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EFFECTS OF PROCAINE ON CALCIUM ACCUMULATION BY THE SARCOPLASMIC RETICULUM OF MECHANICALLY DISRUPTED RAT CARDIAC MUSCLE

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SUMMARY

1. The ability of the sarcoplasmic reticulum of skinned cardiac muscle of the rat to accumulate and release Ca^{2+} was studied in the presence and absence of procaine.

2. Ca^{2+} accumulation was estimated from the magnitude of the caffeine- (30 mM) induced force transient in a weakly Ca^{2+} buffered solution. The relative area under the caffeine-induced force transient was up to 4-fold greater when 5 mM-procaine had been present during the preceding period of Ca^{2+} loading, than that after an equivalent period of Ca^{2+} loading in the absence of procaine.

3. Procaine antagonized the caffeine-induced release of Ca^{2+} when present in the Ca^{2+} releasing solution, however, the ability of procaine to attenuate the caffeine-induced Ca^{2+} release diminished as the extent to which the sarcoplasmic reticulum was loaded with Ca^{2+} increased.

4. In the presence of 1 mM-Mg^{2+} procaine also markedly attenuated the small spontaneous force oscillations (5–10 % P_0) associated with the cyclic release and reuptake of Ca^{2+} by the sarcoplasmic reticulum. When the Mg^{2+} concentration was reduced to 0·1 mM, procaine initially suppressed the small spontaneous oscillations in force, however, large force oscillations (40–80 % P_0) of lower frequency were invariably initiated after 20–60 s exposure to 5 mM-procaine.

5. Procaine (5 mM) produced a slight shift ($\sim$ 0·04 pCa unit) of the force–pCa relation toward lower Ca^{2+} concentrations. This effect is too small to influence in any substantial way the apparent effects of procaine on the sarcoplasmic reticulum.

6. The results indicate that whilst procaine is indeed able to suppress Ca^{2+} release under certain circumstances, in its presence the net accumulation of Ca^{2+} by the sarcoplasmic reticulum can be markedly enhanced.

INTRODUCTION

The best documented effect of procaine on muscle, apart from its actions at the sarcolemma, is an inhibition of Ca^{2+} release from the sarcoplasmic reticulum (Endo, 1977). Contractures induced by the application of caffeine or hypertonic fluids, or by

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rapid cooling, can be blocked by procaine and some other local anaesthetics in both cardiac (Chapman & Miller, 1974; Chapman & Léoty, 1976; Chapman, 1978; Hunter, Haworth & Berkoff, 1982) and skeletal muscles (Feinstein, 1963; Bianchi & Bolton, 1967; Lüttgau & Oetliker, 1968; Isaacson, Hinkes & Taylor, 1970; Lännergren & Noth, 1973; Caputo, 1976). It is generally believed that these contractures result from the release of Ca^{2+} from the sarcoplasmic reticulum and that this release is inhibited by these local anaesthetics. This action is not necessarily a general property of local anaesthetics, however, since it is not shared by all such compounds (Bianchi & Bolton, 1967).

The ability of procaine to block K^+ contractures, however, is not as firmly established (Etzensperger, 1970; Caputo, 1976; Chapman & Tunstall, 1981) and in skinned muscle fibres procaine is reported to antagonize Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum but not depolarization-induced Ca^{2+} release (Ford & Podolsky, 1972; Thorens & Endo, 1975; Almers & Best, 1976). Furthermore, procaine can itself cause contractures, at least under certain conditions (Bianchi & Bolton, 1967; Isaacson *et al.* 1970; Chapman & Miller, 1974), and has also been reported to potentiate caffeine contractures in some circumstances (Chapman & Léoty, 1976).

These latter observations clearly indicate that procaine may affect the sarcoplasmic reticulum in other ways besides simply inhibiting Ca^{2+} release and in the present study we have examined the effects of procaine on the ability of the sarcoplasmic reticulum of functionally skinned cardiac muscle to accumulate and release Ca^{2+}. The results show that procaine can not only inhibit Ca^{2+} release in this preparation but is also able to markedly enhance the net accumulation of Ca^{2+} by the sarcoplasmic reticulum.

METHODS

Fibre preparation

Hearts from Long Evans (hooded) rats that had been killed by cervical dislocation were rapidly excised and flushed with oxygenated physiological saline. A portion of ventricular tissue was minced into small pieces (1–3 mm^3) that were then homogenized in high relaxing solution (see below) using a blade type microhomogenizer. The homogenization procedure disaggregated the tissue into fragments ranging in size from that of a portion of a single cell upwards. Fragments with a diameter around 50 μm and a length of 0·5–1·0 mm were selected from the homogenate for use in the experiments. The sarcolemmae of the cells within these bundles are disrupted by the homogenization procedure which effectively removes the sarcolemmal diffusion barriers leaving a preparation that is effectively skinned whilst still retaining the functional integrity of the sarcoplasmic reticulum (Fabiato & Fabiato, 1972; Kerrick & Best, 1974; Donaldson & Hermanson, 1978; Su & Kerrick, 1979; Best, 1983; Wendt & Stephenson, 1983).

Force measurement

Force was measured using a piezo-resistive force transducer (AME AE801). The preparation was impaled at one end with a sharpened stainless steel needle that was attached, with shellac, to the original peg of the transducer element, and held at the other end by a second needle impaled through it. The holding needle and transducer needle were mounted on separate micromanipulator arms allowing independent movement of each needle in any direction. The attachment of the preparation to the impaling needles was extremely firm. No evidence of slippage was ever observed and if the preparation was subjected to extreme stretch it would usually tear between the needles rather than break free from one of them. After being attached to the force measuring apparatus the preparations

were stretched until resting force was just noticeable (< 5% of maximum Ca^{2+} activated force). Where possible, microscopic observation revealed that under these conditions the average sarcomere length was around 2·2 μm. Under maximum Ca^{2+} activation these preparations developed forces up to 75 mN/mm² (cross-sectional area estimated from diameter, assuming uniform cylindrical geometry).

Solutions

Experimental solutions were prepared in the manner described in detail previously (Moisescu & Thieleczek, 1978; Stephenson & Williams, 1981). Three basic stock solutions were used. High relaxing (HR) solution which contained 50 mM-EGTA (ethyleneglycol-bis-(β-aminoethylether)*N*,*N*′-tetra-acetic acid), low relaxing (LR) solution containing 50 mM-HDTA (hexamethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid) and to which could be added small amounts of EGTA (usually 0·01–0·1 mM), and activating (A) solution which contained Ca and EGTA in equimolar concentrations. These solutions all contained (mM): K^+, 117; Na^+, 36; Mg^{2+}, 1; TES (2-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)aminoethane sulphonic acid), 60; ATP, 8; creatine phosphate, 10 and creatine kinase, 15 u./ml. The pH of all solutions was 7·10 ± 0·01 at the experimental temperature of 22 °C and the ionic strength *ca.* 225 mM. In some experiments the free Mg^{2+} concentration of the solutions was reduced to 0·1 mM.

To study steady-state Ca^{2+}-activated force, activating solutions with various free-Ca^{2+} concentrations were obtained by mixing the HR and A solutions in appropriate proportions. These solutions were strongly buffered (50 mM-total EGTA) for Ca^{2+}. To study force responses due to Ca^{2+} movements associated with the sarcoplasmic reticulum, weakly buffered solutions were required. These were prepared by mixing small amounts of HR and A solutions with the LR solution to give final total EGTA concentrations of around 0·01 to 0·1 mM. The EGTA concentration needs to be kept very low in these experiments so that small amounts of Ca^{2+} that might be released from the sarcoplasmic reticulum are able to effectively raise the Ca^{2+} concentration surrounding the myofilaments and so give rise to a measurable force response, rather than this Ca^{2+} being absorbed by the solution buffer system. Relative and absolute Ca^{2+} and EGTA concentrations were determined in individual solutions at the end of each experiment using pH metric titration techniques (Stephenson & Williams, 1981). The apparent affinity constants used for computing free ion concentrations in the solutions were those previously determined in this laboratory for the appropriate experimental conditions (see Moisescu & Thieleczek, 1978; Stephenson & Williams, 1981).

Solutions were prepared fresh at the beginning of each experiment and to study the effects of procaine the solutions were split into appropriate aliquots, one of the which served as the control whilst procaine (5 mM) was added solid (as a chloride salt) to the other. This procedure ensured that the control and with-drug solutions were always identical in composition except for the presence of the drug itself. In the concentrations employed procaine did not alter the pH of the solutions or interact in any other way to alter the free-Ca^{2+} concentration within the solutions. The solutions were housed in milled Perspex vials (volume 3 ml) and a solution change was effected by transferring the preparation from one vial to another. This could be accomplished in 2–3 s.

RESULTS

The behaviour of preparations of the type used in this study in solutions weakly buffered for Ca^{2+} is illustrated in the lower panel of Fig. 1 and can be contrasted to the typical steady-state force response observed in the presence of strong Ca^{2+} buffering (upper panel). When the Ca^{2+} buffering capacity of the solution is low spontaneous oscillations in the force are observed. These force oscillations were observed in all preparations investigated ($n = 26$) and typically occurred at a frequency of around 0·5 Hz with peak amplitudes of 5–10% of the maximum Ca^{2+} activated force. Their amplitude is progressively decreased by increasing concentrations of EGTA in the bathing solution. In general the oscillations were no longer observed once the EGTA in the solution reached 0·1–0·2 mM. They were also totally

abolished by treatment of the preparation with Triton X-100 (1 % v/v, 30–60 min). These force oscillations are presumed to reflect cyclic release and re-uptake of Ca^{2+} by the sarcoplasmic reticulum.

The ability of the sarcoplasmic reticulum to accumulate Ca^{2+} was tested by placing the preparations in a low level, weakly Ca^{2+}-buffered solution for a fixed period of

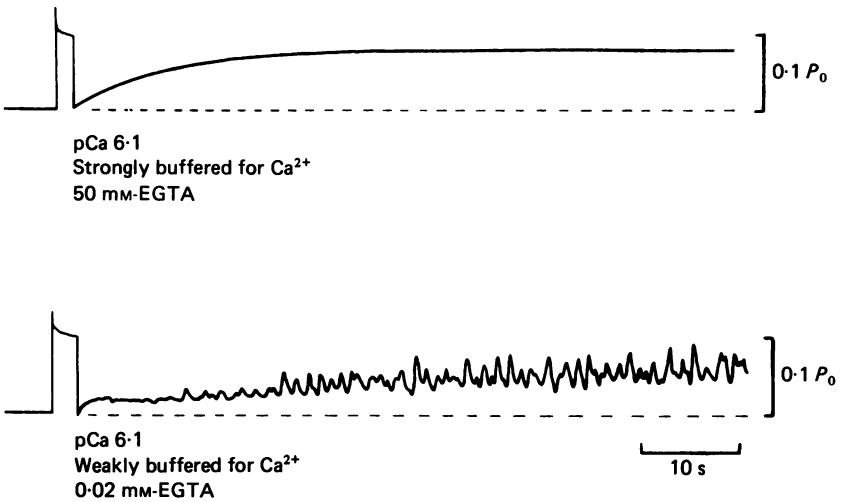

Fig. 1. Representative force responses from the same functionally skinned cardiac preparation (diameter 65 μm, length 0·7 mm) when transferred from a low relaxing (LR) solution either to a solution strongly buffered for Ca^{2+} (total EGTA 50 mM; pCa 6·1, upper trace) or to a solution weakly buffered for Ca^{2+} (total EGTA 0·02 mM; pCa ~ 6·1, lower trace). The dashed lines represent the base line of zero active force.

time. Ca^{2+} stored within the sarcoplasmic reticulum was then released by exposing the preparations to 30 mM-caffeine in LR type solution. This high concentration of caffeine was chosen to ensure that all the releasable Ca^{2+} would be released by this technique in each experimental run (Endo, 1977). Within each experiment the EGTA concentration in this Ca^{2+} releasing solution was constant and was adjusted to between 0·02 and 0·15 mM (pCa > 7) to allow for the force transient to return to base line within 2 min. The magnitude of the force transient subsequent to caffeine exposure was taken as an index of the amount of Ca^{2+} released and all comparisons between with-procaine and control responses are made within individual preparations exposed to the two conditions. To ascertain that the recorded force transients were due to a release of Ca^{2+} and not to an effect of caffeine on the apparent Ca^{2+} sensitivity of the contractile apparatus (Wendt & Stephenson, 1983) the preparations were subsequently treated with the non-ionic detergent Triton X-100 (1 % v/v, 30–60 min) and the Ca^{2+} loading/release procedures repeated. In all instances the force transients previously observed upon application of caffeine were abolished by the detergent treatment.

Prior to being placed in the Ca^{2+} loading solution the preparations were always taken through a set sequence of solution changes designed to empty the sarcoplasmic

reticulum of all its releasable Ca^{2+} and ensure that each loading sequence commenced from the same initial content. This sequence consisted of, (1) maximal activating solution containing caffeine and high EGTA (to release Ca^{2+}); (2) HR solution containing caffeine (to lower the Ca^{2+} concentration whilst preventing re-uptake); (3) HR solution without caffeine (to wash-out caffeine); (4) LR solution with < 0·1 mM-EGTA (to lower the EGTA concentration); (5) second rinse in LR solution.

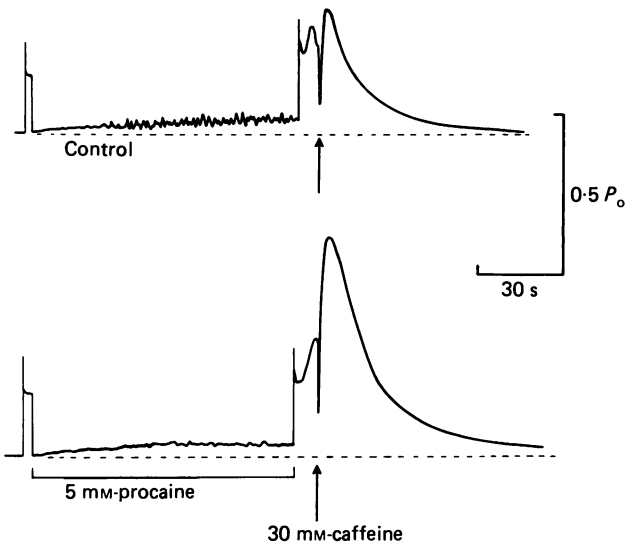

Fig. 2. Force responses obtained with the same cardiac preparation (diameter 60 μm, length 0·9 mm) when loaded with Ca^{2+} for an equal time in a solution weakly buffered for Ca^{2+} (total EGTA 0·02 mM; pCa ~ 6·1) in the absence (upper trace) and presence (lower trace) of 5 mM-procaine. The arrows under the traces indicate the moment the preparation was transferred to the same 30 mM-caffeine containing LR solution (total EGTA 0·05 mM; pCa > 7). The magnitude of the ensuing force transient is a measure of the amount of Ca^{2+} released, by caffeine, from the sarcoplasmic reticulum.

Typical force transients recorded upon releasing Ca^{2+} stored in the sarcoplasmic reticulum by application of caffeine are shown in Fig. 2. In the upper panel the preparation was placed in a low level, weakly Ca^{2+} buffered activating solution for 90 s following which caffeine was applied to release any Ca^{2+} that had been accumulated in the sarcoplasmic reticulum over that period. In the lower panel the sequence has been repeated in the same preparation using the same loading solution except that it now contained in addition 5 mM-procaine. Two features are apparent. First in the presence of procaine the spontaneous force oscillations are substantially attenuated and secondly the force transient upon application of caffeine is very much larger following the period of Ca^{2+} loading in the procaine containing solution. This latter observation shows that over an equivalent period of time in a solution of identical Ca^{2+} concentration the sarcoplasmic reticulum of this preparation has accumulated substantially more Ca^{2+} when procaine was present.

Experiments of this type were repeated for various exposure times to the Ca^{2+} loading solutions and the results obtained with nine preparations from different hearts

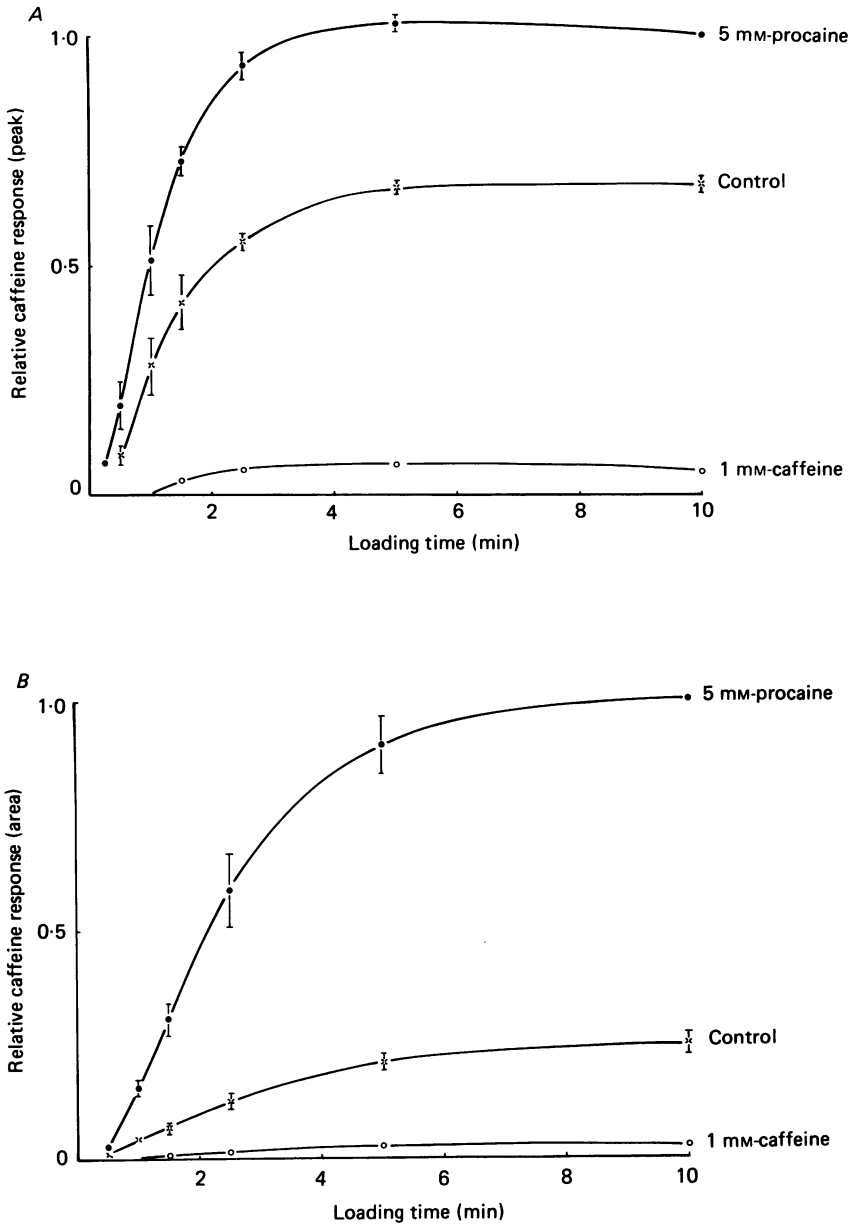

Fig. 3. The time course of Ca^{2+} accumulation by the sarcoplasmic reticulum in a solution weakly buffered for Ca^{2+} (total EGTA 0·02 mM; pCa ~ 6·1) in the absence of drugs (×), in the presence of 5 mM-procaine (●) and in the presence of 1 mM-caffeine (○). The amount of Ca^{2+} accumulated is judged from the relative peak (*A*) or the relative area (*B*) of the force transient induced by 30 mM-caffeine at the end of the Ca^{2+} loading period (see also Fig. 2). The results are from paired with- and without-drug experiments performed on nine preparations for procaine, with caffeine having been also tested in four preparations and are expressed, within each preparation, relative to the response obtained after 10 min of Ca^{2+} loading in the presence of procaine. The vertical bars indicate the S.D. of mean for each given loading period and were too small to be shown on the 1 mM-caffeine data.

are presented in Fig. 3. For all loading times both the peak height and area of the subsequent caffeine-induced force transient were greater when procaine had been present in the loading solution. It should be noted that the apparently slow time course of Ca^{2+} accumulation in these experiments is largely due to diffusional limitations coupled with Ca^{2+} sequestration by the preparation in these solutions with very weak Ca^{2+} buffering capacity. Also shown in Fig. 3 is the effect of including a

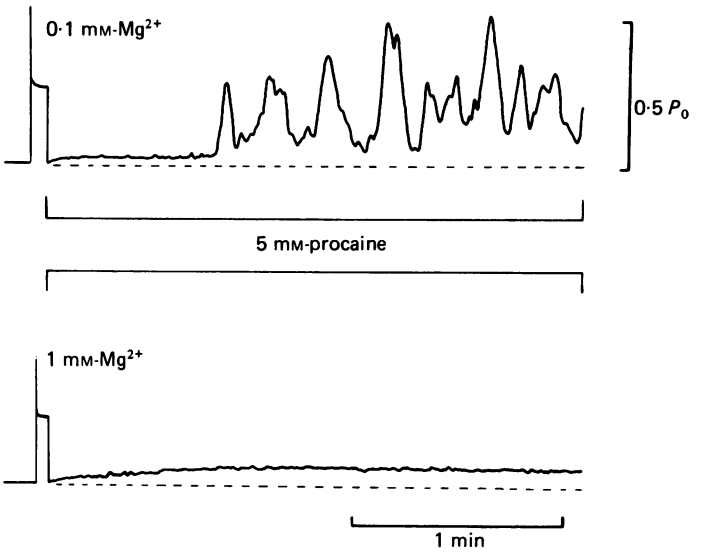

Fig. 4. Typical force responses from the same skinned cardiac preparation (diameter 60 μm, length 0·8 mm) when transferred from LR solutions containing 0·1 (upper trace) or 1 mM-Mg^{2+} (lower trace) to corresponding solutions weakly buffered for Ca^{2+} (total EGTA 0·02 mM) and containing 5 mM-procaine. The pCa of the solutions was adjusted to give similar levels of background force in both situations and was ~ 6·1 for the 1 mM-Mg^{2+} solution and ~ 6·4 for the 0·1 mM-Mg^{2+} solution.

low concentration (1 mM) of caffeine in the Ca^{2+} loading solution (four preparations). This almost entirely abolished the capacity of the sarcoplasmic reticulum to accumulate any Ca^{2+}.

One of the observations from Fig. 2 was that procaine attenuated the spontaneous release of Ca^{2+} from the sarcoplasmic reticulum. To characterize this further the free-Mg^{2+} concentration of the solutions was reduced from the usual 1 mM to 0·1 mM. Fig. 4 presents an example of a consistent observation (six preparations) made under these circumstances. In the presence of 1 mM-Mg^{2+}, procaine attenuated the normal spontaneous force oscillations as illustrated in the lower panel and also in Fig. 2. In 0·1 mM-Mg^{2+} solutions small spontaneous force oscillations were also observed. These were initially suppressed by procaine, however, large force oscillations were invariably initiated after 20–60 s exposure to procaine. These large force oscillations generally had amplitudes of 40–80 % of the maximum Ca^{2+} activated force and occurred at a frequency of around 4–5/min. They were never observed in the low-Mg^{2+} solutions in the absence of procaine. Exposure to procaine in the presence of 1 mM-Mg^{2+} also

occasionally led to large spontaneous contractions, however, only after prolonged exposure (> 15 min). These large contractions generally occurred in isolation or in small groups of two or three separated by several minutes.

Since it is believed that procaine is able to antagonize the caffeine-induced release of Ca^{2+} from the sarcoplasmic reticulum a series of experiments was conducted in which the preparations were loaded with Ca^{2+} for a certain time in a weakly

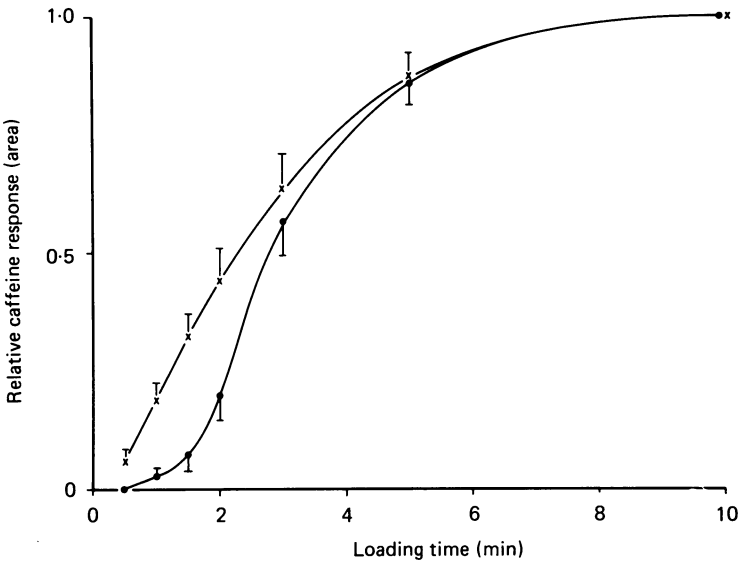

Fig. 5. The effect of the presence of 5 mM-procaine in the 30 mM-caffeine containing Ca^{2+}-releasing solution on the magnitude (relative area) of the caffeine-induced force transient following different times of Ca^{2+} loading in solutions weakly buffered for Ca^{2+} (total EGTA 0·02 mM, pCa ~ 6·1) without procaine. The crosses (×) indicate the Ca^{2+} release induced by 30 mM-caffeine alone whilst the filled circles (●) indicate that induced by 30 mM-caffeine in the presence of 5 mM-procaine. Results are the means ± S.D. of mean from paired experiments on five preparations.

Ca^{2+}-buffered solution and the accumulated Ca^{2+} then released by transferring the preparation to the caffeine containing LR type solution (as in Fig. 2 upper panel). The sequence was then repeated in the same solutions except that the 30 mM-caffeine solution contained in addition 5 mM-procaine. The mean results from five preparations for various times of Ca^{2+} loading are shown in Fig. 5. The resulting force transient upon exposure to caffeine was suppressed when procaine was added to the caffeine solution only when the preceding loading period was relatively brief. For longer periods of Ca^{2+} loading, when presumably the amount of Ca^{2+} in the sarcoplasmic reticulum was progressively greater, the 5 mM-procaine was no longer able to significantly antagonize the caffeine-induced release of Ca^{2+}. It is worth noting that when the preparations are transferred to the caffeine Ca^{2+}-releasing solution, following a period of loading in a procaine containing solution, they would be expected to carry with them some procaine. Consequently any antagonism of the

caffeine-induced Ca^{2+} release by procaine would tend, if anything, to lead to an underestimation of the amount of Ca^{2+} accumulated in the preceding Ca^{2+} loading period.

To assess whether a direct effect of procaine on the contractile apparatus might be contributing to the observed difference in the force transients presumed to be reflecting Ca^{2+} release from the sarcoplasmic reticulum we determined the effect of procaine on steady-state Ca^{2+} activated force in solutions strongly buffered for Ca^{2+}.

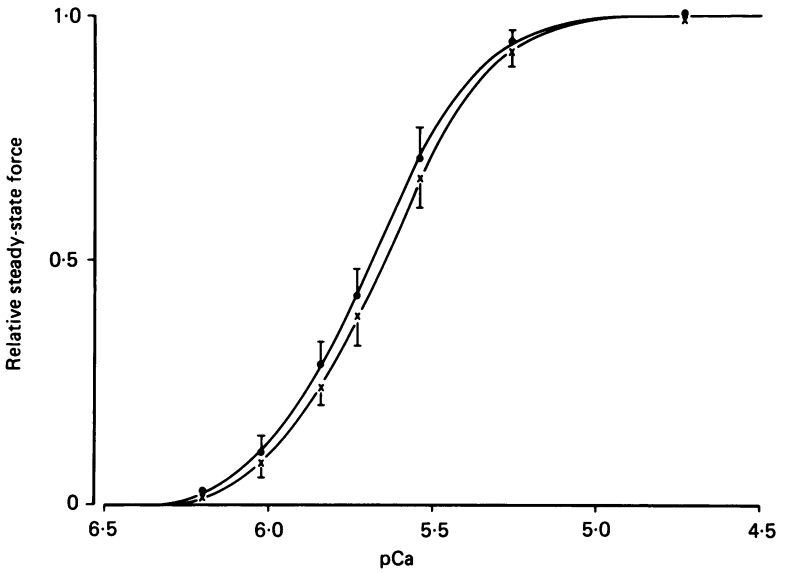

Fig. 6. Isometric force–pCa curves for skinned cardiac preparations activated in solutions strongly buffered for Ca^{2+} (50 mM-EGTA; 1 mM-Mg^{2+}; see Wendt & Stephenson, 1983) in the absence (×) and presence (●) of 5 mM-procaine. Vertical bars indicate the absolute scatter of the results which were obtained in paired experiments on three preparations. The curves were drawn by eye.

A series of activating solutions were prepared and split into equal aliquots to one of which was added 5 mM-procaine. The relation between relative steady-state force and pCa of the bathing solution was then determined in the control set of solutions and the procaine containing solutions within the same preparation. In each of the three preparations so tested 5 mM-procaine produced a slight (~ 0·04 pCa unit) leftward shift in the force–pCa relation as shown in Fig. 6. The maximum force was not affected by this concentration of procaine. The effect is clearly too small to affect in any substantial way the apparent effects of procaine on Ca^{2+} movements associated with the sarcoplasmic reticulum shown in Figs. 2 and 3.

DISCUSSION

The most striking feature of the results obtained in the present study is the marked enhancement by procaine of the amount of Ca^{2+} accumulated by the sarcoplasmic reticulum. This observation was invariant. In every preparation, following all

durations of Ca^{2+} loading, the subsequent caffeine-induced release of Ca^{2+} was always much larger when procaine had been present during the period of Ca^{2+} loading. The mechanisms whereby procaine enhances Ca^{2+} accumulation are unclear. There is no evidence to suggest that procaine is able to enhance the active uptake of Ca^{2+}. On the contrary, in isolated sarcoplasmic reticulum vesicles it may at high concentrations inhibit the uptake process (Johnson & Inesi, 1969; Wilcox & Fuchs, 1969). Inhibition of the spontaneous cyclic Ca^{2+} release seen under the control experimental conditions may contribute to a greater net accumulation. It has been suggested that the sarcoplasmic reticulum of rat cardiac muscle is particularly 'leaky' (Nayler, Dunnett & Burian, 1975) and if procaine were to reduce the leak of Ca^{2+} from the sarcoplasmic reticulum whilst not interfering with its uptake then over a given interval of time this should result in a greater net accumulation. It is perhaps worth noting, however, that in preliminary experiments we have observed similar effects of procaine in preparations of rabbit cardiac muscle in which the sarcoplasmic reticulum is presumed not to be as 'leaky'. Consequently stabilization of an unusually high leak of Ca^{2+} from the sarcoplasmic reticulum may not be the principal explanation for the observed enhancement of Ca^{2+} accumulation.

Another mechanism whereby procaine could enhance Ca^{2+} accumulation may be related to its ability to block K^+ selective channels in the sarcoplasmic reticulum. It has recently been shown that there are K^+ selective channels in the sarcoplasmic reticulum membrane of skeletal muscle that can be blocked by several compounds including procaine (Coronado & Miller, 1982). The sarcoplasmic reticulum of cardiac muscle appears also to contain similar K^+ channels (Montgomery, Tomlins & Williams, 1983) raising the possibility that procaine may, through interfering with the K^+ conductance, modify the movement of Ca^{2+} across the sarcoplasmic reticulum membrane. A preliminary report by Fink & Stephenson (1985) suggests that a variety of K^+-channel blockers, including procaine, are able to increase Ca^{2+} accumulation by the sarcoplasmic reticulum of amphibian skeletal muscle. However, whether such an action of procaine could be implicated in the enhanced Ca^{2+} accumulation observed in the present experiments remains entirely speculative.

In apparent contrast to the results obtained in this study Ford & Podolsky (1972) reported that procaine (2 mM) did not alter the rate at which skinned fibres of frog semitendinosus muscle accumulated ^{45}Ca over a 30 s period in weakly Ca^{2+} buffered solutions. Whether the difference between this result and the enhanced Ca^{2+} accumulation observed in our cardiac preparations reflects some fundamental differences between cardiac and skeletal sarcoplasmic reticulum or merely some differences in the experimental protocols (e.g. the time over which Ca^{2+} uptake was followed) cannot be said with certainty. Whilst skinned skeletal muscle fibres can show spontaneous contractions in solutions weakly buffered for Ca^{2+} (Endo, Tanaka & Ogawa, 1970; Ford & Podolsky, 1972) these appear to be less readily initiated than in cardiac preparations and certainly occur at a much lower frequency than that observed in the present study. If inhibition of spontaneous Ca^{2+} release is the principal mechanism whereby procaine allows a greater net accumulation of Ca^{2+} then this may account for a less marked effect in this regard in skeletal muscle fibres.

The present results clearly show that procaine attenuates the spontaneous cyclic contractions that occur in preparations of mechanically disrupted cardiac muscle of

the rat in the presence of low-EGTA concentration in the bathing solutions. If these contractions are due, as we believe, to cyclic release and re-uptake of Ca^{2+} by the sarcoplasmic reticulum this would be consistent with procaine acting to inhibit the release of Ca^{2+}. Similar spontaneous force oscillations have previously been observed in skinned cardiac preparations (e.g. Fabiato & Fabiato, 1972, 1975*a*; Kerrick & Best, 1974; Endo & Kitazawa, 1978) and are in general held to be due to Ca^{2+} release from the sarcoplasmic reticulum. This release probably occurs through a Ca^{2+}-induced mechanism although these cyclic contractions may not necessarily represent a manifestation of the Ca^{2+}-induced Ca^{2+} release mechanism proposed to play a physiological role in excitation contraction coupling in cardiac muscle (Fabiato, 1985). Spontaneous cyclic release of Ca^{2+} from the sarcoplasmic reticulum may also occur in intact, undamaged cardiac cells at rest (Stern, Kort, Bhatnagar & Lakatta, 1983; Wier, Kort, Stern, Lakatta & Marban, 1983) although the possible physiological relevance of this remains uncertain (Stern *et al.* 1983; Fabiato, 1985).

It is clear from the results presented in Fig. 5 that procaine is also able to antagonize, to some extent, the caffeine induced release of Ca^{2+} from the sarcoplasmic reticulum in these preparations. Again this is consistent with procaine's ability to block caffeine contractures in intact muscle (Feinstein, 1963; Bianchi & Bolton, 1967; Lüttgau & Oetliker, 1968; Isaacson *et al.* 1970; Chapman & Miller, 1974; Hunter *et al.* 1982). It is interesting to note, however, that procaine's antagonism of the caffeine-induced Ca^{2+} release decreased as the extent to which the sarcoplasmic reticulum was filled with Ca^{2+} increased. It is presumably likely that a significant antagonism of the caffeine-induced Ca^{2+} release may have been observed following the longer Ca^{2+} loading times if the concentration of procaine had been raised above 5 mM. Nevertheless these observations suggest that the efficacy of the Ca^{2+} release mechanism is influenced by the amount of Ca^{2+} that is within the sarcoplasmic reticulum.

The occurrence of the large spontaneous force oscillations in the presence of procaine and low free-Mg^{2+} concentration may also relate to the Ca^{2+} content within the sarcoplasmic reticulum. Mg^{2+} appears to exert an inhibitory influence on Ca^{2+}-induced Ca^{2+} release (Fabiato & Fabiato, 1975*b*; Endo, 1977) and consequently in the presence of low free-Mg^{2+} concentration Ca^{2+} release is more easily initiated. The large force oscillations shown in Fig. 4 may occur when the Ca^{2+} content within the sarcoplasmic reticulum reaches a critical level at which the release mechanism is activated and Ca^{2+} release is initiated despite the presence of procaine. In the absence of procaine these large contractions never occurred since presumably the Ca^{2+} load within the sarcoplasmic reticulum did not reach a sufficient level to initiate release. When the Mg^{2+} concentration is higher Ca^{2+} release is less easily initiated and the large force oscillations do not occur.

As well as its marked influence on Ca^{2+} accumulation by the cardiac sarcoplasmic reticulum procaine also appears to slightly enhance the apparent Ca^{2+} sensitivity of the contractile apparatus (see Fig. 6). Almers & Best (1976) observed a similar slight leftward shift of the force–pCa relation of frog skeletal muscle fibres in the presence of 2 mM-tetracaine. This effect is, however, small and could not be implicated in any way in the apparent effects on the sarcoplasmic reticulum described here.

The present results provide further evidence that effects on Ca^{2+} movements

associated with the sarcoplasmic reticulum are amongst procaine's actions on muscle. Procaine can suppress Ca^{2+} release from the sarcoplasmic reticulum under certain circumstances but may also act to markedly enhance Ca^{2+} accumulation. The question remains open whether or not this previously undocumented ability of procaine to enhance net accumulation of Ca^{2+} by the sarcoplasmic reticulum in skinned cardiac muscle of the rat may have implications for the interpretation of procaine's actions in intact muscle preparations, particularly in situations of prolonged exposure to this drug.

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