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Ca^{2+}-movements in muscle modulated by the state of K^+-channels in the sarcoplasmic reticulum membranes

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Abstract. 1. A procedure has been developed to load Ca^{2+} reversibly by the sarcoplasmic reticulum (SR) of mechanically skinned muscle fibres from the toad *Bufo marinus* under controlled conditions and was employed to investigate the effects of conditions known to reduce the K^+ conductance located in the SR-membrane during Ca^{2+}-loading on the amount of Ca^{2+} releasable by caffeine. 2. The amount of releasable Ca^{2+} was markedly increased compared to controls when 4-aminopyridine (4AP) (6 μM to 2 mM), tetraethylammonium (TEA), decamethonium (0.5 mM) or procaine (1 mM) were present in the Ca^{2+}-loading solutions. All these substances are known to act as SR-K^+-channel blockers. 3. The increased amount of releasable Ca^{2+} in the presence of the K^+-channel blocker 4AP was observed both at low (0.3 mM) and at higher (1 mM) Mg^{2+} concentrations and was not affected by verapamil (20 μM), a known Ca^{2+}-channel blocker of the sarcolemma nor by the Na^+-K^+ pump inhibitor, ouabain (1 mM). 4. In the presence of 0.1–5 μM ruthenium red, a known inhibitor of Ca^{2+} induced Ca^{2+} release from the SR, the amount of releasable Ca^{2+} was greatly increased by up to 300%. Addition of between 50 μM and 1.6 mM 4AP to ruthenium red Ca^{2+} loading solution modified differently the amount of releasable Ca^{2+}, suggesting that the mechanism of action of 4AP is different from that of ruthenium red. 5. When all K^+ ions in the loading solution were replaced by the less permeant Na^+ ions the amount of releasable Ca^{2+} ions was also increased. 6. These results indicate that the amount of releasable Ca^{2+} from the SR is consistently modified under conditions aimed at interfering with the state of SR-K^+-channels, suggesting that SR-K^+-channels may play an important physiological role in the modulation of excitation-contraction coupling. One possible mechanism involving SR-K^+-channels which could explain our results is discussed.

Key words: Skeletal muscle – Sarcoplasmic reticulum – K^+ conductance – Ca^{2+} loading – Skinned muscle fibres – K^+ channel blockers

Introduction

K^+ selective channels in the surface membrane of muscle and other cells are important in directly and indirectly determining many membrane-linked cellular processes (Stefani and Chiarandini 1982; Stanfield 1983). Sarcoplasmic reticulum (SR) membranes also have K^+-selective channels (Miller 1978; McKinley and Meissner 1978; Coronado and Miller 1982; Montgomery et al. 1983) and like the K^+-channels in the surface membrane of muscle, they show voltage dependent gating (Labarca et al. 1980) and can be blocked by specific organic and inorganic cations (Coronado and Miller 1982; Miller 1983). The properties of these SR-K^+-channels were mainly studied using reconstituted systems in planar-bilayer membranes and this makes it difficult to predict their behaviour in their native environment (Miller 1983). Clearly, from such experiments it is impossible to ascribe a physiological function to these SR-K^+-channels (Oetliker 1982; Miller 1983), though it has been suggested that they may act simply as passive pathways for charge compensation during Ca^{2+} release, by allowing K^+ ions to move across the SR membranes in the opposite direction to Ca^{2+} ions (Miller 1978; McKinley and Meissner 1978; Somlyo et al. 1981). In mechanically skinned fibre preparations, the sarcoplasmic reticulum is left largely intact (see e.g. Stephenson 1981 for a review) and in this study we examined the effect of decreasing SR-K^+-conductance on the Ca^{2+}-loading capacity of the SR using these preparations. The results show that the amount of releasable Ca^{2+} from the SR by caffeine is significantly increased when the conditions in the environment are changed such as to decrease the K^+-conductance of the SR membrane during Ca^{2+}-loading. The finding strongly suggests that the SR-K^+-channels may play an important physiological role in modulating Ca^{2+} movements across the SR membranes. Some of the results have been published in abstract form elsewhere (Fink and Stephenson 1984).

Methods

The experiments were carried out on twitch muscle fibres from the iliofibularis muscle of the toad *Bufo marinus*. The muscle fibres were skinned under paraffin oil, as previously described (Moisescu and Thieleczek 1978; Stephenson and Williams 1981) and mounted on a force transducer (AME 875). The length of the preparations was then adjusted to an average sarcomere length of 2.2–2.3 μm (determined by laser diffraction). The dimensions of the preparations were in the range 30–60 μm for the diameter (measured in paraffin oil saturated with water, immediately after skinning) and 1.5–3.5 mm for the length. Further details of

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the force measuring system are given elsewhere (Stephenson and Williams 1981; Fink et al. 1986a, b).

Solutions. Experimental solutions were prepared in the manner described in detail previously (Ashley and Moisescu 1977; Moisescu and Thieleczek 1978; Stephenson and Williams 1981) and contained (mM) Na^+ 36; K^+ 125; Hepes (N-2-hydroxyethyl-piperazine-N′-2-ethanesulfonic acid) 60; ATP 8; creatine phosphate 10; creatine kinase 15 U/ml; ionic strength ($I/2$) 233; pH 7.10 ± 0.01 at room temperature (22–25° C). Three basic types of solutions were used: high relaxing (HR) solution which contained 50 mM $EGTA^{2-}$ (ethylene glycol bis (β-aminoethyl ether) N,N′-tetraacetate), low relaxing (LR) solution which contained 49.9 mM $HDTA^{2-}$ (hexamethylene diamine-N,N,N′,N′-tetraacetate) and 0.1 mM $EGTA^{2-}$, and maximum activating solution (HA) which contained 50 mM $CaEGTA^{2-}$ (pCa = $-\log [Ca^{2+}] \sim 4.7$). The apparent affinity constants used for calculation of the free ionic concentrations were those previously determined in this laboratory (Stephenson and Williams 1981; Fink et al. 1986a). The concentration of ionized Mg^{2+} in solutions was either 0.3 mM or 1 mM to cover the range of concentrations reported for intact amphibian twitch fibres (Hess and Weingart 1981; Baylor et al. 1982; Maughan 1983; Alvarez-Leefmans et al. 1985). All experiments were carried out at room temperature (22–25° C).

Ca^{2+} Loading and Ca^{2+} release procedure. The basic procedure by which Ca^{2+} ions were loaded into the SR and then released is outlined in Fig. 1. This procedure is a modification of the procedures which are commonly used in experiments on sarcoplasmic reticulum in skinned muscle preparations (Endo et al. 1970; Fabiato and Fabiato 1975; Stephenson 1981; Thieleczek 1982). The first release of Ca^{2+} (R1) in Fig. 1 was obtained by transferring the freshly skinned muscle fibre preparation from paraffin oil to a Ca^{2+} release solution containing 0.3 mM Mg^{2+} and 30 mM caffeine (mixture of solutions HR and LR in the proportion of 1:56). The magnitude of the force transient (force peak and area under the force transient) can be used as an indicator of the amount of Ca^{2+} stored in vivo. Soon after this force transient came close to the baseline, maximum force (P_{max}) was obtained by activation in solution HA (pCa = 4.7) with 30 mM caffeine. This step ensured that the SR had released all Ca^{2+} releasable by caffeine before starting a new loading/release cycle (Endo 1977) and also was used to correct the Ca^{2+}-release responses for slow deterioration of maximum force. After a maximal response, the fibre was relaxed for 60 s in HR solution which lacked caffeine and contained 50 mM EGTA (pCa > 9) and in which the sarcomere length was checked by laser diffraction (see Methods). Then it was equilibrated for 120 s in solution LR (0.1 mM EGTA, pCa > 8) to allow for a sudden increase in myofibrillar Ca^{2+} concentration in the next step, when the preparation was transferred into a strongly Ca^{2+}-buffered loading solution (Moisescu 1976) obtained from a mixture of solutions HR and A to give a pCa = 6.5 (standard Ca^{2+}-loading solution). At this Ca^{2+} level our preparations developed less than 10% of P_{max} and the ionized Ca^{2+} was expected to reach at least 95% of its final concentration in preparations within 0.2 s under our experimental conditions (Moisescu and Thieleczek 1978).

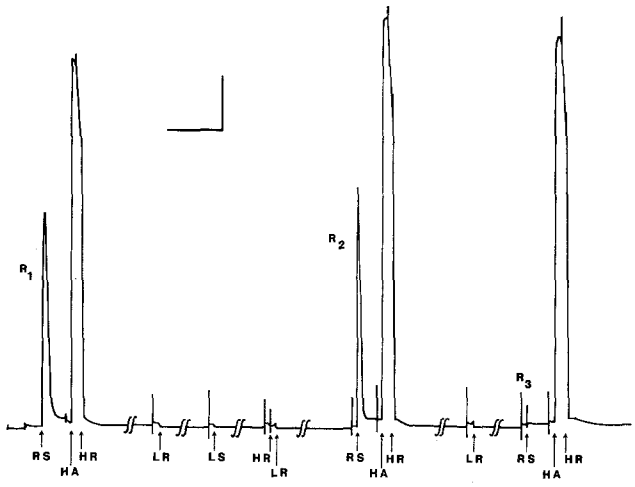

Fig. 1. Procedure for loading the sarcoplasmic reticulum with Ca^{2+} and inducing Ca^{2+} releases with caffeine in mechanically skinned muscle fibres. The trace is a pen recording of isometric force developed by a skinned toad muscle fibre. The freshly skinned preparation was transferred into a Ca^{2+} release solution (*RS*) which resulted in the first Ca^{2+} release (*R1*) due to Ca^{2+} stored in the SR before skinning. Then maximum Ca^{2+}-activated force was obtained by activating in solution (*HA*). After relaxation for 1 min in high relaxing solution (*HR*), the fibre was equilibrated for 2 min in the low relaxing solution (*LR*) to allow a sudden increase in myofibrillar Ca^{2+} concentration when the preparation was loaded for 1 min in strongly Ca^{2+}-buffered loading solution (*LS*). The loading of the SR was rapidly stopped by briefly dipping the fibre in HR solution before it was washed for 2 min in LR solution. Then the preparation was again placed in the RS solution and the ensuing amount of Ca^{2+} released (*R2*) was very similar to the first release (*R1*) judging from the magnitude of the force responses. After equilibration in LR solution for 4 min the fibre did not show a force transient (*R3*) in RS-solution. The absence of a force transient indicates that the SR significantly loaded Ca^{2+} only in the loading solution, and that no releasable Ca^{2+} was in the SR at the end of each cycle. The sarcomere spacing of the fibre was 2.2 μm, the diameter 45 μm and the length 1.9 mm. *Calibration bars*: vertical 0.1 mN; horizontal 30 s

In these experiments the sarcoplasmic reticulum was loaded for 60 s and the loading was rapidly terminated by dipping the preparation in the HR solution containing 50 mM EGTA (pCa > 9) for about 2 s. Thereafter, the preparation was equilibrated and washed for 120 s in LR solution (pCa > 8) to remove the high concentration of the Ca-EGTA buffer which was important to maintain a constant $[Ca^{2+}]$ during loading. Care was taken to keep all time intervals as accurately as possible. The second Ca^{2+} release in Fig. 1 (R2) was induced in the same caffeine containing solution as for R1. This Ca^{2+}-release (R2) was very similar in size and time course to the first release (R1), indicating that with this procedure the amount of Ca^{2+} loaded by the SR was approximately similar to that in vivo.

In order to check that the SR did not load Ca^{2+} significantly other than in the loading solution and that the SR did not contain significant amounts of Ca^{2+}, releasable by caffeine, at the end of each cycle, we routinely checked for a Ca^{2+} release after equilibration in LR solution for 4 min. As it can be seen from Fig. 1, no force transient can be elicited in the caffeine Ca^{2+}-releasing solutions under these

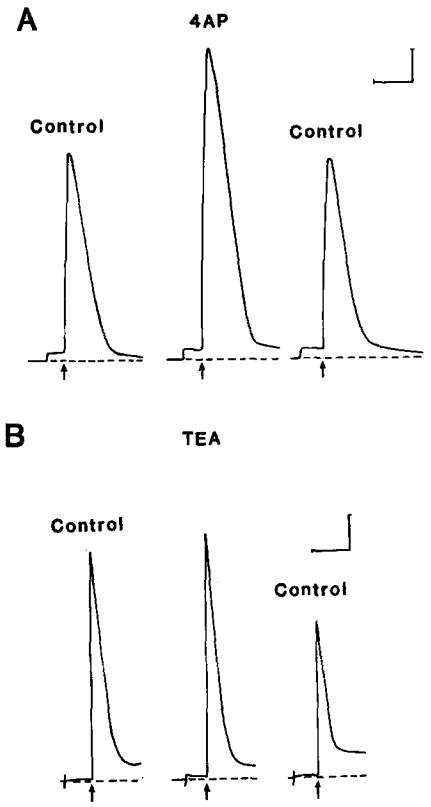

Fig. 2. Ca^{2+}-releases after loading in the presence of the K^+-channel blockers 4AP (**A**) and TEA (**B**). The protocol to load and release Ca^{2+} by sarcoplasmic reticulum was the same as described in Fig. 1 for the controls and was similar for the middle traces (for details, see Methods). Note that Ca^{2+} releases after loading in 1.6 mM 4AP (**A**) and 10 mM TEA (**B**) are all significantly higher than the control releases with the same preparation before and after the respective treatment. *Calibration bars*: vertical $10 \pm 1\%$ P_{max} for all three control maxima; horizontal 5 s

conditions. The cycle shown in Fig. 1 was finished with a maximum contraction in solution HA.

The protocol used to assess the amount of releasable Ca^{2+} loaded by the sarcoplasmic reticulum under given conditions was the same for the test experiments (middle traces in Fig. 2) and for the controls (see below and Fig. 2). In the test experiments the fibres were first equilibrated (2 min) with the blocking agents in the low relaxing solution (LR) and then transferred for 1 min into equivalent SR Ca^{2+}-loading solutions containing the respective K^+-channel blocker. For example, the solutions containing 4-aminopyridine (4 AP; Sigma, St. Louis, MO, USA) and tetraethyl ammonium (TEA; Aldrich Chemical Company, Amersham, GB) were similar to their corresponding standard solutions used for controls, with the exception that 1.6 mM and 10 mM K^+ were replaced by 1.6 mM 4AP and 10 mM TEA respectively. Decamethonium bromide (0.5 mM, Sigma, St. Louis, MO, USA) and procaine hydrochloride (1 mM, Sigma, St. Louis, MO, USA) were added directly to the Ca^{2+}-loading solutions. The loading of Ca^{2+} was always terminated by dipping the fibres into normal, blocker free HR solution and this was followed by equilibration (2 min) in the same LR solution used also for the controls (see below) before immersion in the RS solution. This was particularly important in order to avoid possible interactions of drugs with the contractile apparatus itself or with the SR during Ca^{2+}-release (e.g. block of Ca^{2+}-channels by procaine, Endo 1977).

Analysis of results. The relative amount of releasable Ca^{2+} from the SR was estimated from the areas under the force transients during the Ca^{2+} release (Endo et al. 1970; Fabiato and Fabiato 1975). The results are expressed relative to the maximum Ca^{2+}-activated force and to average control values before and after a particular response from the same fibre ($\pm$ SD). The validity of this approach was tested by running series of three successive control responses with individual fibres and by expressing the values of the middle responses as a percentage of the average control values before and after. The result obtained was $98.5 \pm 4.1\%$ ($n = 5$) indicating that the control procedured used gives an accurate estimate of the various responses under test conditions. In separate experiments we also evaluated the relative changes of the peak forces of the Ca^{2+} transients. Using the same control procedure we obtained a value of $105.4 \pm 2.5\%$ ($n = 5$) for the normalized peak forces.

Results

To test for the effect of changes in the K^+-conductance of SR membranes on the Ca^{2+}-uptake, we initially used the better known K^+-channel blockers 4-aminopyridine (4 AP) and tetraethylammonium (TEA) (Stanfield 1983; Fink and Wettwer 1978). In these experiments we first released all the releasable Ca^{2+} stored in the SR with caffeine (Fig. 1, R1 + first maximum contraction in the presence of caffeine) and then in sequence we loaded Ca^{2+} in the absence of the drug for 1 min, released it all with caffeine (control before), loaded Ca^{2+} in the presence of the drug for 1 min while maintaining all other conditions identical to the controls, released all Ca^{2+} with caffeine in the same solution as for controls (response), loaded Ca^{2+} in the absence of the drug for 1 min and released it all with caffeine (control after). This cycle (control, response, control) could be repeated several times with one preparation without alteration in the pattern of the response. The normalized peak forces (with respect to the maximum force) after loading in the presence of 1.6 mM 4AP and 10 mM TEA were greater by about 58% (Fig. 2A) and 30% (Fig. 2B) respectively than the average control values. The areas under the force transients were also greatly increased. They increased by 82% for 1.6 mM 4AP and 24% for 10 mM TEA (Fig. 2A,B). Results obtained with 8 preparations and different concentrations of 4AP are shown in Fig. 3. An important observation was that 4AP had a pronounced effect on SR-Ca^{2+}-loading starting from very low concentrations. A significant increase in Ca^{2+}-release over the control was observed after loading in the presence of 4AP at a concentration of only 6 μM (Fig. 3). The 4AP results were similar in the presence and absence of 1 mM ouabain (Fig. 3). This discards the possibility that the enhanced Ca^{2+}-uptake by 4AP may be due to changes in the residual electrical activity in fragments of the transverse tubular systems in the skinned fibre preparations (Donaldson 1985). Averaged results $\pm$ SD ($n = 3$) for experiments with 10 mM TEA were $31\% \pm 14\%$ for increases in normalized peaks and $26\% \pm 14\%$ for increases in normalized areas.

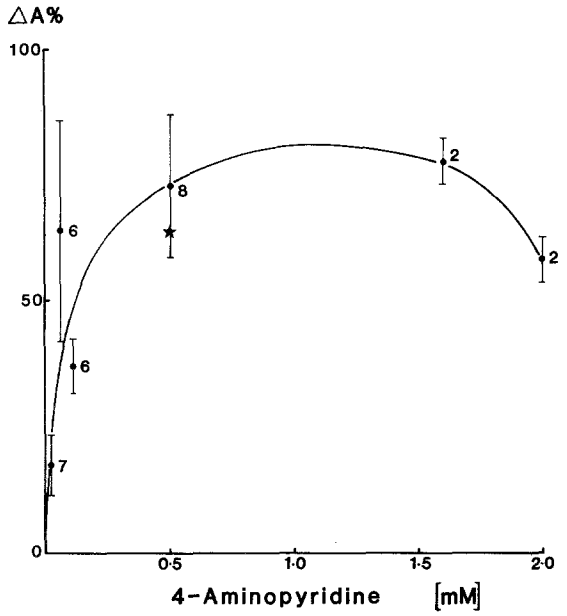

Fig. 3. Increase of Ca^{2+}-loading by 4-aminopyridine in skinned muscle fibres. The dose-response dependence was obtained following application of various 4AP concentrations in the loading solution LS. Ca^{2+} from the SR was always released in 4AP free RS standard solution. All solutions contained 0.3 mM Mg^{2+}. On the ordinate are percentage increases in the area under the caffeine induced force transients (AR; ●) after SR-Ca^{2+}-loading at different 4AP concentrations. All areas were normalized with respect to corresponding maximum forces and the nett increases were obtained by comparing the 4AP results with the averages of the controls before and after each 4AP treatment. The data were collected from 8 preparations. The ★ represents results obtained with 0.5 mM 4AP, when all solutions contained in addition 1 mM ouabain to inhibit the Na-K transport ATPase in the damaged transverse tubular system

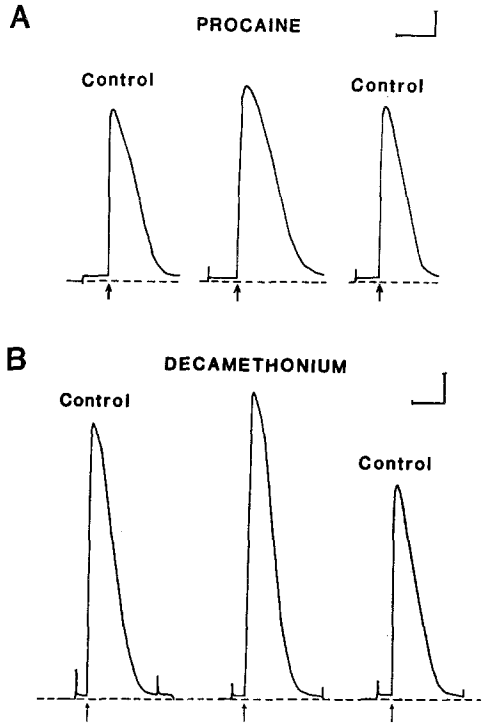

Fig. 4. Ca^{2+}-releases after loading in the presence of the SR-K^+ channel blocker procaine (**A**) and decamethonium (**B**). The same procedures for loading and releasing Ca^{2+} under control and test conditions were used as described in Figs. 1 and 2. The solutions with procaine had 1 mM procaine added to the counterpart standard solutions, the solutions with decamethonium had 0.5 mM decamethonium bromide added. *Calibration bars*: vertical 10 ± 1% P_{max} for all three control maxima; horizontal 5 s (**A**) and 8 s (**B**)

To determine which component of the overall Ca^{2+}-loading process is mainly affected by 4AP, we modified the ionic composition of the Ca^{2+}-loading solution from that used in Fig. 3. The Ca^{2+}-concentration in the loading solution was decreased by half (pCa = 6.8) and solutions containing either 0.3 or 1 mM free Mg^{2+} were used. In all these experiments we found that 0.5 mM 4AP always enhanced the net amount of Ca^{2+} loaded, compared to the controls. The increases in the peak forces during Ca^{2+}-release were 42% ± 32% (± SD, $n = 4$; 1 mM free Mg^{2+} solution; loading at pCa 6.5) and 19% ± 6% (± SD, $n = 2$; low Ca^{2+} solution; pCa 6.8; 0.3 mM free Mg^{2+}). These results suggest that the effect of blocking the SR-K^+-channels during Ca^{2+}-loading is maintained for conditions when the total amount of Ca^{2+} loaded in the SR differs greatly.

In addition to 4AP and TEA we have also used other drugs known to block SR-K^+-channels: decamethonium, a specific SR-K^+-channel blocker and procaine, a drug which is also known to block Ca^{2+}-channels (Labarca et al. 1980). When 1 mM procaine was used in the Ca^{2+}-loading solution (see Fig. 4A), the increase in the caffeine-induced force response was about 12% for the peak forces and 50% for the area under the force transients, compared to controls. From our experiments with 0.1 mM decamethonium present during Ca^{2+} loading, the normalized peak force developed by the fibre during test release was larger by 19% and the area by 20% as shown in Fig. 4B, with average increases of 18% ± 1% for the peak and 10% ± 8% for the area (± SD; three fibres). In the presence of 0.5 mM decamethonium during Ca^{2+}-loading, the normalized peak force increased by 29% ± 3%(± SD; $n = 3$) and the area under the force transient by 45% ± 5% (± SD; $n = 3$), compared to controls.

We have also carried out experiments to determine whether the effects we observed on Ca^{2+} movement by K^+-channel blockers can also be produced by the commonly used Ca^{2+}-channel blocker, verapamil (Reuter 1983). We carried out Ca^{2+}-loading experiments (at 1 mM Mg^{2+}) in the presence of 20 μM verapamil and found that, if anything, it slightly decreased the amount of Ca^{2+} loaded compared with control experiments done in the absence of the drug. In contrast, when both 1 mM 4AP and 20 μM verapamil were present during loading, the value of the peak and the area under the transient were increased significantly compared to controls, similar to the results when only 4AP was used (ratios between results for 4AP + verapamil and 4AP alone: peak forces 1.01; areas 1.20). These results indicate that the effects we observed on Ca^{2+}-movements K^+-channel blockers were not brought about by blocking Ca^{2+}-channels similar to those found in the sarcolemma.

In order to test if 4AP interferes directly with Ca^{2+}-channels located in the sarcoplasmic reticulum membrane, we have also studied the effect of Ruthenium Red (RR, Sigma), a more specific SR-Ca^{2+}-channel blocker (Volpe et

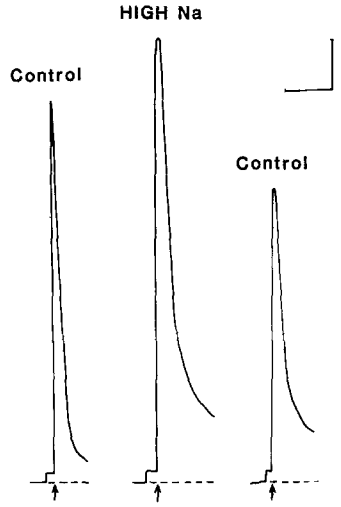

Fig. 5. Ca^{2+}-release after loading in a sodium rich solution in which all K^+ was replaced by Na^+. The same protocol for loading and releasing Ca^{2+} was used as described in Fig. 1 and Methods. The low relaxing and SR loading solutions containing the higher level of Na^+ were similar with the exception that 125 mM K^+ was replaced by 125 mM Na^+. *Calibration bars*: vertical $10 \pm 1\%$ P_{max} for all three control maxima; horizontal 24 s for the first and 20 s for the other two traces

al. 1986), on the Ca^{2+} loading in the presence and in the absence of 4AP. When only RR was present during Ca^{2+} loading, (0.1 to 5 μM RR), the amount of releasable Ca^{2+} markedly increased in a dose-dependent manner by up to 300% (see also Fink and Stephenson 1986). Addition of 50 μM to 1.6 mM 4AP to the RR Ca^{2+}-loading solutions modified differently the amount of Ca^{2+} loaded. Thus lower concentrations of 4AP generally reduced the observed RR effects. However, increasing the 4AP concentration increased the net amount of Ca^{2+} loaded compared to that obtained when only RR was present. Furthermore, the 4AP concentration at which one could notice a potentiating effect on the Ca^{2+} loading in the presence of RR was lower at lower RR concentrations. These results suggest that the two drugs can enhance the amount of releasable Ca^{2+} from the SR via different mechanisms. Most likely, 4AP affects Ca^{2+}-loading indirectly by binding to SR-K channels, whereas RR affects Ca^{2+}-loading more directly by blocking the release of Ca^{2+} through the SR-Ca^{2+}-channels. If both drugs were to act via the same mechanism then one would expect to obtain an additive effect in the presence of both drugs, which was not always the case.

We also interfered with the K^+ conductance of the SR-membrane by using a different approach. We replaced all the K^+ ions in the loading solution by an equal amount of Na^+ ions which are less permeant and should significantly reduce the rate of flow of monovalent ions through the SR-K^+-channels (Coronado et al. 1980; for additional effects of Na^+ on the SR see De Meis 1981). Therefore, when loading in high Na^+ concentration, one would expect a similar effect to that observed when loading in the presence of K^+ blockers. This was found to be the case (Fig. 5). After loading in a solution containing an increased level of Na^+, the caffeine induced Ca^{2+}-release (in the standard K^+ solution RS) was greater than the control value. The peak force was about 20% greater and the area under the force transient was 68% larger when loading in the presence of an increased Na^+ level than in the control releases.

Discussion

The procedure outlined in Fig. 1 has several advantages compared with the procedures previously used (Endo et al. 1970; Fabiato and Fabiato 1975; Stephenson 1981; Thieleczek 1982): it allows (i) the accurate control of Ca^{2+}-loading conditions for times longer than a few seconds, by producing sudden changes in pCa in the whole preparation; (ii) correction of the force responses for slow deterioration; (iii) complete release of stored Ca^{2+}, releasable by caffeine, from the SR before starting a new cycle of Ca^{2+}-loading and release; (iv) the ionic environment of the myofilaments to be changed during Ca^{2+}-loading and release by the SR.

The main conclusion from the experiments described in this paper is that the ability of the SR to load Ca^{2+} can be increased by decreasing the K^+-conductance of the SR membranes. We lowered the SR-K^+-conductance during SR-Ca^{2+}-loading by using four chemically unrelated drugs known to act as K^+-channel blockers (4AP, TEA, decamethonium and procaine; Coronado et al. 1980) and found consistently that the SR loaded more Ca^{2+} in the presence of the drugs than in their absence. We also lowered K^+-conductance by replacing some of the K^+ in the Ca^{2+}-loading solution by the less permeant Na^+ ion, which also resulted in increased Ca^{2+}-loading by the SR. One should be aware that some of the drugs used can also have different effects other than blocking the SR-K^+-channels in our preparations (e.g. see Methods). However, the evidence from all test and control experiments done clearly points out that SR-K^+-channels can modulate the SR-Ca^{2+}-movements and therefore suggests a possible physiological role for the SR-K^+-channels.

Theoretically, the net increase in releasable Ca^{2+} in these experiments could be due to the effects of decreased K^+ conductance on the increased loading Ca^{2+} ability by the SR, on the Ca^{2+}-release channels or on the Ca^{2+} pump. Our results with ruthenium red strongly indicate that the action of the K^+-channel blockers is not via a modification of the Ca^{2+}-release mechanism from the SR (see Results). Experiments with isolated SR membrane systems also failed to show an effect of K^+-channel blockers on the unidirectional Ca^{2+} release and Ca^{2+} uptake by the SR (Volpe et al. 1983). Therefore, the most likely explanation of our results is that the decrease of SR-K^+ conductance somehow increases the ability of the SR to load Ca^{2+}. Our findings are consistent with the assumption that during Ca^{2+}-uptake and release, ionic counter-currents are necessary to preserve electroneutrality (Oetliker 1982). From X-ray microanalysis data (Somlyo et al. 1981), it appears that it is mainly a movement of cations, such as K^+, Mg^{2+} and probably H^+ that balances the movement of Ca^{2+} ions. Then, if the balance between cationic currents is disturbed by blocking the K^+-channel, this would result in a forced outflow of H^+ (and Mg^{2+}) during Ca^{2+}-uptake and in an increased pH and decreased Mg^{2+} in the SR lumen. This condition could affect Ca^{2+}-loading (Lea and Ashley 1978), for example, by increasing the apparent Ca^{2+}-binding affinity of the numerous Ca^{2+}-binding sites in the SR.

The experiments with the SR-K^+-channel blockers also indicate that some of these drugs elicit marked effects on the SR properties of mechanically skinned fibres at much lower concentrations than the K_D values reported for the SR-K^+-channels incorporated in lipid bilayers (Coronado and Miller 1982). 4AP was already potent at a concentration of 6 μM (Fig. 3) compared with a K_D value of 58 mM, TEA was potent at 10 mM compared with a K_D value of 85 mM and procaine was potent at a concentration of 1 mM compared with a K_D value of 26 mM. This strongly suggests that these SR-K^+-channel blockers are more effective when acting on intact SR membranes bathed in physiological solutions than when acting on K^+-channels from fragmented SR vesicles incorporated in lipid bilayers and bathed in a less physiological environment.

Now, if SR-K^+-channel are important in modulating Ca^{2+} movements across the SR membranes in the living muscle, then, based on the skinned fibre experiments, one would expect that the Ca^{2+}-transient would decrease in height when the SR-K^+-channels were blocked. This is because an increased ability of the SR to sequestrate Ca^{2+} should result in a reduced Ca^{2+}-transient for twitch responses when only part of the Ca^{2+} stored in the SR is released. Experiments with the Ca^{2+}-sensitive photoprotein aequorin (Lyster et al. 1985) in the presence of 1–10 mM 4AP, strongly support this view. The Ca^{2+}-transient initially increased, as would be expected, from the prolongation of the action potential by 4AP. However, after long incubation times (20–60 min), the Ca^{2+}-transient decreased markedly (see also similar observations on Ca^{2+}-transients reported by Miledi et al. 1984), who used 3,4-diaminopyridine and Arsenazo III). The delayed action of 4AP is consistent with its diffusion into the muscle fibre and action on the SR membranes.

In conclusion, all our experimental findings on skinned and living muscle fibres demonstrate that movement of Ca^{2+} across the SR-membrane can be modified by altering the SR-K^+ conductance.

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