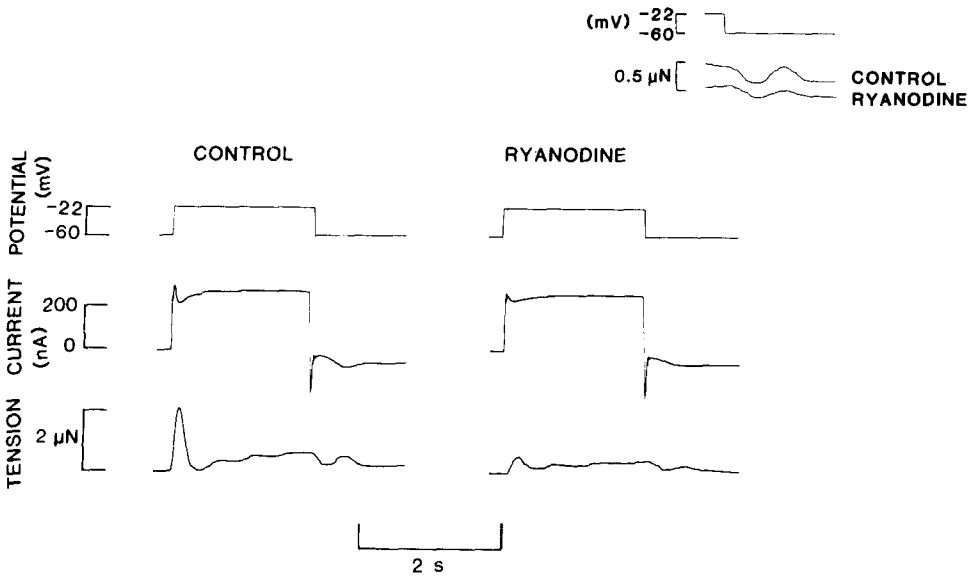

FIGURE 6. The onset of action of ryanodine. Original records. Top: membrane potential; middle: current; bottom: tension. In each panel, a 2-s-long voltage-clamp pulse was applied from −60 to −22 mV at 0.1 Hz. Left: control; right: ~3 min after the addition of 0.5 μM ryanodine. The inset shows same records of tension expanded. All traces are the average of four records. K_o^+ was absent throughout.

min to abolish the oscillations. This is consistent with other reports that ryanodine acts slowly (Hillyard and Procita, 1959). Low concentrations of ryanodine (0.1–0.5 μM) also completely abolished the spontaneous tension oscillations.

We examined the effects of ryanodine during the onset of action and found that it decreased the magnitude of the AC, the TI, and the oscillations, as well as the twitch, but did not change the oscillation frequency. For the control, the AC latency was 467 ± 37 ms and the magnitude was 1.25 ± 0.38 μN; in the presence of ryanodine (just before the AC was abolished), the AC latency was 480 ± 43 ms and the magnitude was 0.52 ± 0.29 μN (mean ± SEM for six experiments). A representative result is shown in Fig. 6, where the inset shows more clearly that the AC magnitude decreased by ~50%, but its latency was unchanged after 3 min exposure to 0.5 μM ryanodine.

DISCUSSION

Previous work has shown that Ca-dependent oscillations are abolished by drugs that interfere with SR Ca metabolism. In the present paper, we have confirmed that caffeine, tetracaine, and ryanodine can abolish the oscillations. However, when applied at lower concentrations, the drugs have very different effects on the oscillations. Caffeine increases the frequency, tetracaine decreases it, and ryanodine has little effect.

One possible complication with the interpretation of these results is that we do not know which of the effects results from a direct action of the drugs on the oscillatory mechanism, as opposed to being secondary to changes of $[Ca^{2+}]_i$ produced by the SR. In order to investigate this point, we compared the ac-

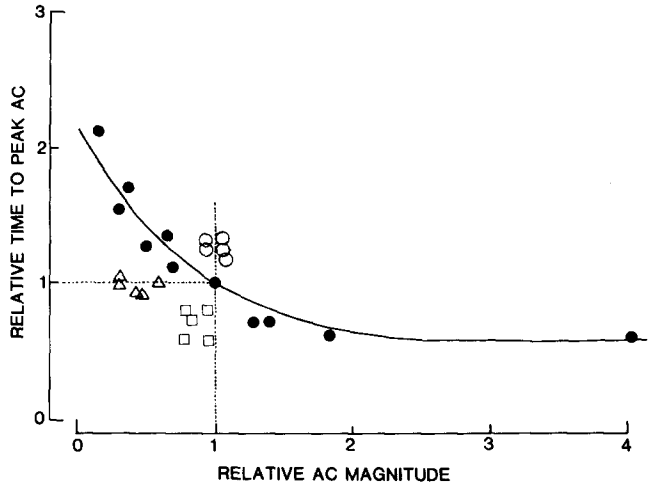

FIGURE 7. The relationship between AC magnitude and latency for changing $[Ca^{2+}]_o$ and for the addition of drugs. The latency of the AC was measured from the moment of repolarization to the peak of the AC. The solid circles represent points obtained by changing $[Ca^{2+}]_o$. The line through these points was drawn by eye. The points for caffeine (□), tetracaine (○), and ryanodine (△) came from different preparations. All points were normalized relative to the AC magnitude and latency, which were obtained in a solution containing 0 K^+, 2 mM Ca^{2+} just before the drug was added.

tions of the drugs with the effects of changing $[Ca^{2+}]_i$ directly either by varying $[Ca^{2+}]_o$ or during inhibition of the Na-K pump. The results, which are summarized in Fig. 7, showed that maneuvers that elevated $[Ca^{2+}]_i$ increased both the frequency and the magnitude of the oscillations. This direct correlation between frequency and magnitude was not seen for any of the drugs used, as indicated by the fact that the points for caffeine, tetracaine, and ryanodine lie off the control line. Indeed, caffeine increased the frequency while decreasing the magnitude. Although when tetracaine was applied at high enough concentrations, it decreased both the magnitude and the frequency of the oscillations, when used at lower concentrations, it decreased the frequency but had little effect on the magnitude. Again, this cannot be explained simply in terms of an

effect on $[Ca^{2+}]_i$. Similarly, the fact that ryanodine decreased the magnitude with little effect on the frequency is inconsistent with its principal action being to change $[Ca^{2+}]_i$. Therefore, our conclusion is that the effect of all three drugs must be directly on the oscillatory mechanism.

Further analysis of the mechanism of the effects of the drugs on the oscillation requires some understanding of their method of production by the SR. It appears likely that Ca is taken up into the SR by an energy-dependent Ca-ATPase and that release of Ca from the SR occurs either by the opening of specific Ca channels or by a nonspecific increase in permeability. The experiments of Fabiato and Fabiato (1975) suggest that Ca is released from the SR by an intracellular Ca trigger only when the Ca concentration inside the SR has increased to a threshold level. In order to produce an oscillation rather than a maintained release, the release must be terminated, perhaps by a $[Ca^{2+}]_i$-dependent inactivation (Fabiato, 1983). The oscillations are the product of repeated cycles of Ca uptake and release.

Pharmacological Interventions

CAFFEINE Caffeine is a drug that is thought to promote release of Ca from the SR in both skeletal (Weber and Hertz, 1968) and cardiac muscle (Chapman and Miller, 1974; Fabiato and Fabiato, 1975; Blayney et al., 1978). We find, in agreement with Glitsch and Pott (1975) and Capogrossi et al. (1984), that caffeine decreases the oscillation amplitude but increases its frequency. This result can be explained if caffeine lowers the threshold for Ca release from the SR. In this case, the Ca concentration in the SR will rise to the threshold level more quickly and this will increase the frequency. However, because the threshold is reached sooner, the SR has had less time to load up and therefore less Ca will be released and so the oscillation amplitude will decrease. It is worth noting that the decrease of amplitude is probably larger than it appears, as caffeine increases the sensitivity of the myofilaments to Ca (Wendt and Stephenson, 1983).

TETRACAINE Whereas caffeine is thought to promote release of Ca from the SR, tetracaine is thought to inhibit the release (Johnson and Inesi, 1969; Almers and Best, 1976; Chapman and Leoty, 1981). We have shown that tetracaine has the opposite effect on the frequency of the oscillations to that of caffeine. The effects of caffeine and tetracaine on the amplitude of the oscillations are different. The simplest hypothesis, that tetracaine has the opposite mode of action to caffeine, i.e., increasing the threshold for release, could not explain our results. It could explain the decrease in frequency we observed, by taking longer to reach threshold and thus producing peaks in $[Ca^{2+}]_i$ less often. However, it would then also be expected to increase the oscillation amplitude, but we saw little change in this. There is also evidence from work on isolated skeletal SR (Johnson and Inesi, 1969; Kurebayashi et al., 1982) that tetracaine decreases the rate of Ca uptake by the Ca-ATPase. Therefore, a plausible mechanism of action for tetracaine is to increase the time taken to reach threshold by decreasing the rate of Ca uptake, and probably also decreasing the rate of release. Unlike caffeine, tetracaine has no effect on the sensitivity of the myofibrils to Ca (Almers and Best, 1976). As explained above (see Results), we can also rule out the possibility that tetracaine is acting purely as a local anesthetic.

RYANODINE The mode of action of ryanodine is harder to ascertain because it is irreversible in action, and we found that all concentrations eventually abolished the oscillations. However, during the onset of action, using 0.1–10 μM, ryanodine progressively decreased the amplitude but left the frequency of the oscillations unaffected. This is in contrast to the results of Capogrossi et al. (1984), who found a decrease in the frequency of their spontaneous contractile waves in papillary muscles. The action of ryanodine has been attributed to inhibition of Ca release from the SR (Sutko et al., 1979; Sutko and Willerson, 1980; Sutko and Kenyon, 1983), although other effects have been reported, such as an action on the sarcolemma or T-tubules (Penefsky and Kahn, 1970), or Ca leak from the SR (Hilgemann et al., 1983; Hunter et al., 1983). Total inhibition of Ca release from the SR can explain the eventual abolition of the oscillations. However, it is difficult to see how the present results could be due to a leak of Ca from the SR. The decrease in the amplitude with no change in frequency could be due to early preferential block of some of the SR, so that if the oscillations seen are the sum of unit oscillators at the same frequency, selective block of some units will reduce the amplitude but will not alter the frequency.

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REFERENCES

Allen, D. G., D. A. Eisner, and C. H. Orchard. 1984. Characterization of oscillations of intracellular calcium concentration in ferret ventricular muscle. *J. Physiol. (Lond.).* 352:113–128.

Allen, D. G., D. A. Eisner, J. S. Pirolo, and G. L. Smith. 1985. Calcium transients during "calcium overload" in ferret ventricular muscle. *J. Physiol. (Lond.).* 357:57P. (Abstr.)

Almers, W., and P. M. Best. 1976. Effects of tetracaine on displacement currents and contraction of frog skeletal muscle. *J. Physiol. (Lond.).* 262:583–611.

Blayney, L., H. Thomas, J. Muir, and A. Henderson. 1978. Action of caffeine on calcium transport by isolated fractions of myofibrils, mitochondria and sarcoplasmic reticulum from rabbit heart. *Circ. Res.* 43:520–526.

Capogrossi, M. C., A. Fraticelli, and E. G. Lakatta. 1984. Ca^{2+} release from the sarcoplasmic reticulum: different effects of ryanodine and caffeine. *Fed. Proc.* 43:820. (Abstr.)

Chapman, R. A., and C. Leoty. 1981. The effects of tetracaine on the membrane currents and contraction of frog atrial muscle. *J. Physiol. (Lond.).* 317:475–486.

Chapman, R. A., and D. J. Miller. 1974. The effects of caffeine on the contraction of the frog heart. *J. Physiol. (Lond.).* 242:589–613.

Deck, K. A., R. Kern, and W. Trautwein. 1964. Voltage clamp technique in mammalian cardiac fibers. *Pflügers Arch. Eur. J. Physiol.* 280:50–62.

Eisner, D. A., and W. J. Lederer. 1979. Inotropic and arrhythmogenic effects of potassium depleted solutions on mammalian cardiac muscle. *J. Physiol. (Lond.).* 294:255–277.

Eisner, D. A., and W. J. Lederer. 1982. Effects of caffeine on the transient inward current in cardiac Purkinje fibres. *J. Physiol. (Lond.)*. 322:48–49P. (Abstr.)

Eisner, D. A., W. J. Lederer, and S.-S. Sheu. 1983. The role of intracellular sodium activity in the antiarrhythmic action of local anaesthetics in sheep cardiac Purkinje fibres. *J. Physiol. (Lond.)*. 340:239–257.

Eisner, D. A., and C. J. Nieman. 1984. The effects of low concentrations of caffeine on Ca^{2+}-dependent oscillations in sheep Purkinje fibres. *J. Physiol. (Lond.)*. 353:124P. (Abstr.)

Fabiato, A. 1983. Calcium-induced release of calcium ions from the cardiac sarcoplasmic reticulum. *Am. J. Physiol.* 245:C1–C14.

Fabiato, A., and F. Fabiato. 1975. Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. *J. Physiol. (Lond.)*. 249:469–495.

Glitsch, H. G., and L. Pott. 1975. Spontaneous tension oscillations in guinea-pig atrial trabeculae. *Pflügers Arch. Eur. J. Physiol.* 358:11–25.

Hilgemann, D. W., M. J. Delay, and G. A. Langer. 1983. Activation-dependent cumulative depletions of extracellular free calcium in guinea pig atrium measured with antipyralazo III and tetramethylmurexide. *Circ. Res.* 53:779–793.

Hillyard, I. W., and L. J. Procita. 1959. The effect of ryanodine on the contractile strength of mammalian cardiac (atrial) muscle. *J. Pharmacol. Exp. Ther.* 127:22–28.

Hunter, D. R., R. A. Haworth, and H. A. Berkoff. 1983. Modulation of cellular calcium stores in the perfused rat heart by isoproterenol and ryanodine. *Circ. Res.* 53:703–712.

Johnson, P. N., and G. Inesi. 1969. The effect of methylxanthines and local anaesthetics on fragmented sarcoplasmic reticulum. *J. Pharmacol. Exp. Ther.* 169:308–314.

Karagueuzian, H. S., and B. G. Katzung. 1982. Voltage-clamp studies of transient inward current and mechanical oscillations induced by ouabain in ferret papillary muscle. *J. Physiol. (Lond.)*. 327:255–271.

Kass, R. S., W. J. Lederer, R. W. Tsien, and E. Weingart. 1978. Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. *J. Physiol. (Lond.)*. 281:187–208.

Kass, R. S., and R. W. Tsien. 1982. Fluctuations in membrane current driven by intracellular calcium in cardiac Purkinje fibers. *Biophys. J.* 38:259–269.

Kort, A. A., and E. G. Lakatta. 1984. Calcium-dependent mechanical oscillations occur spontaneously in unstimulated mammalian cardiac tissues. *Circ. Res.* 54:396–404.

Kurebayashi, N., Y. Ogawa, and H. Harafuji. 1982. Effect of local anaesthetics on calcium activated ATPase and its partial reaction with fragmented sarcoplasmic reticulum from bullfrog and rabbit skeletal muscle. *J. Biochem. (Tokyo)*. 92:915–920.

Lederer, W. J., and R. W. Tsien. 1976. Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibres. *J. Physiol. (Lond.)*. 263:73–100.

Matsuda, H., A. Noma, Y. Kurachi, and H. Ogawa. 1982. Transient depolarization and spontaneous voltage fluctuations in isolated single cells from guinea pig ventricles. Calcium mediated membrane potential fluctuations. *Circ. Res.* 51:142–151.

Orchard, C. H., D. A. Eisner, and D. G. Allen. 1983. Oscillations of intracellular Ca^{2+} in mammalian cardiac muscle. *Nature (Lond.)*. 304:735–738.

Palade, P., R. D. Mitchell, and S. Fleischer. 1983. Spontaneous calcium release from sarcoplasmic reticulum. *J. Biol. Chem.* 258:8098–8107.

Penefsky, Z. J., and M. Kahn. 1970. Mechanical and electrical effects of ryanodine on mammalian heart muscle. *Am. J. Physiol.* 218:1682–1686.

Rosen, M. R., H. Gelband, and B. F. Hoffman. 1973. Correlation between effects of ouabain on the canine electrocardiogram and transmembrane potentials in isolated Purkinje fibers. *Circulation.* 47:65–72.

Sutko, J. L., and J. L. Kenyon. 1983. Ryanodine modification of cardiac muscle responses to potassium-free solutions. Evidence for inhibition of sarcoplasmic reticulum calcium release. *J. Gen. Physiol.* 82:385–404.

Sutko, J. L., and J. T. Willerson. 1980. Ryanodine alteration of the contractile state of rat ventricular myocardium. Comparison with dog, cat, and rabbit ventricular tissues. *Circ. Res.* 46:332–343.

Sutko, J. L., J. T. Willerson, G. H. Templeton, L. R. Jones, and H. R. Besch. 1979. Ryanodine: its alteration of cat papillary muscle contractile state and responsiveness to inotropic interventions and a suggested mechanism of action. *J. Pharmacol. Exp. Ther.* 209:37–47.

Tsien, R. W., R. Weingart, and R. S. Kass. 1978. Digitalis: inotropic and arrhythmogenic effects on membrane currents in cardiac Purkinje fibers. *In* Biophysical Aspects of Cardiac Muscle. M. Morad, editor. Academic Press, Inc., New York. 345–368.

Valdeolmillos, M., and D. A. Eisner. 1985. The effect of ryanodine on calcium-overloaded sheep cardiac Purkinje fibers. *Circ. Res.* 56:452–456.

Weber, A. M., and R. Hertz. 1968. The relationship between caffeine contracture of intact muscle and the effect of caffeine on isolated reticulum. *J. Gen. Physiol.* 52:750–759.

Wendt, I. R., and D. G. Stephenson. 1983. Effects of caffeine on Ca-activated force production in skinned cardiac and skeletal muscle fibers of the rat. *Pflügers Arch. Eur. J. Physiol.* 398:210–216.

Wier, W. G., A. A. Kort, M. D. Stern, E. G. Lakatta, and E. Marban. 1983. Cellular calcium fluctuations in mammalian heart: direct evidence from noise analysis of aequorin signals in Purkinje fibers. *Proc. Natl. Acad. Sci. USA.* 80:7367–7371.