Caffeine and the Myoplasmic Calcium Removal Mechanisms in Cut Frog Skeletal Muscle Fibres

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Abstract. Antipyrylazo III myoplasmic calcium transients were recorded in cut skeletal muscle fibres of the frog (*Rana esculenta*), using the double vaseline-gap voltage-clamp system. Intracellular calcium removal mechanisms were analysed, using a slightly modified model taken from the literature. Parameter values reported here are generally consistent with those obtained by the original model. Caffeine (0.5 mmol l^{-1}) moderately enhanced the overall myoplasmic calcium removal. In particular, the rate constant of the non-saturable uptake increased by 51 % on the average, but there was a considerable fiber-to-fiber variation. The kinetic features of the binding sites representing the saturable uptake did not change singificantly while the concentration of the available sites decreased by 36 %. It is concluded that the caffeine-induced changes of the calcium removal components can be explained by supposing an increased resting myoplasmatic Ca^{2+} concentration in the presence of the drug.

Key words: Cut skeletal muscle fibres — Myoplasmic calcium transients — Calcium removal mechanisms — Caffeine

Introduction

Caffeine is one of the most frequently used pharmacological tools for studying skeletal muscle function. The drug has two basic effects: in high concentrations it evokes contracture without modifying the electrical properties of the muscle membrane, while in subthreshold doses it potentiates the twitches elicited by electrical stimulation (Axelsson and Thesleff 1958; Sandow et al. 1964). Numerous studies have been performed during recent years aimed at revealing the cellular and subcellular actions causing the above changes. There are data indicating that the drug modifies contractile activation (Lüttgau and Oetliker 1968) although neither the electrical properties of the T-tubules (Delay et al.

1986) nor the intramembrane charge movement seem to be altered (Kovács and Szűcs 1983).

Another line of investigations deals with the caffeine-induced changes of the intracellular calcium homeostasis. Some data show an increase of the resting myoplasmic Ca^{2+} level even in the presence of low caffeine doses (Konishi et al. 1985; Konishi and Kurihara 1987; Simon et al. 1989) while other authors could not detect the same change (Lopez et al. 1983; Delay et al. 1986). Studies on isolated sarcoplasmic reticulum (SR) vesicles indicate that caffeine may induce calcium release (Weber and Herz 1968; Ogawa 1970), probably by potentiating the Ca^{2+}-induced Ca^{2+} release mechanism (Endo 1975). Applied in higher concentrations, the drug can decrease the calcium uptake of the SR vesicles (Weber 1968; Weber and Herz 1968).

Some recent works have led to the conclusion that under physiological conditions the most probable site of the caffeine action is a step in the electromechanical coupling process. Some data indicate that this step may be a biochemical link at the level of the triads (Kumbaraci and Nastuk 1982; Herrmann 1986). A similar conclusion was drawn by Delay et al. (1986) who studied this question using the metallochromic indicator dyes Antipyrylazo III and Arsenazo III to reveal the caffeine effects on intracellular calcium concentration changes. Antipyrylazo III and fura-2 calcium transients were analysed by Simon et al. (1989) who reported that the voltage control of the SR calcium release channels was lost in the presence of caffeine, probably due to the appearance of the Ca^{2+}-induced Ca^{2+} release mechanism.

The experiments presented in this paper used the method introduced by Melzer et al. (1984; 1986) to model the removal of calcium ions from the myoplasmic space. The results indicate that caffeine influences the overall removal only to a small extent because the capacity of the saturable uptake is decreased while the rate of the non-saturable uptake is increased in the presence of the drug. These changes are elicited probably by an elevation of the resting myoplasmic Ca^{2+} concentration. Preliminary results have been presented elsewhere (Csernoch et al. 1986).

Materials and Methods

Preparation and solutions

Experiments were carried out on single fibres isolated from the semitendinosus muscles of frog (*Rana esculenta*). The muscle was prepared in Ringer solution (all the concentrations are given as mmol.l^{-1} unless otherwise indicated; NaCl 115; KCl 2.5; $CaCl_2$ 1.8; TRIS sodium maleate buffer 2); then a relaxing solution was applied (K-glutamate 120; $MgCl_2$ 2; EGTA 0.1; TRIS sodium maleate buffer 5). When the relaxiation following the K-contracture had been completed,

the fibres were cut and mounted in a double vaseline-gap chamber, their sarcomere length being set longer than 3.4 μm by stretching.

After completing the separation, the relaxing solution in the middle pool was replaced by the external solution (TEA-sulphate 75; Cs_2SO_4 10; $CaSO_4$ 8; tetrodotoxin 3.1×10^{-7} mol.l^{-1}; TRIS sodium maleate buffer 5) and that in the open end pools by the internal solution (Cs-glutamate 108; $MgCl_2$ 5.5; EGTA 0.1; $CaCl_2$ 0.0082; TRIS sodium maleate buffer 4.5; TRIS caesium maleate buffer 13.2; ATP 5; glucose 5.6). All the solutions had pH of 7.0 $\pm$ 0.1. Caffeine dissolved in the external solution was applied into the middle pool. The experiments were carried out at low temperature (2 –5 °C). Other details are given in previous papers (Kovács et al. 1983; Csernoch et al. 1987).

Experimental setup and recording of data

The resting potential of the fibres was set at −90 mV using a voltage-clamp circuit. Optical signals (ΔI) accompanying depolarizing pulses were measured using a photodiode (PV-100, EG&G) and a track-and-hold circuit (for details see Kovács and Szűcs 1983; Csernoch et al. 1987).

ΔI transients were recorded on-line by a microcomputer system (HT-680X, HTSZ, Hungary) using a 10-bit analog-to-digital converter. The sampling interval was 200 μs and five consecutive points were added to get data points with 1 ms intervals (in some cases 400 μs sampling rate and, consequently, a final resolution of 2 ms was used). To improve signal-to-noise ratio, the transients were averaged; the number of the necessary repetitions was 2 – 8. The recorded transients consisted of 255 data points and were stored permanently on a hard-disk system (SZM 5400, IZOT, Bulgaria). Timing of the pulses was also controlled by the microcomputer.

Calculation of myoplasmic calcium transients

Changes in the myoplasmic calcium concentration accompanying depolarizing pulses were monitored using the metallochromic indicator dye Antipyrylazo III. The dye was applied in 1 mmol.l^{-1} concentration into the solution in the open end pools. Calcium-specific changes in the transmitted light intensity were calculated from the optical signals using appropriate K_D (17 500 μmol^2) and ε_{720} molar extinction coefficient (8.2×10^3 mol^{-1} cm^{-1}) values (Kovács et al. 1983). For the calculations, the formation of calcium : dye complexes in a 1 : 2 ratio only was supposed.

ΔI transients measured at 720 nm contain a component which originates from intrinsic absorbance change and thus distorts the time course of the calculated true calcium signals. To minimize this effect, ΔI signals recorded at 720 nm were corrected for the intrinsic component with the help of pure intrinsic absorbance transients measured at 850 nm using a procedure described by Melzer et al. (1986). This method was also supposed to abolish most of the possible artifacts when stretching was not sufficient to completely eliminate movement. Our optical set-up did not allow parallel recording at the two wavelengths, so the appropriate signals were evoked consecutively using the same pulse parameters.

Statistics

Scatter of the means was calculated as standard error (S.E.). The significance of the differences was determined using Student's paired t-test.

Results

Components of myoplasmic calcium removal

Our procedure for studying removal of calcium from the myoplasmic space is an adaptation of different methods introduced previously (Kovács et al. 1983; Melzer et al. 1984). According to these approaches, Ca^{2+} released from the

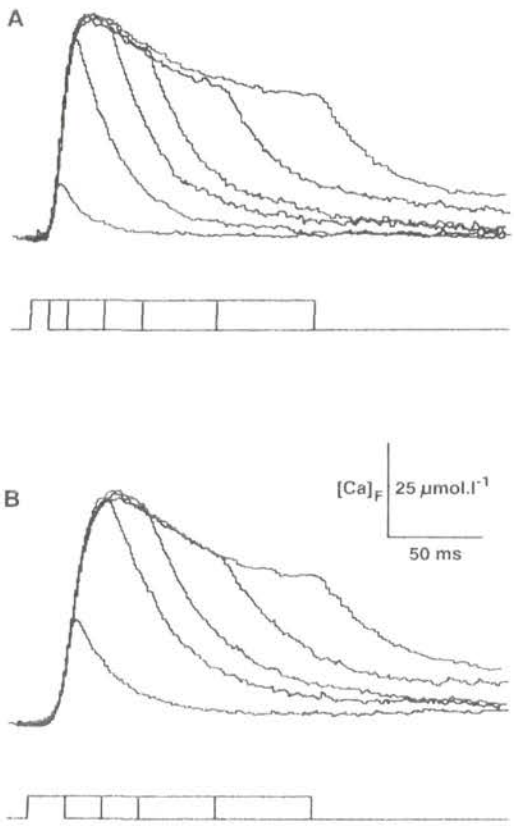

Fig. 1. Changes of the calcium concentration in the fast equilibrating pool ($[Ca]_f$) under control conditions (*A*) and in the presence of 0.5 mmol l^{-1} caffeine (*B*). The transients were evoked by depolarizing pulses to −13.4 mV (control) and to −32.6 mV (caffeine), respectively. Duration of the pulses is indicated below the traces. The original myoplasmic calcium transients were sampled with the usual 1 ms rate except for those with 150 ms duration, where 2 ms sampling was used. *F* was determined for the individual signals supposing $F_1 = 15$ and calculating F_2 from the actual myoplasmic concentration of Antipyrylazo III. The original records were obtained averaging 2–6 sweeps. Fiber 51016; *s* (sarcomere length) = 3.50 µm; *T* (temperature) = 3.7–4.7 °C.

sarcoplasmic reticulum can be found either in free form in the myoplasm ($[Ca^{2+}]$) or bound to different intracellular sites. The concentration of calcium being in instantaneous equilibrium with the myoplasmic free Ca^{2+} ($[Ca]_f$; Fig. 1) can be calculated as a linear function of the myoplasmic calcium transients, supposing that the binding sites are far from saturation:

$$[Ca]_f = F[Ca^{2+}] \qquad (1)$$

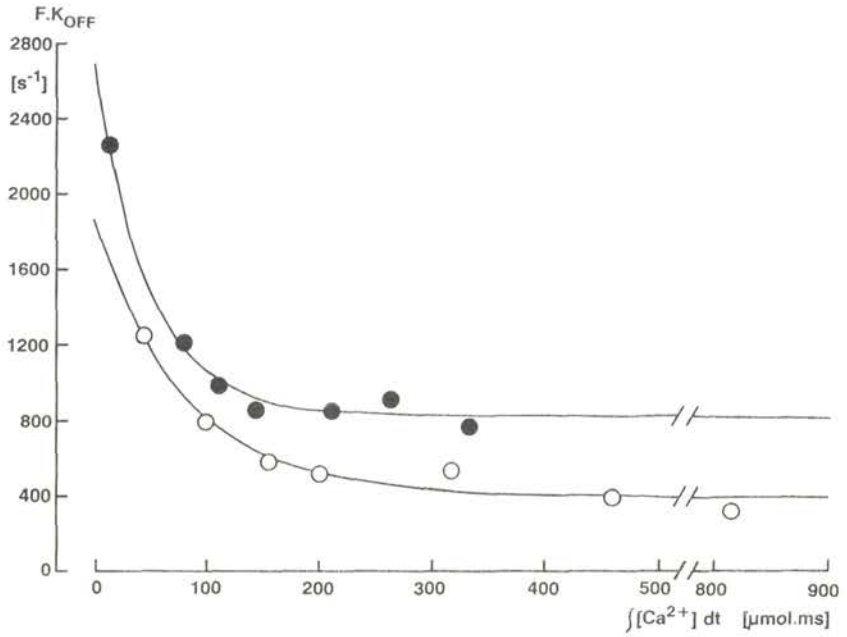

Fig. 2. Decay rate constants of calcium transients as a function of the time integral of the transients. Depolarizing pulses to −23.7 mV for 10, 20, 30, 40, 70, 100 and 150 ms under control conditions (○) and to −42.6 mV for the same durations in the presence of 0.5 mmol l^{-1} caffeine (●) were applied. Data points were fitted using Eq. (4), the best fit parameters for the control set are given in Table I, for changes due to the caffeine treatment see Table II. Calculated theoretical curves are also shown. Fiber 51120; $s = 3.43$ μm; $T = 2.3$–3.7 °C.

where F is the proportionality factor, which has a component (F_D) due to the presence of the dye and an intrinsic component (F_i) resulting from intracellular binding sites. F_i can be determined using the volume expansion concept (Kovács et al. 1983).

Calcium removal in this model has two components, a saturable and a non-saturable uptake; the latter is described by its rate coefficient (k_{ns}) in the equation

$$dU/dt = k_{ns}[Ca^{2+}] + dCa_{sat}/dt \tag{2}$$

where U stands for the overall calcium removal from the myoplasmic space. The saturable uptake is supposed to be a first order binding site, hence

$$dCa_{sat}/dt = k_{ON}(N - Ca_{sat})[Ca^{2+}] - k_{OFF}Ca_{sat} \tag{3}$$

where N denotes the resting concentration of the free binding sites, Ca_{sat} is the concentration of those already having bound calcium, and k_{ON} and k_{OFF} are the corresponding rate coefficients. The removal parameters (k_{ns}, Nk_{ON} and k_{OFF}) can be derived from the falling phase of the calcium transients, because during repolarization the release is already turned off.

Calculation of calcium removal supposing $k_{OFF} = 0$

To characterize the calcium removal system, we used the method 2 of Melzer et al. (1987) with a slight modification. The original approach supposes that there is no backflow of calcium from the saturable binding sites, i.e. $k_{OFF} = 0$ in Eq. (3). In this case the other parameters of the uptake systems can be evaluated by evoking calcium transients using depolarizing pulses of constant amplitude but of various duration (Fig. 1) and examining the dependence of the decay rate constant of the falling phases (K_{OFF}) on the integral of the signals.

Fig. 2 shows an example of this analysis. K_{OFF} was calculated by fitting a single exponential plus constant (Ca_{OFF}) to the calcium signals starting 15 ms after turning off the pulse or after the peak of the calcium transient (in the case of short pulses when the intracellular calcium concentration increase reaches its maximum following repolarization). The time integral of the myoplasmic calcium transients was also calculated using the ON part of the signals together with the early OFF part (up to $1/K_{OFF}$ ms). To take into account the existence of the fast equilibrating calcium pool, the K_{OFF} values were multiplied by the corresponding F factor and plotted as a function of the integrals and the data points were fitted using the equation

$$F K_{OFF} = k_{ns} + k_{s\,max} \exp\{\int [Ca^{2+}]\,dt\,(-k_{s\,max}/N)\} \tag{4}$$

where $k_{s\,max}$ is the maximum rate of the saturable uptake, while the other symbols bear their usual meaning. It follows from Eq. (3) that the saturable uptake rate (k_s) is maximal when $Ca_{sat} = 0$. In this case

$$k_{s\,max} = k_{ON} N \tag{5}$$

while k_s at a given time of a depolarizing pulse can be obtained from Eq. (4) using the running integral of that particular calcium transient. On the basis of the above considerations, Eqs. (2) and (3) may be simplified:

$$dU/dt = (k_{ns} + k_s)[Ca^{2+}]. \tag{6}$$

This method of calcium removal calculation is illustrated in Fig. 3.

Determination of k_{OFF}

Omission of k_{OFF} from the analysis makes method 2 suitable for calculating

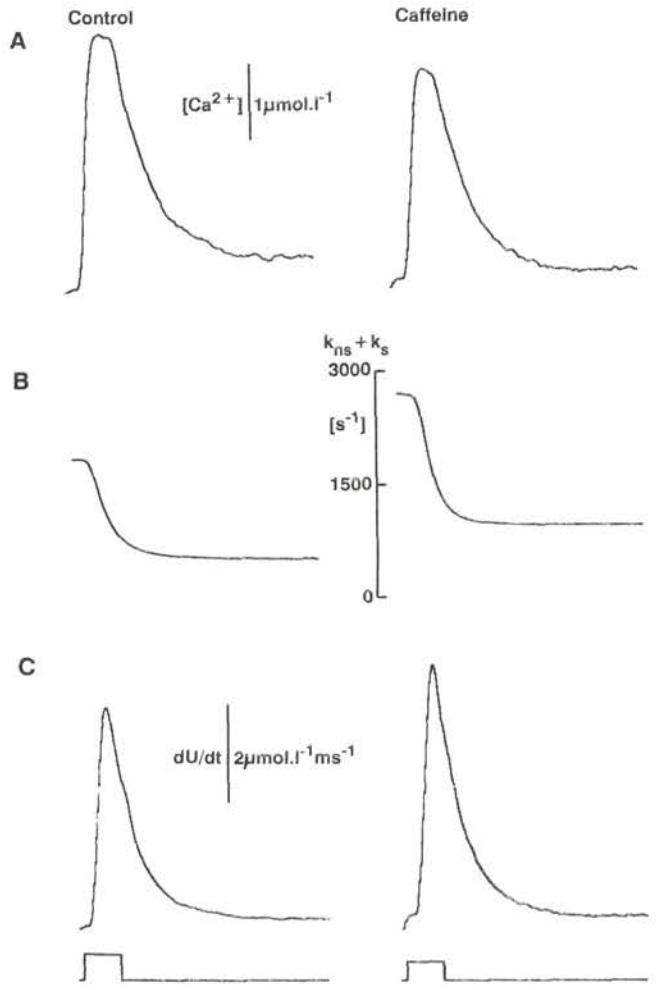

Fig. 3. Calcium removal calculation supposing $k_{OFF} = 0$ without caffeine and after the application of the drug (0.5 mmol $.l^{-1}$). The upper panel (*A*) shows myoplasmic calcium transients evoked by 40 ms depolarizing pulses (indicated at the bottom) to −23.7 mV under control conditions, and to −42.6 mV in the presence of caffeine. The middle panel (*B*) illustrates how the sum of the saturable and non-saturable reuptake rate ($k_{ns} + k_s$) changes during the sampled period. The lower panel (*C*) gives the change of the overall removal rate (dU/dt), calculated point by point as a product of the myoplasmic calcium transients (*A*) and the combined uptake rate time course (*B*). Same fiber as in Fig. 2.

intracellular calcium movements only at the membrane potential to which the fiber was depolarized by the pulses of different duration (Melzer et al. 1987). To

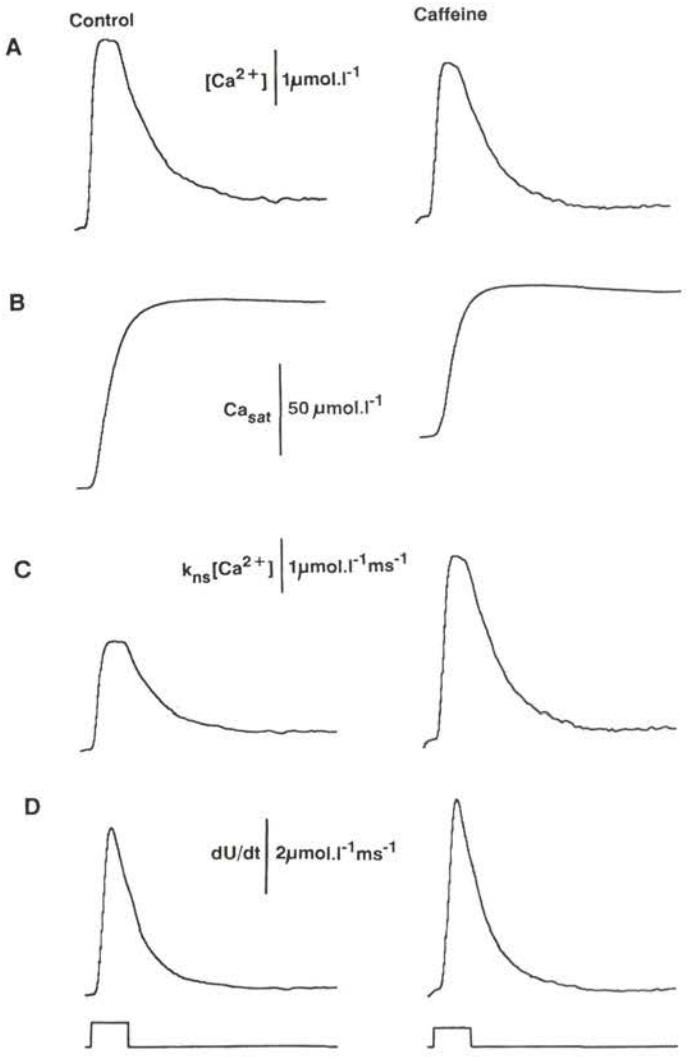

Fig. 4. Calcium removal analysis supposing backflow of calcium from the saturable binding sites under control conditions and following 0.5 mmol $.l^{-1}$ caffeine treatment. Panel *A* shows the same myoplasmic calcium transients as in Fig. 3. Panel *B* gives the amount of calcium bound to the sites representing the saturable uptake (Ca_{sat}). Panel *C* represents the rate at which the calcium is taken back by the non-saturable uptake component. These curves were calculated by multiplying the myoplasmic calcium transients by the corresponding k_{ns} value. Panel *D* illustrates the time course of the overall removal rate, calculated as the time derivative of Ca_{sat} (panel *B*) plus $k_{ns}[Ca^{2+}]$ (panel *C*). Same fiber as in Figs. 2 and 3.

Table 1. Parameter values of the removal system

Fiber ref.	k_{ns} s^{-1}	N $\mu mol \cdot l^{-1}$	k_{ON} $\mu mol \cdot s^{-1}$	k_{OFF} s^{-1}	Dye $\mu mol \cdot l^{-1}$
51014	650.3	83.5	20.55	2.28*	464—534
51016	1080.0	66.3	52.00	2.94*	488—655
51017	672.0	79.9	21.71	0.50	274—339
51023	596.8	52.2	15.23	0.08	405—553
51031	249.6	145.7	5.19	0.93	346—400
51112	638.3	54.1	25.30	0.65	378—434
51113	858.7	85.2	38.08	1.04	347—387
51114	711.9	69.0	25.83	0.93	427—484
51119	781.6	77.8	15.69	0.60	371—427
51120	396.4	115.9	12.51	0.96	363—410
Mean	663.6	83.0	23.21	1.09	
±S. E.	72.7	9.0	4.26	0.27	

"Dye" represents the change of Antipyrylazo III concentration while the depolarizing pulses of constant amplitude and different durations were applied. Mean ±S. E. value for k_{OFF} when two exceptionally high values (marked with × in the Table) are omitted: 0.71 ± 0.11 s^{-1}.

achieve a more comprehensive characterization of the calcium removal, we determined k_{OFF} from the individual myoplasmic calcium transients. During repolarization, when calcium is no longer released from the SR, the change in $[Ca]_F$ can be described using Eqs. (1), (2) and (3) as

$$d[Ca]_F/dt = -\{k_{ns} + k_{ON}(N - Ca_{sat})\}[Ca^{2+}] + k_{OFF} Ca_{sat}. \quad (7)$$

It is reasonable to suppose that at the end of the recording the final myoplasmic calcium level (Ca_{OFF}) reflects a state where the saturation of the binding sites is not changing (i.e. $Ca_{sat} = Ca^*_{sat}$ = constant). In this case

$$Ca_{OFF} = k_{OFF} Ca^*_{sat}/\{k_{ns} + k_{ON}(N - Ca^*_{sat})\} \quad (8)$$

and

$$F K_{OFF} = k_{ns} + k_{ON}(N - Ca^*_{sat}). \quad (9)$$

In Eqs. (5), (8) and (9) all the parameters can be determined either directly from the calcium transients (K_{OFF}, Ca_{OFF}) or using Eq. (4) (k_{ns}, $k_{s\,max}$, N). This allows the calculation of Ca^*_{sat}, k_{ON} and k_{OFF}, which, in turn, enables the evaluation of the calcium removal by integrating numerically Eq. (3) and using Eq. (2). Fig. 4 illustrates this version of the analysis in which the extent of the non-saturable uptake and the amount of calcium bound to the saturable binding sites is calculated separately.

Evaluation of the uptake parameters under control conditions

The removal system was characterized on 10 fibres, the parameter values obtained during the analyses are given in Table I. In our experiments we were not able to determine F_i in each and every fiber, consequently, a constant value of 15 was applied throughout. This value was found in previous control experiments (L. Csernoch, L. Kovács, G. Szücs, unpublished observation) and is close to that reported by Melzer et al. (1986). We can conclude that despite the simplifications, our k_{ns}, N and k_{ON} parameter values are in agreement with those obtained by Melzer et al. (1986) who used a more sophisticated fitting method. The same conclusion does not hold for k_{OFF} which was more than three times higher in our experiments.

Effects of caffeine on the removal system

Evaluation of the parameter values of the uptake components both before and after the application of 0.5 mmol . l^{-1} caffeine was succesful in 5 fibres. Calcium concentration changes in the fast equilibrating pool obtained in a typical experiment are shown in Fig. 1. Under control conditions depolarizations to −13.4 mV were applied to evoke the calcium signals with different pulse durations. In the presence of caffeine the transients at this membrane potential grew considerably, so the fiber was depolarized to −32.6 mV only. To get $[Ca]_F$ signals, the myoplasmic Ca^{2+} concentration changes calculated from the ΔI records were multiplied by the corresponding F values. When determining F for a given transient, only changes in the dye concentration were taken into account (F_D) and the constant F_i value of 15 was used also for caffeine-treated fibres.

Fig. 1 shows that the calcium concentration changes in the fast equilibrating pool were the same before and after the application of caffeine despite the fact that in the latter case the depolarizing pulses were smaller by approximately 20 mV. With this protocol characteristics of the removal were studied at the same degree of saturation and supposing a similar depletion of the calcium stores. Recording of both the control and the caffeine-modified signals was done as close to the solution change as possible to minimize any unknown effect of increasing dye concentration on the myoplasmic calcium transients.

Fig. 2 illustrates how the decay rate constants of calcium transients recorded in another experiment depended on the amount of the calcium released. The application of 0.5 mmol . l^{-1} caffeine increased the decay rate of the calcium transients indicating a stimulating effect on the calcium removal. Percentile changes in parameter values of the uptake components are given in Table II. The k_{ns} rate constant of the non-saturable uptake increased by approximately 51 % while the concentration of the saturable binding sites decreased by about 36 %. The k_{OFF} rate constant remained unchanged following caffeine treatment, k_{ON} increased by about 23 %, but this change did not prove to be statistically significant.

Table 2. Effects of 0.5 μmol.l^{-1} caffeine on the removal parameters

Fiber ref.	k_{ns}	N	k_{ON}	k_{OFF}	Dye μmol.l^{-1}
51016	133.2	80.1	85.5	128.2	892—996
51113	103.6	33.2	122.9	86.5	488—517
51114	235.9	46.5	181.4	43.0	581—627
51119	76.5	82.4	58.0	156.7	552—580
51120	206.6	77.5	166.7	91.7	547—572
Mean	151.2	63.9	122.9	101.2	
±S.E.	30.3	10.1	23.4	19.4	
p	n.s.	< 0.05	n.s.	n.s.	

Parameter values obtained in the presence of the drug are expressed as percentages of the corresponding values measured under control conditions. Statistical analysis was done using the original absolute data. "Dye" gives the change of Antipyrylazo III concentration when the depolarizing pulses were applied following caffeine treatment.

To illustrate the caffeine-induced changes of the calcium removal, Fig. 3 shows an example of the analysis in which k_{OFF} was supposed to be 0. The upper panel (*A*) gives myoplasmic [Ca^{2+}] transients the peak value of which is only moderately smaller in the presence of the drug, although the depolarizing pulse was smaller by about 20 mV. The middle panel (*B*) shows the sum of the saturable and non-saturable uptake rates as a function of time. The combined uptake rate showed a higher initial value but decreased to approximtely the same level under the caffeine action. The lower panel (*C*) indicates that in the presence of the drug the overall removal rate has a somewhat higher peak value.

Fig. 4 presents the more realistic removal calculation, starting from the same [Ca^{2+}] transients as in Fig. 3 (panel *A*). The saturable uptake is shown as the change in the amount of the calcium bound to the intracellular sites representing this uptake component (Ca_{sat}; panel *B*). Under the influence of the drug, there is a higher presaturation of the binding sites indicating a smaller free capacity. On the contrary, the non-saturable uptake brings back more calcium in the presence of caffeine (panel *C*). The sum of the two uptake components gives the overall removal rate (panel *D*), which allows essentially the same conclusion as in Fig. 3.

Comparison of the dU/dt records calculated by the two approaches (not shown) revealed that k_{OFF} affects only the later part of the transients. By this time the relative importance of the calcium backflow becomes greater because the non-saturable uptake decreases, but the saturable binding sites are still occupied. The reappearance of some Ca^{2+} in the myoplasm has to be balanced by a somewhat higher removal rate when k_{OFF} is supposedly 0.

The above results suggest that caffeine hardly modifies the kinetic features of the saturable binding sites but reduces their concentration, and this results in a decrease of the saturable uptake capacity. The lower concentration of the available binding sites in the presence of caffeine can be explained by supposing an elevation of the resting myoplasmic [Ca^{2+}] concentration and consequently, a higher resting saturation of the binding sites. The behavior of the non-saturable removal component exhibited a very marked fiber-to-fiber variation in the presence of caffeine. In the fiber shown in Figs. 2—4 the increase of k_{ns} overbalanced the decreased saturable uptake and this resulted in a slightly increased overall removal rate under the drug effect. A similar conclusion can be drawn on the basis of the average values presented in Table II.

Discussion

Measurement of the myoplasmic calcium concentration changes

The results presented in this paper have been based on the application of the metallochromic indicator dye Antipyrylazo III for monitoring myoplasmic calcium concentration changes. Since the introduction of this method (Kovács et al. 1979) the interpretation of the measured absorbance changes has become more and more intricate, due to problems related to the formation of calcium: dye complexes with different stoichiometry (Hollingworth et al. 1987) and to the binding of Antipyrylazo III to intracellular sites (Maylie et al. 1987). These factors seem to give rise to uncertainties in our calculations. Fortunately, a part of the possible error can be regarded as systematic, i.e. it appears as a linear scaling of the calcium transients, and consequently, does not cause severe complications in interpreting the caffeine effects.

Non-linear distortion of the calcium signals can be brought about by the increasing intracellular dye concentration during the experiment. To minimize this effect, we tried to keep the duration of the measurements as short as possible and to choose a pulse sequence which makes the comparisons more reliable. Moreover, during the subsequent analysis of the transients, the change of the Antipyrylazo III concentration was taken into account.

When discussing possible errors of the method, the interaction between caffeine and the dye must also be considered. In fact, there are data suggesting the formation of caffeine-Antipyrylazo III complex, but the difference spectrum of this reaction is flat in the range where calcium-specific absorbance changes are recorded (Best and Abramcheck 1985). Furthermore, any decrease of the free dye concentration due to the complexation with caffeine would lead to an underestimation of the myoplasmic calcium transients only if the bound form

of the dye cannot react with calcium. In this case however, F_D is overestimated, so the two errors may be mutually exclusive when calculating the amount of calcium in the fast equilibrating pool. On the other hand, the caffeine-induced changes in the myoplasmic calcium transients proved to be the same regardless of whether measured with Antipyrylazo III or with fura-2 (Simon et al. 1989); so the caffeine-Antipyrylazo interaction does not seem to introduce errors relevant to the evaluation of the calcium transients.

Modelling of myoplasmic calcium removal

Our method for analysing intracellular calcium movements is the same as that introduced by Melzer et al. (1986, 1987). We calculate the same removal parameters but in a slightly different way, combining the original methods 1 and 2. We chose this approach because we were not able to use an overall fitting procedure. The parameter values presented in this paper are generally consistent with those reported by Melzer et al. (1986) although k_{OFF} is more than three times higher in our case.

There are several possibilities to account for this difference. To get characteristic k_{OFF} for a given fibre, we treated the individual calcium transients separately and averaged the calculated values as a final step. During the determination of Ca_{OFF} one has to bear in mind that the late part of the calcium transients may be biassed by intrinsic absorbance changes, movement artifacts, changes in myoplasmic Mg^{2+} concentration and slow drifts of the track-and-hold amplifier. The relative contribution of these possible sources of error may vary from fiber to fiber and from signal to signal depending, for example, on stretching. Consequently, the correction method cannot always entirely abolish distortion of the signals and this may lead to an overestimation of k_{OFF}. The importance of the fiber-to-fiber variation is rendered likely by the fact that ignoring two exceptionally high k_{OFF} values in Table I brings our data close enough to those published by Melzer et al. (1986). Despite all the above uncertainties, sarcoplasmic calcium release waveforms calculated on the basis of our removal analysis showed close resemblance both in their absolute magnitude and in their time course to those reported previously, proving the validity of our method (L. Csernoch, L. Kovács, B. Nilius, G. Szücs, unpublished observation).

Calcium removal in the presence of caffeine

Caffeine does not substantially change the overall removal of calcium from the myoplasmic space because it decreases the saturable uptake capacity but usually increases the non-saturable uptake rate. Although the identification of the model parameters with biochemical processes is still questionable, the change of k_{ns} may reflect a modification of the SR calcium pump. There are data indicating

an inhibitory effect of caffeine on the calcium pump (Weber 1968; Weber and Herz 1968; Sorenson et al. 1986) although species differences and the use of higher caffeine doses render the interpretation difficult. On the other hand, the caffeine-induced increased resting myoplasmic Ca^{2+} concentration (see below) may exert a stimulating effect on the SR calcium pump (Kovács et al. 1989). The different combinations of these opposing effects may explain the non-definite caffeine effect on the non-saturable uptake rate.

The decrease of the saturable uptake seems to be a consequence of the smaller number of available binding sites following caffeine treatment. This effect of the drug can be explained supposing an elevation of the resting myoplasmic $[Ca^{2+}]$ concentration of about 20.8 ± 5.1 nmol.l^{-1} (estimated from the parameter values given in Tables I and II). Such an increase is consistent again with a part of previous data (Konishi et al. 1985; Konishi and Kurihara 1987; Simon et al. 1989). Considering the magnitude of the reported calcium concentration changes, the reason for studies with negative results (Lopez et al. 1983; Delay et al. 1986) may be, among others, insufficient sensitivity of the applied methods.

The possible immediate cause of the higher resting Ca^{2+} level may be a calcium release-inducing effect of caffeine (e.g. Weber and Herz 1968; Su and Hasselbach 1984) although variations in the experimental conditions apparently cause contradictions. Recent data (Simon et al. 1989) suggest that such an increased calcium outflow is connected to the appearance of the Ca^{2+}-induced Ca^{2+} release mechanism following caffeine treatment.

In conclusion, the effects of caffeine on the myoplasmic calcium removal in skeletal muscle fibres can be understood supposing a higher resting Ca^{2+} concentration. This change may explain both the decreased saturable and the increased non-saturable uptake capacity. The balance of the two opposite effects may be, however, influenced by other factors, resulting in a considerable variability of the non-saturable uptake rate. As a net result of the different actions, the overall calcium removal rate is not changed markedly following caffeine treatment.

Acknowledgements. We are indebted to Miss R. Őri for skilled technical assistance. This work was sponsored by the Hungarian OTKA Grant (No. 119).

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Final version accepted October 31, 1989