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A possible role of sarcoplasmic Ca^{2+} release in modulating the slow Ca^{2+} current of skeletal muscle

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Abstract. Ca^{2+} channels are regulated in a variety of different ways, one of which is modulation by the Ca^{2+} ion itself. In skeletal muscle, Ca^{2+} release sites are presumably located in the vicinity of the dihydropyridine-sensitive Ca^{2+} channel. In this study, we have tried to investigate the effects of Ca^{2+} release from the sarcoplasmic reticulum on the L-type Ca^{2+} channel in frog skeletal muscle, using the double Vaseline gap technique. We found an increase in Ca^{2+} current amplitude on application of caffeine, a well-known potentiator of Ca^{2+} release. Addition of the fast Ca^{2+} buffer BAPTA to the intracellular solution led to a gradual decline in Ca^{2+} current amplitude and eventually caused complete inhibition. Similar observations were made when the muscle fibre was perfused internally with the Ca^{2+} release channel blocker ruthenium red. The time course of Ca^{2+} current decline followed closely the increase in ruthenium red concentration. This suggests that Ca^{2+} release from the sarcoplasmic reticulum is involved in the regulation of L-type Ca^{2+} channels in frog skeletal muscle.

Key words: Ca^{2+} release – Sarcoplasmic reticulum – Slow Ca^{2+} current – Skeletal muscle

Introduction

L-type Ca^{2+} channels are present in cardiac, smooth and skeletal muscle cells as well as in neurons. They are characterised by a high voltage threshold for activation and their sensitivity to dihydropyridines, which led to their classification as dihydropyridine receptors. Ca^{2+} channels are regulated by many hormones, neurotransmitters and a variety of intracellular second messengers including Ca^{2+} itself (review [38]). The effects of Ca^{2+} on the activity of the dihydropyridine-sensitive Ca^{2+} channels can be subdivided into both positive and negative feedback mechanisms. It has been shown that Ca^{2+} can cause a down-regulation of the Ca^{2+} channel activity by enhancing the rate of channel inactivation [13, 18, 24]. On the other hand, a rise in the intracellular Ca^{2+} concentration can produce a reversible potentiation of the L-type Ca^{2+} current in cardiac and smooth muscle [20, 33, 34]. It has been suggested that the release of Ca^{2+} from intracellular stores and a subsequent activation of a Ca^{2+}- and calmodulin-dependent protein kinase, which causes phosphorylation of the channel molecule, is involved in this up-regulation process [20, 34].

In skeletal muscle fibres, dihydropyridine-sensitive Ca^{2+} channels are found mainly in the membrane of the transverse tubular system [1]. Dihydropyridine receptors function not only as Ca^{2+} channels but also as voltage sensors in excitation/contraction coupling and give rise to intramembraneous charge movement (for a recent review of the similarities between L-type Ca^{2+} channels and skeletal muscle voltage sensors see [27]). At the so-called triadic junctions, the t-tubules are in close association with the terminal cisternae of the sarcoplasmic reticulum (SR), the intracellular Ca^{2+} store. Here, the membranes of both systems are separated only by a narrow, 10- to 20-nm wide gap. Ca^{2+} release channels (ryanodine receptors) are localised in the SR membrane facing this *junctional* gap [40]. Upon depolarization, Ca^{2+} release from the SR causes a rapid increase in the Ca^{2+} concentration, which should occur initially in the lumen of the junctional gap and subsequently in the entire myoplasmic space. Since this elevation of the intracellular Ca^{2+} concentration produced a clear potentiation of L-type Ca^{2+} current in both cardiac and smooth muscle it was of interest to investigate whether a similar mechanism is present in skeletal muscle fibres.

In the present study, we have investigated a possible regulatory role of Ca^{2+} on the skeletal muscle Ca^{2+} current by both facilitating and inhibiting sarcoplasmic Ca^{2+} release as well as by using a fast buffer to prevent

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a rapid increase in the myoplasmic Ca^{2+} concentration. Our results suggest that while an increased Ca^{2+} concentration does indeed cause potentiation of the Ca^{2+} current, preventing release or buffering the Ca^{2+} concentration to very low levels leads to its depression.

Some of these results have been presented in abstract form [16].

Materials and methods

Single skeletal muscle fibres were isolated from the semitendinosus muscle of frogs (*Rana esculenta*) which had been killed by decapitation. Following procedures outlined in a previous paper [14] fibre segments were mounted at slack length and voltage-clamped in a double Vaseline gap chamber [26]. The experimental temperature ranged between 16°C and 19°C.

The external solution used in the experiments was of the following composition (in mM): $Ca(CH_3SO_3)_2$ 10, tetraethylammonium methanesulphonate (TEA-CH_3SO_3) 120, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) 2, 4-aminopyridine 1, tetrodotoxin 3.1×10^{-4} (pH 7.4). The cut fibre ends were exposed to the internal solution containing (in mM): caesium glutamate 50, tetraethylammonium glutamate 30, $MgCl_2$ 6.1, CsHEPES 10, Cs_2EGTA 20, Na_2ATP 5, glucose 5.6, antipyrylazo III (APIII) 0.4 (pH 7.0). The free magnesium concentration was calculated to be 1.0 mM in this solution. APIII was used to monitor the myoplasmic Ca^{2+} transients in a rectangular field of the fibre including about 50 sarcomeres. Under our experimental conditions, these spatially averaged Ca^{2+} transients were generally too small to be detectable.

When using caffeine, we added the drug in a concentration of 0.5 mM to the external solution; higher concentrations (0.7–2.0 mM) were also tried but resulted generally in contractures and subsequent deterioration of the fibre despite the intracellular presence of 20 mM EGTA. When the Ca^{2+} release blocker ruthenium red (RR) was added at a concentration of 1.0–4.0 mM, it was found to cause precipitation of the Ca^{2+} indicator dye APIII, which was therefore omitted from the internal solution in these experiments. In the BAPTA experiments, EGTA was replaced by this chelator at either 10 mM or 20 mM. The total $MgCl_2$ concentration was 6.1 mM or 6.4 mM, respectively, under these conditions in order to maintain the free Mg^{2+} concentration constant.

In all RR and some of the BAPTA experiments (6 out of 11) as well as the control experiments (3 out of 5), 0.5 mM anthracene-9-carbonic acid was added to the internal solution in order to block chloride channels. However, the total membrane current remained essentially unaffected in the presence of anthracene-9-carbonic acid, indicating that the chloride currents were rather small under our experimental conditions.

The procedures used for electrical recording and data acquisition were identical to those described in detail in previous papers [14, 15]. In brief, the effective linear capacitance was determined at the beginning of an experiment at a holding potential of 0 mV by 100-ms pulses to +60 mV. During the RR and BAPTA experiments 100-ms pulses from −80 mV to −100 mV were applied at different times in order to monitor changes in the total fibre capacitance. Current signals were filtered by an eight-pole Bessel filter set at a corner frequency appropriate for the digital sampling rate, which ranged from 0.1 kHz to 1 kHz. Recording was started about 10 min after setting the holding potential to −80 mV; this was about 40–70 min after the cut fibre ends were exposed to the internal solution. To correct currents for linear leak and capacitive transients the −P/4 method was used. Control records were averages of eight sweeps applied at intervals of about 5 s while test records were single sweeps.

As previously stated [17] the leak-corrected current consisted of an initial rapid inward phase (see also [3, 4, 10, 21]) followed by a slower and larger phase, which corresponds to the well-characterised dihydropyridine- and phenylalkylamine-sensitive Ca^{2+} current [1, 43]. Only the slow component was affected by the procedures described here and evaluated. In Figs. 2 and 3 the current remaining after complete block of the slow component was subtracted from all records. However, omitting this correction for the fast phase did not affect the results qualitatively.

In the RR experiments we monitored the diffusion of this compounds to the central part of the fibre by photometrical recording of its absorbance; the experimental layout and the recording procedures were similar to those described in earlier publications [14, 26]. However, in the case of BAPTA a direct absorbance measurement was not possible since its spectrum shows a maximum in the ultraviolet range (between 254 nm and 274 nm depending on the intracellular Ca^{2+} concentration; [46]), which was not accessible by our photometric apparatus. Therefore, the increase in the APIII concentration was used to give a rough estimate of the diffusion of the BAPTA-containing internal solution. As described previously [14, 26], the absorbance of both APIII and RR was derived from measurements of transmitted light intensities at 550 nm in the absence and presence of the muscle fibre and corrected for the intrinsic fibre absorbance recorded at 850 nm (where the absorbance of both compounds is negligible; [26] and, for RR, our own cuvette measurements). The concentration was then calculated according to Beer's law using a molar absorption coefficient at 550 nm ($\varepsilon_{550\,nm}$) of $2.55\times10^4\,M^{-1}\,cm^{-1}$ (for APIII; [35]) and $6.3\times10^4\,M^{-1}\,cm^{-1}$ (for RR) respectively. The molar absorption coefficient of RR was derived using the $\varepsilon_{533\,nm}$ of $6.8\times10^4\,M^{-1}s^{-1}$ given by Luft [30] and the ratio between the absorbance at 550 nm and 533 nm. The concentration of both dyes has been referred to myoplasmic water under the assumption that only 70% of the fibre volume was accessible for the dye [5].

Results

Effect of caffeine on the L-type Ca^{2+} current

Figure 1 A demonstrates the current response before and after the application of 0.5 mM caffeine to the bath solution. This concentration of the drug did not produce measurable Ca^{2+} transients under our experimental conditions although it has been shown to facilitate the depolarization-induced Ca^{2+} release when only small concentrations of a chelator were present internally [12, 25, 44].

As can be seen in Fig. 1, in the presence of 0.5 mM caffeine, Ca^{2+} currents were markedly increased. Current potentiation was particularly prominent at more negative membrane potentials: at −20 mV, the average increment in five fibres was by a factor of 2.57 ± 0.57 (mean ± SEM, see Table 1), at −10 mV by a factor 1.70 ± 0.21 and at 0 mV only by a factor of 1.50 ± 0.40.

At more positive potentials the effect of caffeine became more and more obscured by both the activation of an outward current and the reduction in driving force for Ca^{2+}. In addition to the enhancement of the current amplitude, the kinetics of the slow Ca^{2+} current appeared to be altered in the presence of caffeine. Its time course was accelerated, an effect that was also most noticeable at negative membrane potentials. Moreover, the threshold of current activation was shifted towards more negative potentials: in the presence of caffeine, Ca^{2+} currents appeared between −30 and −40 mV, whereas under control conditions the threshold potential for Ca^{2+} current activation was between −20 mV and −30 mV.

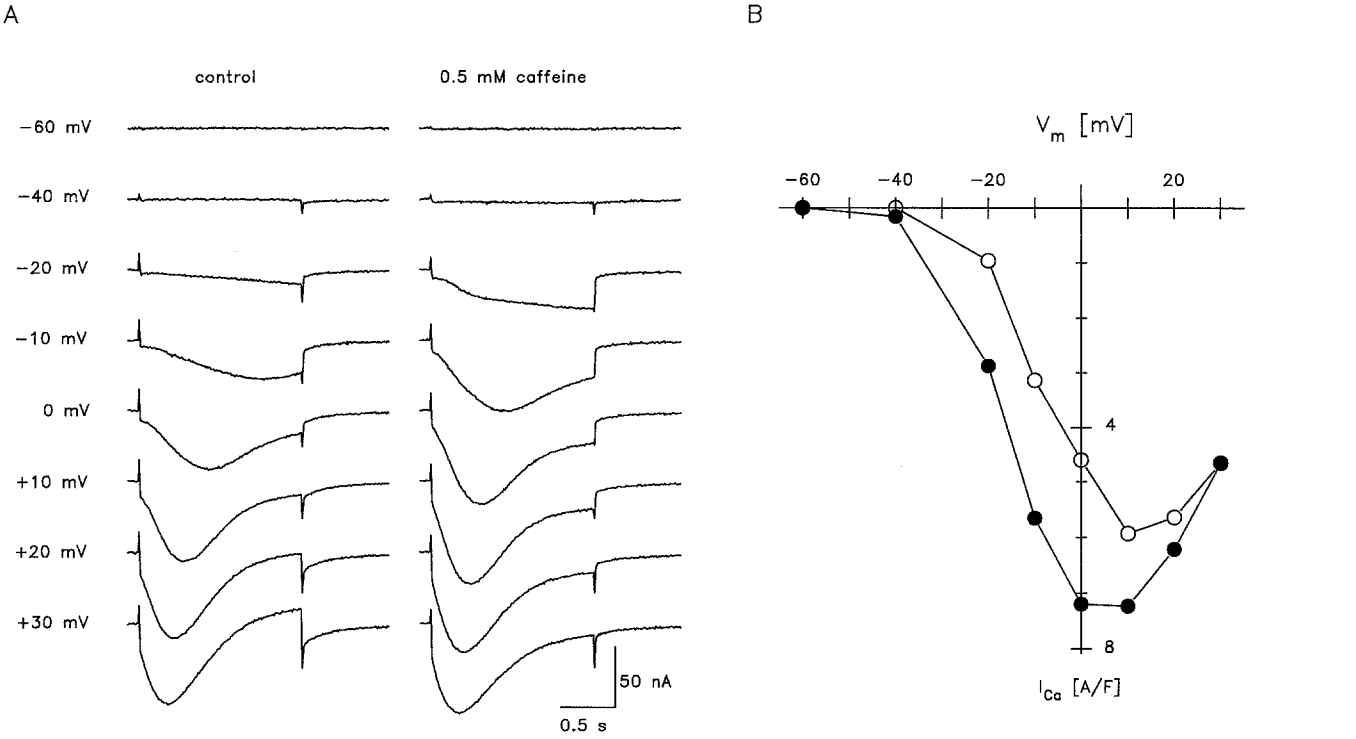

Fig. 1 A, B. Effect of caffeine application on the L-type Ca^{2+} current records in frog skeletal muscle fibres. **A** Ca^{2+} currents were elicited by depolarizations lasting 2 s from a holding potential of −80 mV to potentials ranging between −60 mV and + 30 mV; records on the *left* were obtained in control saline and those on the *right* in the presence of 0.5 mM caffeine. **B** Current/voltage relationship before (○) and after (●) caffeine application; peak current amplitudes were normalised to the fibre capacitance. Fibre 244, segment length 397 µm, diameter 128 µm, temperature 18.0°C

Table 1. Potentiation of Ca^{2+} current amplitude[a]

Fibre no.	Control (nA/nF)	0.5 mM caffeine (nA/nF)	Potentiation
243	0.50	2.42	4.84
244	0.96	2.88	3.00
245	1.13	2.33	2.06
247	1.35	2.44	1.81
248	1.79	2.05	1.15
Mean ± SEM	1.15 ± 0.19	2.42 ± 0.12	2.57 ± 0.57

[a] Currents were elicited by depolarizations from a holding potential of −80 mV to −20 mV. The fast, dihydropyridine-insensitive component of the current has been subtracted and the current normalised to the fibre capacitance

However, in most cases the peak current potential was unaltered at + 10 mV. Although the effect of caffeine on the Ca^{2+} current appeared to be at least partially reversible, washout of caffeine was generally accompanied by an increase in the holding current, which prevented us from studying the reversibility of the caffeine effect.

In a few experiments we attempted to use higher concentrations of caffeine (0.7−2.0 mM). In all these cases a further shift in the threshold for Ca^{2+} current activation towards more negative potentials was observed. However, because of the vigorous contracture of the fibre segment − caused by the caffeine-induced SR Ca^{2+} release − and the concomitant fibre damage, we were not able to study the current potentiation in more detail.

Effects of BAPTA on the L-type Ca^{2+} current

While the resting Ca^{2+} concentration can be kept low by applying EGTA, a rapid Ca^{2+} input is buffered less effectively by this chelator owing to its slow binding kinetics [41]. Therefore, Ca^{2+} release from the SR may still produce significant Ca^{2+} transients in the junctional gap despite the presence of EGTA. To improve "kinetic" buffering, the chelator BAPTA was substituted for EGTA in the internal solution. BAPTA has faster Ca^{2+}-binding kinetics than EGTA [46] and is thus able to suppress effectively the rapid initial part of the intracellular Ca^{2+} transients, which is not markedly affected by EGTA [41]. Jacquemond et al. [22] report that BAPTA also affects Ca^{2+} release from the SR by eliminating the initial peak of the rate of release but left the maintained steady level unaffected. When BAPTA was present in the internal solution a considerable decrease in Ca current amplitude was observed. At a concentration of 10 mM, BAPTA caused a partial inhibition ($n = 4$), and at 20 mM (as in Fig. 2) a complete inhibition of the Ca^{2+} current ($n = 5$), leaving only the fast current unaffected. In two further experiments with 20 mM BAPTA no Ca^{2+} currents could be elicited, indicating that a complete block was already established at the start of the experiment (which was 46 min and 70 min after exposing the cut fibre ends to the BAPTA-containing internal solution, respectively).

The experiments of the kind shown in Fig. 2 were designed to monitor the time course of current decline

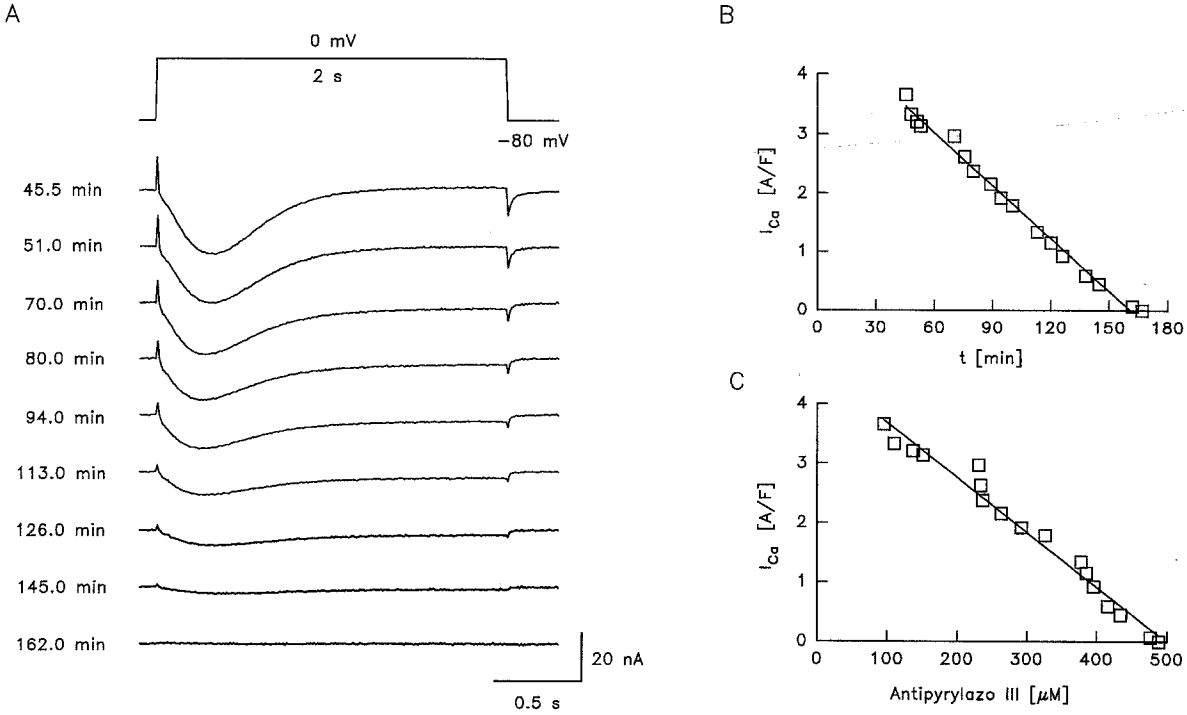

Fig. 2 A–C. Effect of intracellular application of 20 mM BAPTA on the slow L-type Ca^{2+} current. **A** Slow Ca^{2+} currents caused by depolarizing steps from −80 mV (holding potential) to 0 mV at different times (indicated on the *left*) after the cut fibre ends were exposed to an internal solution containing 20 mM BAPTA. During the diffusion of the BAPTA internal solution into the fibre segment, the current peak amplitude declined from 75.3 nA to 0. **B** Time dependence of Ca^{2+} current run-down in the presence of BAPTA-containing internal solution. The decline of the current was linearly related to the time after exposure to the BAPTA internal solution (correlation coefficient $r = 0.995$). **C** Relation between current decline and the increasing concentration of the internal solution as estimated from the intracellular antipyrylazo III concentration, which was determined by microspectrophotometry. Ca^{2+} current amplitudes show an inverse linear relationship with the increasing dye concentration ($r = 0.988$). Current amplitudes given in **B** and **C** are normalised to the capacitance. Fibre 308, segment length 522 μm, diameter 174 μm, temperature 16.1°C

while simultaneously recording the diffusion of the internal solution via the increase in the myoplasmic APIII concentration. The end-pool concentration of APIII was 0.4 mM. Ca^{2+} currents were elicited by depolarizations from −80 mV to 0 mV at intervals of at least 5 min in order to exclude insufficient restoration from Ca^{2+} current inactivation. As can be seen in Fig. 2 A, the slow Ca^{2+} current became gradually smaller with time, eventually becoming completely blocked after 162 min of exposure to BAPTA. No significant change in the current kinetics was observed. In control experiments with EGTA-containing internal solution but otherwise the same design as the BAPTA experiments, a comparable reduction in Ca^{2+} current was never observed. The ratio of the Ca^{2+} current amplitude at the start and the end ($I_{Ca,start}/I_{Ca,end}$) of these experiments (lasting between 162 min and 407 min) was 1.11 ± 0.12 (mean ± SEM, n = 5), indicating no change in current amplitude in the presence of EGTA.

The decrease in Ca^{2+} current amplitude shown in Fig. 2 was linearly related to the time of exposure to BAPTA (Fig. 2 B) with a correlation coefficient r = 0.995 as well as to the intracellular APIII concentration which served as an indicator of the diffusion of the internal solution ($r = 0.988$; Fig. 2 C). In experiments where 10 mM BAPTA was used, the reduction of the Ca^{2+} current started only after a notable lag phase during which the current amplitude remained virtually constant.

A possible explanation for these results may be a rapid depolarization-induced rise of intracellular Ca^{2+} in the vicinity of the Ca^{2+} channel, caused by Ca^{2+} release from the SR, which in turn enhances the current activation and is prevented by BAPTA but not by EGTA.

Effect of ruthenium red on the L-type Ca^{2+} current

In a second approach to interfere with intracellular Ca^{2+} release, we perfused skeletal muscle fibre segments internally with RR, which acts probably by directly blocking the SR Ca^{2+}-release channel [42]. In addition, RR has been reported to suppress optical signals related to the depolarization-induced Ca^{2+} transient in intact [6] and cut skeletal muscle fibres [11].

Internal solution containing RR as well as 20 mM EGTA was diffused into the muscle fibres through the cut ends. In agreement with earlier reports the diffusion of RR was found to be extremely slow (e. g. experiment in Fig. 3; even after 86 min of RR exposure to the cut ends none was detected in the central part of the fibre). Baylor et al. [6] report a diffusion coefficient of 0.030×10^{-6} cm^2 s^{-1} for this dye, which is about 30 times smaller than could be expected for a freely diffusible molecule of the same size. This is probably due to binding of RR to myofilaments and other myoplasmic constituents as suggested by an electron-microscopic in-

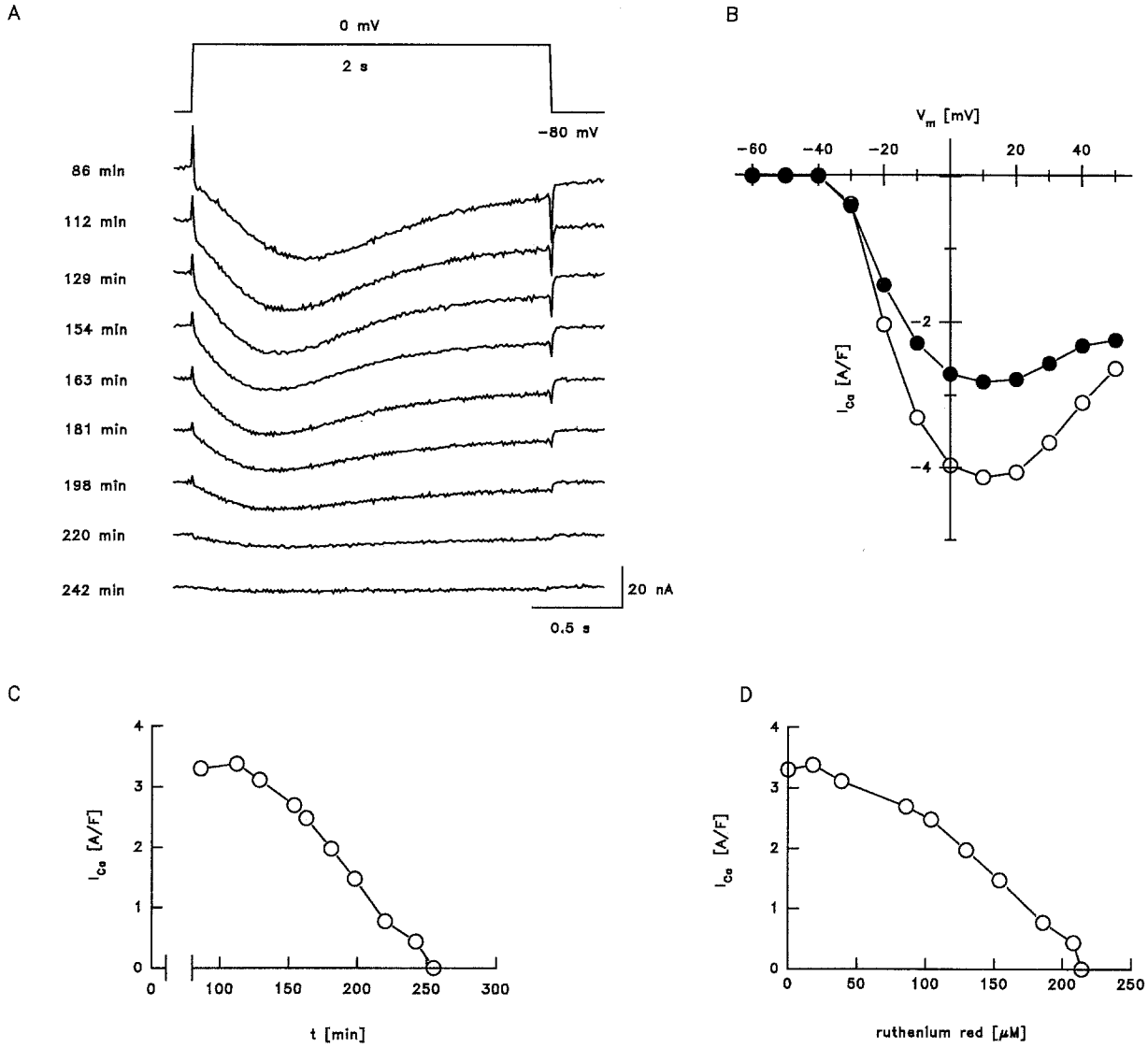

Fig. 3 A–D. Effect of ruthenium red (RR) on L-type Ca^{2+} currents in frog skeletal muscle fibres. The dye was present at 1.8 mM in the internal solution, which also contained 20 mM EGTA. **A** Ca^{2+} current records obtained by the voltage-clamp pulse shown at the top at different times (indicated on the *left*) after exposure to RR. During the 4-h period of drug application the peak amplitude of the L-type Ca^{2+} current decreased from 37.2 nA to almost 0. **B** Current/voltage relationship 100–120 min after (○) and 170–190 min after exposure to RR (●). Note the clear reduction of the Ca^{2+} current without a major change in the voltage dependence. **C** Time course of Ca^{2+} current decline in the presence of RR. The time given on the *abscissa* is the time after the application of the RR-containing internal solution. **D** Concentration dependence of current run-down. The myoplasmic RR concentrations on the abscissa were determined microspectrophotometrically in the central part of the muscle fibre using an $\varepsilon_{550\,nm}$ of $6.3\times10^4\ M^{-1}\ s^{-1}$. Current amplitudes given in **B, C** and **D** are normalised to fibre capacitance. Fibre 273, segment length 422 μm, diameter 210 μm, temperature 16.5°C

vestigation of cut skeletal muscle fibres perfused with RR for 2–3 h (Feldmeyer and Jung, unpublished results).

The experiment illustrated in Fig. 3 shows that RR caused a decrease of the slow component of the current response (the L-type Ca^{2+} current) in a fashion similar to that observed in the presence of BAPTA. This decrease was manifest at all membrane potentials (Fig. 3 B). Under control conditions without RR, a similar change in the current/voltage relationship was not observed.

As can be seen in Fig. 3 D, Ca^{2+} current reduction was clearly dependent on the RR concentration in the fibre: at low RR concentrations the current remained virtually constant; when the RR concentration exceeded 40 μM, a decline of its amplitude became noticeable. With further increase in the RR concentration, the Ca^{2+} current became progressively smaller and was blocked completely at a total myoplasmic RR concentration of 214 μM. As mentioned above, in control experiments without RR a similar reduction in the current amplitude was never observed.

In two further experiments, RR caused a decrease of the slow Ca^{2+} current to almost zero; in two other experiments, the Ca^{2+} current was already inhibited at the start of the experiment. Interestingly, in one experi-

ment RR did not diffuse into the fibre; under this conditions no decrease in the Ca^{2+} current was observed.

Discussion

The results of the present study show that various manipulations that should affect the Ca^{2+} transients caused by Ca^{2+} release from the SR modify the amplitude of the L-type Ca^{2+} current. A decrease in Ca^{2+} release, caused either by blocking the release channels with RR or by effectively buffering Ca^{2+} with BAPTA, resulted in a reduction of the Ca^{2+} current amplitude (recently confirmed by Gonzáles et al. [19]). In contrast, 0.5 mM caffeine caused an increase of the calcium current and – by revealing small currents at negative potentials – a negative shift of the voltage threshold for current activation. This low concentration of caffeine has been shown to produce a significant potentiation of the voltage-dependent calcium release from the SR [12, 25, 44] suggesting that the increase in Ca^{2+} current described here could be related to this calcium release potentiation. A possible explanation for our results is an involvement of SR Ca^{2+} release in the regulation of the activity of skeletal muscle Ca^{2+} channels. On the other hand, 20 mM EGTA, which in most fibres led to a virtual elimination of Ca^{2+} transients (as measured with the Ca^{2+} indicator APIII), affected the current only marginally. However, it seems likely that a much larger transient than can be detected photometrically persists close to the release sites of each sarcomere in the narrow gap between the transverse tubule system and SR. Strong contractures at higher caffeine concentrations (see above) indicate that the SR remained loaded with Ca^{2+} in the presence of 20 mM EGTA. Indirect evidence for Ca^{2+} binding to a site on the dihydropyridine receptor as a result of large local Ca^{2+} transients was recently provided by studying the γ-component of intramembraneous charge movement [39]. Methods for direct measurements of these local changes of Ca^{2+} are not yet available.

A well-known form of L-type Ca^{2+} current regulation in skeletal muscle is through the cAMP-dependent protein kinase [3, 36]. It may be argued that the effect of caffeine described here is associated with its inhibitory action on the cAMP-degrading phosphodiesterase and thus via the protein kinase A pathway. However, the concentrations of caffeine used in the present experiments cause only minor inhibition of the *purified* phosphodiesterase ($\approx$ 10% [7]). Therefore, it seems unlikely that caffeine acts indirectly via protein-kinase-A-dependent phosphorylation of the channel molecule.

Caffeine has been described to cause inhibition of the L-type Ca^{2+} current in a number of preparations including heart and smooth muscle cells as well as neurons [28, 29, 47]. This inhibitory effect of caffeine has been attributed to its direct blocking mechanism on the Ca^{2+} channel [29, 47] and was only partially reversible [29]. Lipp et al. [28] describe a more complex pattern for the effect of caffeine on the Ca^{2+} current: after an initial decrease the Ca^{2+} current increases again and becomes eventually larger than the control current. The authors suggest that this potentiation resulted from the slowdown of current inactivation caused by caffeine-induced Ca^{2+} release from the SR and inhibition of the SR Ca^{2+} pump. However, the caffeine concentrations used in these studies were much higher than those in the present study (5–30 mM as compared to 0.5 mM). Since low concentrations of caffeine (0.1–1 mM) resulted only in a minor reduction of the Ca^{2+} current in one of these studies [47], any direct blocking action of caffeine on the Ca^{2+} current is likely to be negligibly small under our experimental conditions.

One possible explanation for the effect of Ca^{2+} release on the Ca^{2+} current is an indirect action of Ca^{2+} on enzymes that phosphorylate the Ca^{2+} channel, as proposed by Armstrong et al. [2]. It has been suggested for both the heart and smooth muscle that a Ca^{2+}/calmodulin-dependent protein kinase (CaMPK) is involved in the up-regulation of the L-type Ca^{2+} current [20, 34]. Moreover, in inside-out patches excised from GH3 cells, CaMPK shifts Ca^{2+} channels into a mode with prolonged openings [2]. In skeletal muscle, CaMPK activity is associated with the part of the SR that faces the t-tubular membrane (*junctional* SR [9]) and is thus in the vicinity of the dihydropyridine receptor. In addition, this receptor has been shown to be a substrate of CaMPK-dependent phosphorylation [23] although it failed to cause an increased Ca^{2+} flux in experiments with reconstituted Ca^{2+} channels [8]. However, in these experiments, channels may have suffered from a loss of intracellular constituents involved in Ca^{2+} channel regulation.

Skeletal muscle Ca^{2+} channels are also subject to phosphorylation by protein kinase C [8]. Recently, it has been demonstrated that phosphorylation by this enzyme results in an up-regulation of the Ca^{2+} current by an increase in the channel-open probability [32]. The activity of protein kinase C is dependent on the intracellular Ca^{2+} concentration [37]. Thus, an increase in SR Ca^{2+} release may up-regulate this enzyme and in turn enhance the rate of channel phosphorylation and thus increase the Ca^{2+} current. Low intracellular Ca^{2+} concentrations, on the other hand, would result in a dephosphorylation of the channel and thus a reduction of the current.

Finally, it is possible that Ca^{2+} acts directly by binding to a site on the channel molecule itself. Tsien et al. [45] describe a Ca^{2+}-binding region at the carboxy-terminus of the α_1 subunit of the Ca^{2+} channel. It has been proposed that this site is involved in dihydropyridine binding but one may speculate that binding of Ca^{2+} to the site is important for maintaining channel activity. In this context it is of interest that, in order to record consistently measurable activity of *reconstituted* skeletal muscle Ca^{2+} channels, it was necessary to apply the dihydropyridine agonist BAY K8644 [8, 31]. BAY K8644 may substitute for a positive modulation by rapid local Ca^{2+} transients, caused in intact fibres by release of Ca^{2+} from the SR.

In summary, our data point to a potentiating effect of voltage-dependent Ca^{2+} release on the gating of the skeletal muscle L-type Ca^{2+} channel.

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