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The effects of external potassium, multivalent cations and temperature on caffeine contractures in rat skeletal muscle

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1 The effects of multivalent cations, membrane potential and temperature on caffeine contractures of rat soleus and extensor digitorus longus (e.d.l.) muscles were investigated.

2 The amplitude of the caffeine contracture was depressed by the removal of calcium and by the addition of a high concentration (1 mM) of lanthanum. Low concentrations of lanthanum (0.1–0.5 mM) augmented the caffeine contracture.

3 Low levels of depolarization by potassium (10–40 mM) augmented the amplitude of the caffeine contracture, while higher concentrations of potassium depressed the contracture. Maximum augmentation of the caffeine contracture occurred with a higher concentration of potassium (20 mM vs 10 mM) in the e.d.l. than in the soleus muscle.

4 The amplitude of contractures was directly related to temperature between 22 and 37°C and inversely related to temperature below 22°C.

5 The effects of caffeine in rat skeletal muscle are suggested to be exerted on the sarcolemma and the mechanisms of action are by modification of the processes of activation and inactivation.

Introduction

There is controversy regarding the site of action of caffeine in skeletal muscle. Initial studies by Bianchi (1961) using radioactively labelled caffeine provided evidence that caffeine did not appear to bind to sites on the sarcolemma. Studies on isolated sarcoplasmic reticulum (SR) have been interpreted to indicate that in the intact muscle, caffeine releases calcium from (Ogawa, 1970) and blocks the uptake of calcium by the SR (e.g. Weber & Herz, 1968).

However, studies on frog skeletal muscle have provided evidence that caffeine acts in the transverse tubules (t-tubules) to alter the processes of activation and inactivation (Luttgau & Oetliker, 1968). Later studies on the effects of membrane potential and t-tubule disruption by glycerol treatment also support this suggestion (Foulks *et al.*, 1971; Sakai *et al.*, 1971).

This study investigated the site and mechanisms of caffeine action in mammalian skeletal muscle. The results obtained suggest that in rat skeletal muscle caffeine acts at a sarcolemmal site and exerts its action by altering the processes of activation and inactivation.

Methods

The soleus (10–14 mg) and extensor digitorus longus (e.d.l.) (5–7 mg) muscles of female Wistar rats killed by cranial fracture were used in this study. Muscles were mounted in a physiological salt solution contained in a water jacketed muscle bath and equilibrated for one hour before the start of each experiment. The physiological salt solution had the following composition (mmol l^{-1}): NaCl 118.1, KCl 3.4, $MgSO_4$ 0.8, KH_2PO_4 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.52, glucose 11.1; pH 7.0. Since lanthanum precipitated out of this solution as a bicarbonate salt, a modified saline containing 2 mmol l^{-1} of NaHEPES in place of $NaHCO_3$ and KH_2PO_4 was used. Solutions containing specified concentrations of calcium were prepared by adding 1 mM EGTA and appropriate concentrations of calcium. Solutions containing elevated concentrations of potassium were prepared using K_2SO_4, and the [K] [Cl] product was maintained constant and sucrose was added to keep solutions isotonic with the ordinary physiological saline solution. Solutions were gassed continuously with 95% O_2 plus 5% CO_2, or in the case of the modified physiological saline solution with 100% O_2.

Muscles were placed under a resting tension of 1 g. Changes in tension were monitored by a Stratham UF1 strain gauge transducer, amplified by a Washington FC 135 coupler and recorded on a Servoscribe 2 S pen recorder. Each muscle was exposed to one concentration of caffeine only.

Membrane potential of soleus muscles was measured at room temperature (18–20°C). Fibres were impaled by micro-electrodes (tip resistance c. 20 mΩ) filled with 3 M KCl. The membrane potential was amplified by a Grass P16 d.c. amplifier and displayed on a Telequipment DM 63 variable storage oscilloscope. Each value of membrane potential is the mean of measurements obtained from 10–15 fibres and in at least three muscles for each potassium concentration.

Mean values and standard error of the mean (s.e.mean) were calculated as appropriate. The statistical significance of differences between data was calculated using Student's t test for independent means; and a level of $P < 0.05$ was considered to be significant.

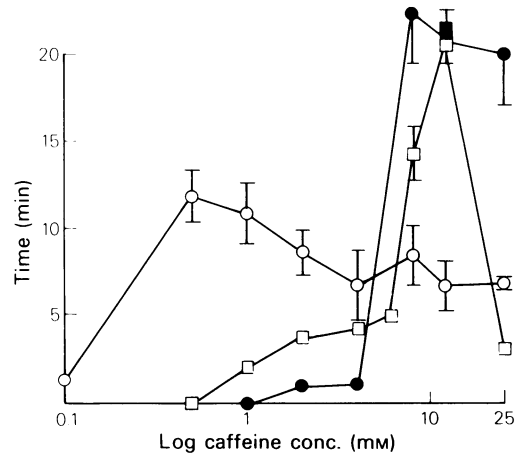

Figure 2 Time to peak tension of caffeine contractures of the soleus at 4 (○), 22 (●) and 37°C (□) and of the e.d.l. at 37°C (■). Values are means ± s.e.means (vertical lines) ($n > 4$).

Results

(a) *Control*

Application of caffeine to the rat soleus muscle evoked contractures whose amplitude and time course depended upon the concentration of caffeine and the temperature. The relationship between caffeine concentration and the peak tension of the contracture at three different temperatures, 4, 22 and 37°C is shown in Figure 1.

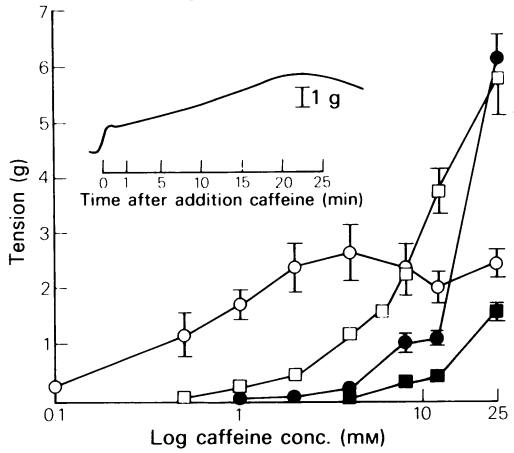

Figure 1 Dose-response curve of the soleus muscle to caffeine at 4 (○), 22 (●), and 37°C (□) and of the e.d.l. at 37°C (■). Values are means ± s.e.means (vertical lines) ($n > 4$). Inset shows biphasic nature of 12 mM caffeine contracture of rat soleus at 37°C.

At 37°C, the contracture threshold was between 0.5 and 1 mM. Concentrations of caffeine up to 6 mM caused monophasic contractures. Above 6 mM caffeine contractures were biphasic (Figure 1 inset), with the amplitude of the first phase 25–40% of the second phase.

The time to first phase tension of the 8, 12 and 25 mM caffeine contractures was 22.7 ± 3.1 ($n = 4$), 21.8 ± 3.0 ($n = 6$) and 10.5 ± 1.5 ($n = 4$) s, respectively. The time to peak tension increased from 2.2 ± 0.3 ($n = 4$) in 1 mM caffeine to 20.5 ± 1.1 ($n = 6$) min in 12 mM, although it fell to 3.2 ± 0.1 ($n = 4$) min in 25 mM caffeine (Figure 2).

At 22 and 4°C, the caffeine contracture developed tension uniphasically. At 22°C, the caffeine contracture threshold was between 1 and 2 mM, and the concentration-effect curve was moved to the right of that at 37°C (Figure 1). Only with 25 mM caffeine were the contracture tensions similar at 22 and 37°C. The time to peak tension was about 1 min at 2 and 4 mM caffeine. With higher concentrations of caffeine, the time to peak tension was over 20 min (Figure 2).

Caffeine had the lowest contracture threshold at 4°C (0.05–0.1 mM), and peak tension was attained in 4 mM caffeine (Figure 1). The times to peak tension with caffeine concentrations up to and including 4 mM were longer and above 4 mM were shorter at 4°C than at 22 or 37°C (Figure 2). In the e.d.l. caffeine was far less potent at all temperatures than in the soleus. The contracture threshold was four times greater and peak tension was at most one quarter that seen in the soleus (Figures 1 and 2).

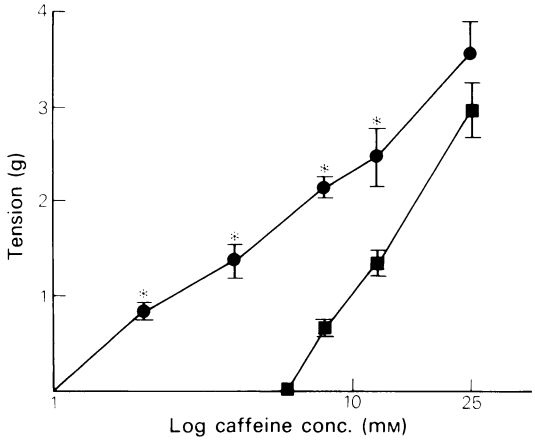

Figure 3 Amplitude of first phase tension of caffeine contractures of rat soleus in normal physiological saline solution (■) and after a two minute pre-exposure to 10^{-9} M calcium solution (●) at 37°C. The values are the means and the vertical lines represent s.e.means. ($n > 4$). Asterisks indicate values significantly different from control values ($P < 0.05$).

(b) *Effect of calcium*

Equilibration of the soleus at 37°C in 10^{-9} M calcium (Ca) for up to 10 min before the addition of caffeine in 10^{-9} M Ca, significantly increased the amplitude of the first phase of the caffeine contracture. Thus, a 2 min incubation in 10^{-9} M Ca caused a large shift to the left of the caffeine concentration-effect curve (Figure 3). Longer periods of equilibration in 10^{-9} M Ca depressed first phase tension. The second phase of the caffeine contracture was always reduced by equilibration in 10^{-9} M Ca (Figure 4). Thus, the peak amplitude of the caffeine contracture was reduced by 64% after 10 min in 10^{-9} M Ca and was virtually abolished after 1 h in 10^{-9} M Ca.

The time to attain first and second phase tension was reduced by equilibration in 10^{-9} M Ca. For example, after 5 min in 10^{-9} M Ca, the first phase rise time of the 12 mM caffeine contracture was reduced to 67% of the control value of 30.8 ± 4.3 s ($n = 4$) while the time to second phase tension was reduced to 46% of the control value of 22.1 ± 2.0 min ($n = 4$).

At 22 and 4°C, equilibration in 10^{-9} M Ca had similar though less pronounced effects, than at 37°C on the subsequent caffeine contracture.

It was possible to restore caffeine contractures in the soleus muscle after they had been abolished completely by a 2 h equilibration in 10^{-9} M Ca at 37°C. After incubation for 2 h in 10^{-9} M Ca, the soleus muscles were re-exposed for 30 min to solutions containing known concentrations of Ca and

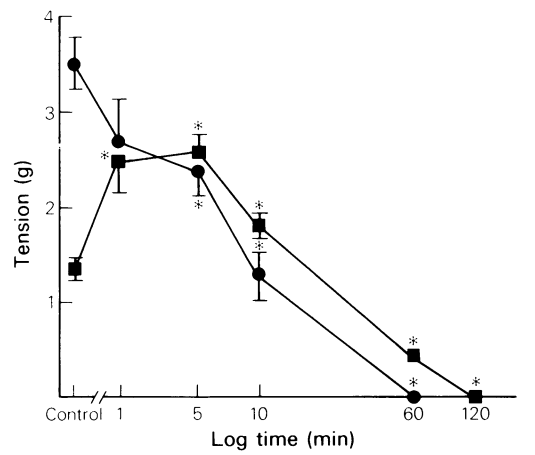

Figure 4 Amplitude of the first (■) and second (●) phases of the 12 mM caffeine contracture as a function of time in 10^{-9} M calcium solution before the addition of caffeine. The values are the means and the vertical lines represent s.e.means ($n > 4$). Asterisks indicate that the value is significantly different from the control value ($P < 0.05$).

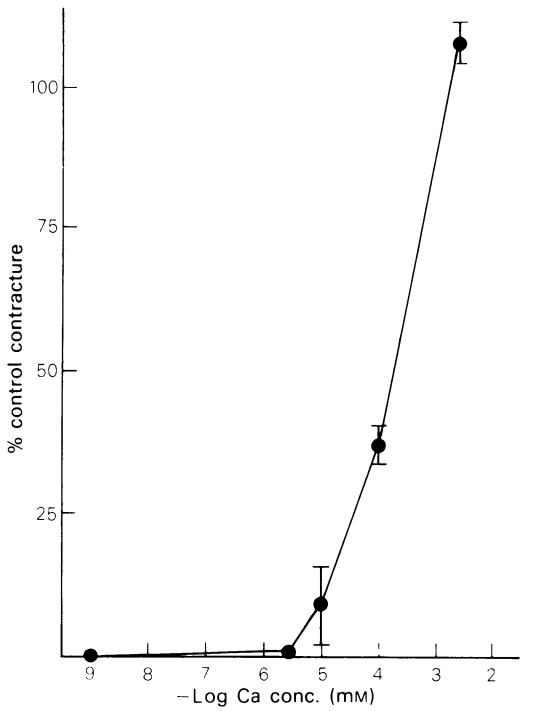

Figure 5 Restoration of the 12 mM caffeine contracture of rat soleus after abolition by a two hour exposure to 10^{-9} M calcium solution as a function of calcium concentration employed. Values are expressed as a percentage of contracture in normal physiological saline solution ($n > 4$), means ± s.e.means (vertical lines).

Table 1 Effect of lanthanum (0.5 mM) on the 12 mM caffeine contracture of rat soleus muscle in normal calcium saline at 37°C

Incubation time in 0.5 mM lanthanum (min)	*Tension of first phase* (g)	*Time to first phase (s)*	*Peak tension* (g)	*Time to peak tension (min)*
0 (control) ($n=4$)	1.4±0.1	30.8±4.3	3.5±0.3	22.1±2.0
2 ($n=3$)	1.5±0.4	29.0±3.0	3.8±0.7	13.5±2.8*
	2.4±0.2*	23.7±0.7	5.3±0.9*	7.6±2.1*

The values shown are means ± s.e.means and the asterisks indicate that the values are significantly different from the control values ($P<0.05$).

then 12 mM caffeine was added in the same concentration of Ca. The threshold for restoration of the caffeine contracture was below 2.9×10^{-6} M Ca (Figure 5). The amplitude of the caffeine contracture increased with the Ca concentration and 50% or full restoration occurred with 1.9×10^{-4} or 2.52×10^{-3} M Ca present, respectively.

In the e.d.l., the reduction of external calcium to 10^{-9} M progressively reduced the amplitude and the time course of the 12 mM caffeine contracture at 37°C. For example, after 10 min in 10^{-9} M Ca, the caffeine contracture amplitude was only 24% of control while the time to peak tension was reduced by almost 92%.

(c) *Effect of lanthanum*

At 37°C, equilibration of the soleus muscle in 0.5 mM La augmented the amplitude of the first and second phases of the 12 mM caffeine contracture by 79 and 52%, respectively and reduced considerably the time taken to attain these values (Table 1). A lower concentration of La (0.1 mM) was without significant effect on the 12 mM caffeine contracture, whilst 1 mM La blocked the caffeine contracture completely.

At both 22 and 4°C, 0.5 mM La had much less effect on the amplitude and time course of the 12 mM caffeine contracture than at 37°C.

The effect of 0.5 mM La on the 12 mM caffeine contracture was also examined by addition of this concentration of La to 10^{-9} M Ca solutions (Table 2). Thus, incubation of the muscle for 2 min in 10^{-9} M Ca with 0.5 mM La added before the addition of caffeine in this solution reduced the augmentation of the first phase to 25%. The amplitude of the second phase in 10^{-9} M Ca was further reduced by the presence of 0.5 mM La. Thus, the augmentation of the contracture tension by La in normal Ca solution was altered to an inhibitory action in the absence of Ca. The time to attain first phase tension in 10^{-9} M Ca was increased 148% by the presence of 0.5 mM La, while the time to second phase tension was little changed.

(d) *Effect of extracellular potassium*

The membrane potentials of soleus fibres corres-

Table 2 Effect of lanthanum (0.5 mM) on the 12 mM caffeine contracture of rat soleus in 10^{-9} M Ca physiological saline at 37°C

Treatment	*First phase of tension* (g)	*Time to first phase of tension* (s)	*Final peak tension* (g)	*Time to final peak tension* (min)
10^{-9} M Ca for 2 min ($n=4$)	2.5±0.3	11.5±1.0	2.7±0.4	18.6±3.1
As above + 0.5 mM La ($n=4$)	1.7±0.2	28.5±1.9*	2.0±0.3	18.9±4.0
10^{-9} M Ca for 10 min	1.8±0.1	17.3±2.5	1.3±0.3	21.7±5.2
As above + 0.5 mM La for final 2 min ($n=4$)	1.8±0.2	20.0±1.6	—	—

The values shown are means ± s.e.means and the asterisk indicates that the value is significantly different from its corresponding control ($P<0.05$).

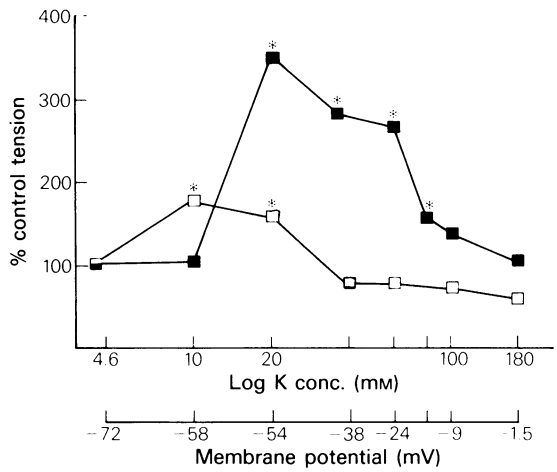

Figure 6 Amplitude of 12 mM caffeine contracture as a function of extracellular potassium after a 1 min exposure to potassium. Mean values are for the soleus at 37°C (□) and for the e.d.l. at 37°C (■). Asterisks indicate that the values are significantly different from the control values ($P < 0.05$).

ponding to different levels of potassium in the bathing solution are shown on the lower abscissa scale of Figure 6. Raising the membrane potential from −72 mV to between −58 and −38 mV increased the amplitude of the 12 mM caffeine contracture (Figure 6). When the membrane potential was increased above −38 mV, the amplitude of the caffeine contracture was decreased. The times to attain first and second phase tension at 37°C were shortened at membrane potentials between −58 and −38 mV. With potentials above −38 mV these times were progressively prolonged.

In the e.d.l., the changes in the amplitude and time course of the caffeine contracture were significantly increased from control by increasing the potassium concentration of the bathing solution to between 20–80 mM, the greatest increase occurring with 20 mM potassium (Figure 6).

Discussion

The modification of the caffeine contracture by membrane potential, multivalent cations and temperature reported in this paper supports the hypothesis that caffeine acts at the sarcolemma, probably on the t-tubule membrane (Luttgau & Oetliker, 1968, Foulks *et al.*, 1972). The mechanisms of caffeine action are suggested to be similar to the activation and inactivation processes which govern the tension development of potassium contractures i.e. a shift of the membrane potential to more positive potentials increases first activation and then inactivation. Thus, the action of caffeine can be thought of as facilitating activation by moving the potassium activation curve closer to the resting membrane potential, retarding the onset of inactivation and moving the inactivation curve to the more positive potentials (Luttgau, 1963). Furthermore, it is suggested that alterations in external K, divalent ions and temperature are affecting the K contractures by altering the activation and inactivation curves. For example, the removal of calcium results in an apparent depolarization of the membrane (Luttgau & Spiecker, 1979) and a rapid shift of the activation curve and a slower shift of the inactivation curves closer to the resting membrane potential. In contrast, the addition of low concentrations (0.1–0.5 mM) of the membrane impermeant cation lanthanum causes an apparent hyperpolarization of the membrane and a shift of the activation and especially the inactivation curves further from the resting membrane potential. With the higher concentration of lanthanum, the shift of the activation curve is more pronounced.

Small depolarizations of 14–18 mV, produced by raising the potassium concentration of the bathing solution to between 10–20 mM, caused large increases in the amplitude of the caffeine contracture, by greatly increasing activation and only slightly changing inactivation. Depolarizations greater than 20–30 mV reduced the amplitude of the caffeine contracture, by producing a large increase in inactivation. Similar findings have been demonstrated in frog twitch muscle (Foulks *et al.*, 1971, Geffner *et al.*, 1975).

The higher threshold and smaller amplitude of caffeine contractures in the e.d.l. than in the soleus is not due to differences in muscle size or the diffusion of caffeine through the muscle (Isaacson *et al.*, 1970). The different responses of the two muscles to caffeine can be readily explained by differences in the process of activation and inactivation. Both the activation threshold and curve are at more positive potentials in the e.d.l. than the soleus (Dulhunty, 1980; 1981). Also, Close (1972) found that the half decay time of contractures (which is indicative of the rate of onset of inactivation) was more rapid in the e.d.l. than in the soleus muscle.

The reduction in the amplitude of the caffeine contracture as the temperature is lowered from 37°C to 22°C can be explained by a shift of the activation curve to more positive potentials and a shift of the inactivation curve to more negative potentials (Caputo, 1972; Foulks & Marishita, 1980; Dulhunty, 1981). As the temperature is lowered to 4°C, the increase in the amplitude of the caffeine contracture is probably caused by the large decrease in calcium uptake by the sarcoplasmic reticulum (Martinosi & Feretos, 1964).

Biphasic caffeine contractures similar to those in the present study have been demonstrated in amphibian whole muscle and single fibre preparations (Bianchi, 1968; Gebert, 1968) and mammalian muscle (Isaacson *et al.*, 1970). This biphasicity is unlikely to be due to several populations of fibres, since biphasic contractures do not occur in the e.d.l. muscle which is composed of a heterogeneous population of fibres (Buchtal & Schmalbruch, 1980). Neither is it due to diffusion of caffeine throughout the muscle, since biphasic contractures occur in frog single fibre preparations. Dulhunty (1980) has shown that peak tension of a potassium contracture was attained before significant depolarization of fibres below the first layer of fibres had occurred. The two phases of the caffeine contracture could indicate that caffeine acts on all the immediately accessible sarcolemmal sites to produce the first phase of tension and then acts on more deeply located, less accessible sites to produce the second phase. The reason for non-biphasicity of contractures in the e.d.l. may be the narrower diameter of t-tubules (Buchtal & Schmalbruch, 1980) which would restrict access to deeply located sarcolemmal sites.

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