Caffeine slows turn-off of calcium release in voltage clamped skeletal muscle fibers

Bruce J. Simon, Michael G. Klein, and Martin F. Schneider
Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, MD 21201

ABSTRACT Myoplasmic free calcium transients $\Delta [Ca^{2+}]$ were monitored with the calcium indicators antipyrylazo III and fura-2 in voltage clamped cut frog skeletal muscle fibers, in the presence and absence of 0.5 mM caffeine. Without caffeine $\Delta [Ca^{2+}]$ began to decline within a few milliseconds of fiber repolarization for pulses of all durations. In caffeine $\Delta [Ca^{2+}]$ continued to rise for 10–60 ms after 10 or 20 ms depolarizing pulses, indicating that the release of calcium from the sarcoplasmic reticulum (SR) continued well after repolarization of transverse tubular (TT) membranes in the presence of caffeine. Caffeine also increased the peak amplitude of $\Delta [Ca^{2+}]$ for all pulses and slowed the decline of $\Delta [Ca^{2+}]$ after pulses of all durations. The rate of calcium release from the SR calculated from $\Delta [Ca^{2+}]$ showed that for 10 ms pulses in caffeine release did not turn off abruptly on repolarization but instead declined to zero with a time constant essentially the same as the time constant for inactivation of SR calcium release during depolarizing pulses in the presence or absence of caffeine. The observed loss of TT membrane potential control of SR calcium release in the presence of caffeine suggests the appearance of a significant component of cytosolic Ca^{2+}-induced calcium release in caffeine.

INTRODUCTION

Electrical depolarization of the membranes of the transverse tubular (TT) system of a skeletal muscle cell initiates the release of calcium from the neighboring sarcoplasmic reticulum (SR), but the mechanism for TT to SR signaling is not established. One class of suggested mechanisms involves a diffusible chemical transmitter, with calcium ions themselves being a possible candidate for the transmitter. This would be consistent with the ability of calcium ions to activate calcium release from both isolated skeletal SR vesicles (1) and skinned skeletal fibers (2) by the process of Ca^{2+}-induced calcium release. However, several lines of evidence indicate that Ca^{2+}-induced calcium release is not involved in the physiological release triggered by TT depolarization in intact skeletal muscle fibers (2).

The drug caffeine is known to increase calcium release from the SR during membrane depolarization of skeletal muscle fibers (3, 4), and to potentiate Ca^{2+}-induced release of calcium from the SR in skinned skeletal fibers (5). Ca^{2+}-induced calcium release could in principle provide an element of positive feedback to calcium release and might thus interfere with TT control of release. We have therefore examined calcium transients in voltage-clamped cut fibers for indications of continued calcium release when the TT is repolarized after depolarizations that initiate release in the presence and absence of caffeine. Under control conditions TT repolarization appeared to rapidly terminate release even when cytosolic $[Ca^{2+}]$ was elevated from the preceding release, indicating that Ca^{2+}-induced calcium release driven by cytosolic $[Ca^{2+}]$ was insignificant. In contrast, we present here the first evidence that in fibers exposed to caffeine the release of calcium from the SR continued well after TT repolarization. This loss of TT control of SR calcium release suggests the appearance of a significant component of Ca^{2+}-induced calcium release in fibers exposed to caffeine.

Dr. Simon's present address is Department of Physiology and Biophysics, F41 University of Texas Medical Branch, Galveston, TX 77550.

METHODS

Single frog ileofibularis muscle fibers were cut at both ends and voltage-clamped in a double Vaseline gap chamber (6) at a holding potential of −100 mV. Two calcium indicators, the absorbance dye antipyrylazo III (AP III) and the fluorescent dye fura-2, entered the fiber by diffusion from the cut end solution (7). The relatively lower affinity calcium indicator AP III was used to monitor cytosolic free calcium transients $\Delta [Ca^{2+}]$ whereas the higher affinity indicator fura-2 was used for monitoring resting $[Ca^{2+}]$ and relatively small changes in $[Ca^{2+}]$ (7). Details of all experimental procedures and the methods for calculating $\Delta [Ca^{2+}]$ and $[Ca^{2+}]$ from the optical measurements were as in (7) and the references cited therein. Fibers were stretched to 3.8–4.1 μm per sarcomere to eliminate movement and the associated optical artifacts.

The internal solution applied to the cut ends of the fibers contained (millimolar) 102.5 Cs^+ glutamate, 5.5 $MgCl_2$, 5 ATP (Na^+ salt), 4.5 Na^+ tris-maleate buffer, 13.2 Cs^+ tris-maleate buffer, 0.1 EGTA, 5

0006-3495/89/04/793/05 $2.00

creatine phosphate (Na^+ salt), 1 AP III, 0.05 fura-2 and 1 g/l glucose. The external solution applied to the intact portion of the fiber in the middle pool contained 75 $(TEA)_2SO_4$, 5 Cs_2SO_4, 7.5 total $CaSO_4$, 5 Na^+ Tris-maleate buffer and 10^{-7} g/ml tetrodotoxin, with or without 0.5 caffeine. Both solutions were adjusted to pH 7.0 at room temperature. Experiments were carried out at 8–10°C.

RESULTS AND DISCUSSION

Effects of caffeine on calcium transients

Fig. 1 presents superimposed calcium transients ($\Delta[Ca^{2+}]$) recorded from a fiber in the presence or the absence of caffeine (0.5 mM) for depolarizations of 30 (*A*) or 120 (*B*) ms (*bottom*). The amplitude of $\Delta[Ca^{2+}]$ for each pulse was greatly increased in the presence of caffeine. In this fiber resting $[Ca^{2+}]$ monitored by fura-2 was increased from 30 to 45 nM in caffeine. Calcium transients were measured before, during and after exposure to 0.5 mM caffeine in 14 fibers. For 30 ms pulses to −20 or −30 mV the peak $\Delta[Ca^{2+}]$ increased 3.2 ± 0.6 −fold (mean ± SEM) relative to the bracketing caffeine-free controls. In the same fibers resting $[Ca^{2+}]$ increased from 38 ± 6 to 52 ± 8 nM in caffeine and returned to 47 ± 9 nM upon removal of caffeine. Changes in resting $[Ca^{2+}]$ of this magnitude were probably beyond the resolution of previous calcium micro-electrode measurements (8). This concentration of caffeine would thus correspond to that

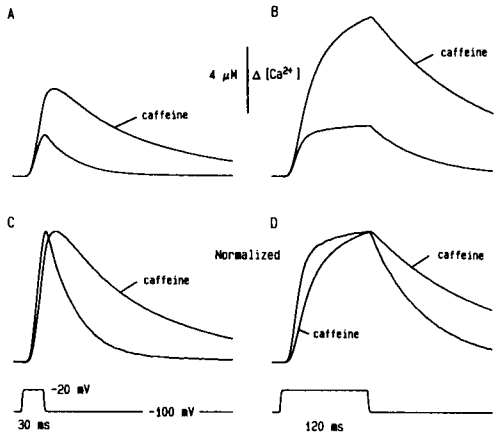

FIGURE 1 The effect of caffeine (0.5 mM) on cytosolic free calcium transients in a voltage clamped frog skeletal muscle fiber. *A*, *B*. Calcium transients for pulses of 30 (*A*) and 120 (*B*) ms (*bottom*) in the absence and presence of caffeine (indicated). *C*, *D*. Records from *A* and *B*, respectively, scaled to the same peak amplitude. Resting $[Ca^{2+}]$ was 30 and 45 nM, respectively, in the absence and presence of caffeine during these records. Control records obtained 19 min before application of caffeine; caffeine records obtained after 2 min in caffeine. Fiber 457, diameter 65 μm, 3.9 μm per sarcomere, 650 μM AP III before caffeine and 790 μM in caffeine.

producing twitch potentiation but not contracture in unstretched fibers (9).

A second effect of caffeine, which has not previously been reported, was the prolongation of the time from the end of the depolarizing pulse until $\Delta[Ca^{2+}]$ began to decline after the pulse. This effect is seen clearly in Fig. 1 *C*, where the records from Fig. 1 *A* (30 ms pulse) are scaled to the same peak amplitude. In the absence of caffeine $\Delta[Ca^{2+}]$ rose steadily throughout the 30 ms pulse and then began to decline abruptly within 6 ms after the pulse. The abrupt decline of $\Delta[Ca^{2+}]$ after repolarization has been a consistent finding (10, 11) and has been taken as evidence that calcium release is tightly regulated by the TT membrane potential under control conditions. In contrast, in the presence of caffeine $\Delta[Ca^{2+}]$ continued to rise for 19 ms after the end of the pulse in this fiber. Because a rise in $\Delta[Ca^{2+}]$ implies that release of calcium from the SR must exceed removal from the myoplasm, the continued rise of $\Delta[Ca^{2+}]$ after the 30 ms pulse in the presence of caffeine provides evidence for continued release of calcium after repolarization in caffeine. In Fig. 1 *D* the $\Delta[Ca^{2+}]$ records for the 120 ms pulse (Fig. 1 *B*) are also scaled to the same peak amplitude. Here $\Delta[Ca^{2+}]$ began to decline abruptly ~4 ms after the pulse both in the presence and the absence of caffeine. Thus the effect of caffeine on prolonging calcium release after repolarization depended on pulse duration, being marked for relatively short pulses but negligible by 120 ms after the start of the pulse.

The relationship between pulse duration and the continued rise of $\Delta[Ca^{2+}]$ after fiber repolarization is further examined in Fig. 2, which presents families of calcium transients from another fiber for pulses of five different durations to the same potential in the absence (Fig. 2 *A*) and the presence (Fig. 2 *B*) of caffeine (0.5 mM). Caffeine greatly increased the amplitude of $\Delta[Ca^{2+}]$ for all pulse durations (note different vertical scales in Fig. 2, *A* and *B*). In the absence of caffeine (Fig. 2 *A*) each $\Delta[Ca^{2+}]$ record began to decline sharply within 4–8 ms after repolarization. This is seen more clearly in Fig. 2 *C*, where the records from Fig. 2 *A* are scaled to the same peak amplitude. The peaks in the $\Delta[Ca^{2+}]$ records for the 10, 20, and 30 ms pulses in the absence of caffeine were about equally spaced at 10 ms intervals. In contrast, in the presence of caffeine (Fig. 2 *B*) the $\Delta[Ca^{2+}]$ records for the shorter pulses continued to rise for a considerable period of time after repolarization, e.g., for 46 ms after the 10 ms pulse. In Fig. 2 *D* the records from Fig. 2 *B* are each scaled to the same peak amplitude. The continued rise in $\Delta[Ca^{2+}]$ after the shortest pulses in caffeine was sufficiently prolonged that the peaks of the calcium transients for the pulses of 10, 20, and 30 ms duration approximately coincided (Fig. 1 *D*), exhibiting no correlation with the time of repolarization. Calcium transients

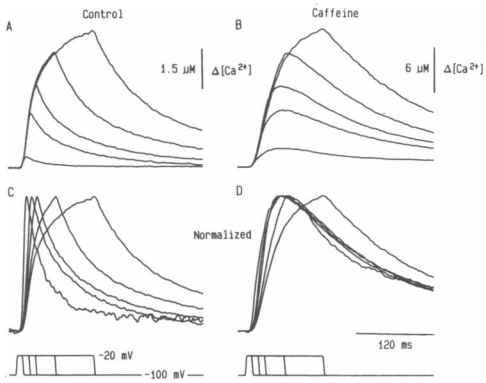

FIGURE 2 Effect of pulse duration on calcium transients in the presence and absence of caffeine. (*A*) Calcium transients for pulses of 10, 20, 30, 60, and 120 ms (*bottom*) recorded 5 min after return to caffeine-free solution after exposure to caffeine. (*B*) Calcium transients for the same pulses recorded after 6 min of exposure to 0.5 mM caffeine. (*C, D*) Records from *A* and *B* scaled to the same peak amplitude. Resting $[Ca^{2+}]$ averaged 53 nM in caffeine and 68 nM in the absence of caffeine during these records. Fiber 458, diam 65 μm, 3.8 μm per sarcomere, 523 μM AP III in caffeine and 575 μM after caffeine.

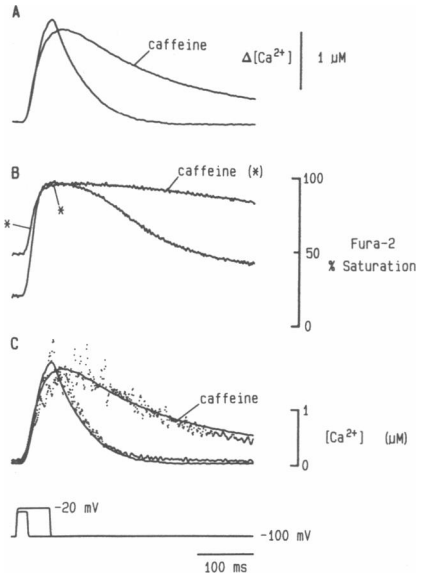

FIGURE 3 Prolonged rise and slowed decay of $\Delta[Ca^{2+}]$ after repolarization in caffeine for pulses giving similar amplitude $[Ca^{2+}]$ transients with and without caffeine. (*A*) $\Delta[Ca^{2+}]$ measured with AP III before and during exposure of the fiber to 0.5 mM caffeine. The depolarizations (*lowermost panel*), to −20 mV for 60 ms before caffeine and to −30 mV for 20 ms during caffeine, were chosen to produce $\Delta[Ca^{2+}]$ of similar peak amplitude. (*B*) Percent saturation of fura-2 for the same depolarizations as in *A*. Percent saturation was calculated from the ratio of the fluorescence signal measured at 380 nm excitation divided by the constant fluorescence intensity at 358 nm excitation (7). (*C*) $[Ca^{2+}]$ transients measured with AP III (same as *A* but with baselines set at resting $[Ca^{2+}]$) superimposed upon the $[Ca^{2+}]$ calculated from the fura-2 ratio in *B* (noisier traces). During the rising phase of the transient in caffeine the points of the $[Ca^{2+}]$ calculated from fura-2 fall below the trace measured with AP III. The parameters for converting the percent saturation of fura-2 to $[Ca^{2+}]$ ($k_{on} = 1.76 \times 10^8$ M^{-1} s^{-1} and $k_{off} = 14.7$ s^{-1}) were determined from several records (7) before exposure to caffeine. Fiber 466, diam 60 μm, 4.1 μm per sarcomere, 690 μM AP III before caffeine, 760 μM during caffeine.

were monitored for 10–20 ms pulses in 12 fibers before, during, and after exposure to 0.5 mM caffeine. In caffeine the peak of $\Delta[Ca^{2+}]$ occurred 29 ± 5 ms after repolarization whereas in the absence of caffeine the peak occurred within 6 ms of repolarization.

A third effect of caffeine was a slowing of the decay of $\Delta[Ca^{2+}]$ after short and long pulses as previously observed (3). The normalized records in Figs. 1 and 2 show that the time course of the decay of $\Delta[Ca^{2+}]$ was slowed after all pulses in the presence of caffeine. All effects of caffeine were completely reversible. In fact, the control records in Fig. 2 were actually obtained 5 min after the return to caffeine-free solution after exposure to caffeine.

The continued rise and slowed decline of $\Delta[Ca^{2+}]$ after repolarization in caffeine (Figs. 1 and 2) were not simply a direct result of the larger $\Delta[Ca^{2+}]$ in the presence of caffeine. In the experiment shown in Fig. 3 we used a shorter and smaller pulse (*bottom*) in caffeine than in control so that the peak amplitude of $\Delta[Ca^{2+}]$ was similar in caffeine and in control (Fig. 3 *A*). The calcium transient in caffeine still exhibited the continued rise and slowed decline after the pulse, even though the peak $\Delta[Ca^{2+}]$ was slightly smaller than in control. Indeed, the continued rise of $\Delta[Ca^{2+}]$ after the depolarization in caffeine was so marked that the peak $\Delta[Ca^{2+}]$ occurred later for the 20 ms pulse in caffeine than for the 60 ms pulse in control conditions. The effects of caffeine on $\Delta[Ca^{2+}]$ were also not due to the small elevation of resting $[Ca^{2+}]$ because in Fig. 2 *B* we observed typical effects of caffeine on $\Delta[Ca^{2+}]$ which were reversed completely upon washing with caffeine-free solution (Fig. 2 *A*) even though resting $[Ca^{2+}]$ increased throughout this experiment.

Caffeine binds weakly to AP III (12), so it was of interest to confirm our observations by examining the simultaneously recorded fura-2 signals. Fig. 3 *B* presents the percent saturation of fura-2 obtained from the fluorescence ratio (−380/358 nm) signals (7) recorded simultaneously with the AP III signals used to calculate the calcium transients in Fig. 3 *A*. The slowed decline of fura-2 saturation in the presence of caffeine qualitatively confirms the slowed decline of $\Delta[Ca^{2+}]$ measured with AP III. Fig. 3 *C* presents superimposed $[Ca^{2+}]$ records obtained from the AP III and fura-2 signals. A single set of parameters determined in the absence of caffeine were used to convert fura-2 saturation to $[Ca^{2+}]$ (7). Although

the fura-2 [Ca^{2+}] records had much more noise than those from AP III due to the near saturation of fura-2 (Fig. 3 *B* and reference 7), they still exhibited essentially the same time course as the simultaneously recorded AP III [Ca^{2+}] signals (Fig. 3 *C*). Because both indicators showed the same effects of caffeine on $\Delta[Ca^{2+}]$, it is unlikely that the observed effects of caffeine were due to an interaction of caffeine with AP III. Comparison of the effects of caffeine on the baselines in Fig. 3 *B* and *C* show that the relatively large increase in the baseline level of fura-2 saturation in caffeine corresponded to only a slight increase of free calcium on the scale of the calcium transients.

Because 0.5 mM caffeine is known to potentiate Ca^{2+}-induced calcium release in skinned fibers (5) but not to affect the TT voltage sensor (reference 3 and B.J. Simon, M.G. Klein, M.F. Schneider, unpublished observations) or the SR calcium pump (13, 14), the simplest explanation for the continued rise and slowed decline of $\Delta[Ca^{2+}]$ after pulses in caffeine is the appearance of a significant component of Ca^{2+}-induced calcium release in the presence of caffeine.

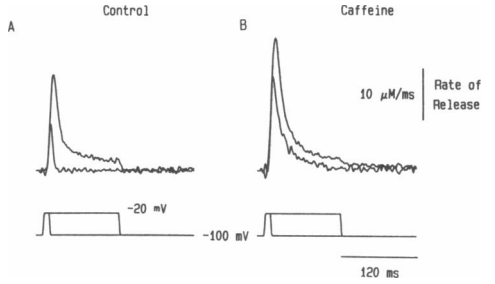

FIGURE 4 Rate of release of calcium from the SR for short and long pulses in the absence (A) and the presence (B) of caffeine. R_{rel} was calculated from the $\Delta[Ca^{2+}]$ records for the 10 and 120 ms pulses in Fig. 2 using the general procedure of Melzer et al (16). The removal model parameter values used to calculate the release were 583* μM parvalbumin calcium/magnesium sites with on and off rate constants of 1.6×10^8 M^{-1} s^{-1} and 1.5 s^{-1} for Ca^{2+} and 4×10^4 M^{-1} s^{-1} and 17.8* s^{-1} for Mg^{2+}, and a maximum pump rate of 1951* μM s^{-1} for the SR calcium pump. The values marked by * were adjusted by least-squares fit to the decay of several $\Delta[Ca^{2+}]$ records (10, 20) obtained before and after caffeine, while the values for the other parameters were set to the assumed values given here. In these calculations the SR pump was assumed to bind calcium at a single site whereas a two site system was used previously (20).

Effects of caffeine on the rate of release of calcium from the SR

The calcium transients in the presence of caffeine provide clear evidence that calcium release continued after repolarization in the presence of caffeine but they do not directly indicate the release time course. We therefore used the calcium transients to calculate the rate of release (R_{rel}) of calcium from the SR using the general procedure of Melzer et al. (15, 16). For the present calculations the rapidly equilibrating calcium binding sites intrinsic to the fiber were assumed to consist of the calcium specific sites on troponin C (assumed to be present at 250 μM and to have on and off rate constants of 1.3×10^8 M^{-1} s^{-1} and 10^3 s^{-1}) and the calcium binding sites on the SR calcium pump (assumed to be present at 200 μM and to equilibrate instantaneously with [Ca^{2+}] with $K_D = 2$ μM). Both the rapidly equilibrating calcium binding sites and the calcium removal system (equals slowly equilibrating sites plus transport systems) were assumed to be unaffected by caffeine. Thus a single set of parameter values, determined from the decay of various $\Delta[Ca^{2+}]$ records (10, 20) taken before addition of caffeine and after washing caffeine from the fiber, were used in the removal model (20) for calculating calcium release in both the presence and absence of caffeine (Fig. 4, legend). Details of the removal model should not influence the release calculation since any removal model that can reproduce the decay of $\Delta[Ca^{2+}]$ is satisfactory for calculating release (16).

Fig. 4 presents superimposed R_{rel} records calculated from the $\Delta[Ca^{2+}]$ records for the 10 and 120 ms pulses in Fig. 2. In the absence of caffeine (Fig. 4 *A*) the 10 ms pulse produced a spike of release which peaked 4 ms after the pulse and rapidly declined to zero. The 120 ms pulse produced a rise in release to an early peak followed by a decline to a much lower steady level. This decline in release during a 100–200 ms pulse under control conditions is now well-documented (15–18) and appears to be due predominately to a calcium-dependent partial inactivation of SR calcium release (19). Comparison of the release records in the presence (Fig. 4 *B*) and absence (Fig. 4 *A*) of caffeine shows that the peak rate of release was increased in caffeine for both pulse durations. A more striking change in the release records was the slow decline of release after the 10 ms pulse. In caffeine R_{rel} for the 10 ms pulse peaked 5 ms after repolarization but no longer turned off rapidly to zero as in the absence of caffeine. Instead R_{rel} declined to zero approximately exponentially over the next 100 ms.

The time course of the relatively slow decline of R_{rel} after the 10 ms pulse in caffeine (Fig. 4 *B*) roughly paralleled the time course of decline of R_{rel} seen during the 120 ms pulse in the absence of caffeine (Fig. 4 *A*). The similarity of time courses suggests that the extra release after the short pulse in the presence of caffeine, which was not turned off by repolarization, may instead have been turned off by an inactivation mechanism similar or identical to that underlying the inactivation of release during long pulses in the absence of caffeine. This possibility is supported by the observation that in caffeine

(Fig. 4 *B*) the decline of release during the 120 ms pulse, which presumably represents inactivation of release during the pulse as in control conditions, closely paralleled the time course of the decline in release after the 10 ms pulse. The two release records in caffeine differed primarily in that after the shorter pulse the release declined to zero, whereas during the longer pulse release declined to a maintained level. Inactivation of the Ca^{2+}-induced calcium release component that appears in caffeine would account for the finding that release in caffeine continues well after short pulses but turns off abruptly after longer pulses.

In conclusion, in the presence of caffeine calcium continues to be released after termination of short pulses that initiate release, consistent with the appearance of a significant component of Ca^{2+}-induced calcium release. The relatively rapid turn-off of release after pulses that elevate cytosolic [Ca^{2+}] in the absence of caffeine suggests that Ca^{2+}-induced calcium release driven by cytosolic [Ca^{2+}] is minimal under control conditions. However, this does not rule out the possibility of Ca^{2+}-induced calcium release driven by locally elevated [Ca^{2+}] in the neighborhood of the release site that dissipates rapidly upon repolarization.

We thank Dr. W. Melzer for helpful comments on the manuscript.

Supported by research grants from the Muscular Dystrophy Association and National Institutes of Health (RO1-NS23346).

Received for publication 14 September 1988 and in final form 12 December 1988.

REFERENCES

1. Meissner, G., E. Darling, and J. Eveleth. 1986. Kinetics of rapid Ca^{2+} release by sarcoplasmic reticulum. Effects of Ca^{2+}, Mg^{2+}, and adenine nucleotides. *Biochemistry*. 25:236–244.
2. Endo, M. 1985. Calcium release from the sarcoplasmic reticulum. *Curr. Top. Membr. Transp.* 25:181–229.
3. Kovacs, L., and G. Szucs. 1983. Effect of caffeine on intramembrane charge movement and calcium transients in cut skeletal muscle fibres of the frog. *J. Physiol. (Lond.)*. 341:559–578.
4. Delay, M., B. Ribalet, and J. Vergara. 1986. Caffeine potentiation of calcium release in frog skeletal muscle fibres. *J. Physiol. (Lond.)*. 375:535–559.
5. Endo, M. 1975. Mechanism of action of caffeine on the sarcoplasmic reticulum of skeletal muscle. *Proc. Jpn. Acad.* 51:479–484.
6. Kovacs, L., E. Rios, and M. F. Schneider. 1983. Measurement and modification of free calcium transients in frog skeletal muscle fibres by a metallochromic indicator dye. *J. Physiol. (Lond.)*. 343:161–196.
7. Klein, M. G., B. J. Simon, G. Szucs, and M. F. Schneider. 1988. Simultaneous recording of calcium transients in skeletal muscle using high and low affinity calcium indicators. *Biophys. J.* 53:971–988.
8. Lopez, J. R., R. L. Alamo, C. Caputo, R. DiPolo, and J. Vergara. 1983. Determination of ionic calcium in frog skeletal muscle fibers. *Biophys. J.* 43:1–4.
9. Konishi, M., and S. Kurihara. 1987. Effects of caffeine on intracellular calcium concentration in frog skeletal muscle fibres. *J. Physiol. (Lond.)*. 383:269–283.
10. Melzer, W., E. Rios, and M. F. Schneider. 1986. The removal of myoplasmic free calcium following calcium release in frog skeletal muscle. *J. Physiol. (Lond.)*. 372:261–292.
11. Rios, E. 1984. Off gating of calcium release in skeletal muscle. *J. Gen. Physiol.* 84:40.
12. Best, P. M., and C. W. Abramcheck. 1985. The metallochromic indicator dye arsenazo III forms 1:1 complexes with caffeine. *Biochem. Biophys. Acta.* 838:179–182.
13. Batra, S. 1974. The effects of drugs on calcium uptake and calcium release by mitochondria and sarcoplasmic reticulum of frog skeletal muscle. *Biochem. Pharmacol.* 23:89–101.
14. Ogawa, Y. 1970. Some properties of fragmented frog sarcoplasmic reticulum with particular reference to its response to caffeine. *J. Biochem.* 67:667–683.
15. Melzer, W., E. Rios, and M. F. Schneider. 1984. Time course of calcium release and removal in skeletal muscle fibers. *Biophys. J.* 45:637–641.
16. Melzer, W., E. Rios, and M. F. Schneider. 1987. A general procedure for determining calcium release from the sarcoplasmic reticulum in skeletal muscle fibers. *Biophys. J.* 51:849–863.
17. Baylor, S. M., W. K. Chandler, M. W. Marshall. 1983. Sarcoplasmic reticulum calcium release in frog skeletal muscle fibers estimated from arsenazo III calcium transients. *J. Physiol. (Lond.)*. 344:625–666.
18. Rios, E., and G. Brum. 1987. Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature (Lond.)*. 325:717–720.
19. Schneider, M. F., and B. J. Simon. 1988. Inactivation of calcium release from the sarcoplasmic reticulum in frog skeletal muscle. *J. Physiol. (Lond.)*. 405:727–745.
20. Brum, G., E. Rios, and M. F. Schneider. 1988. A quantitative model of calcium removal from the myoplasmic solution (Appendix to G. Brum, E. Rios and E. Stefani, Effects of extracellular calcium on the calcium movements of excitation-contraction coupling in skeletal muscle fibres). *J. Physiol. (Lond.)*. 398:467–473.