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Potassium contractures and mechanical activation in rat skeletal muscle: effects of multivalent cations, temperature and tetracaine

R. Anwyl, J.D. Bruton & J.V. McLoughlin

Department of Physiology, Trinity College, Dublin, Dublin 2, Ireland

1 The effects of cations, temperature and tetracaine on potassium-induced contractures of rat soleus and extensor digitorus longus (e.d.l.) muscles were investigated.

2 In the soleus, the threshold for the potassium contracture was lower (10–20 vs 20–40 mM), the peak amplitude was up to fourteen times larger, and the time course was about one half that in the e.d.l. muscle. The extent of inactivation of a test potassium contracture was directly related to the concentration of potassium in the conditioning solution and the period of exposure.

3 Removal of calcium reduced the amplitude and time course of potassium contractures in both preparations. Addition of cobalt (10 mM) reduced the amplitude but prolonged the time course of contractures.

4 Exposure of muscles to tetracaine (10^{-5}–10^{-6} M for 30 min) increased, but higher concentrations reduced, the amplitude of potassium contractures. When present for one minute, tetracaine (1 mM) moved the potassium activation curve to higher, and the potassium inactivation curve to lower, potassium concentrations.

Introduction

Potassium-induced contractures of amphibian skeletal muscle have been the subject of many studies (e.g. Hodgkin & Horowicz 1960; Luttgau & Spiecker, 1979), from which it has been suggested that the development of contracture tension is governed by the interaction of two processes, activation and inactivation (Hodgkin & Horowicz, 1960; Caputo, 1972b).

Tension development of potassium contractures in amphibian muscles is modified by changes in external calcium (Luttgau, 1963; Cota & Stefani, 1981; 1982). Some, but not all, divalent and trivalent cations can substitute for calcium in maintaining the amplitude and to a certain extent the time course of potassium contractures (Frank, 1962; Luttgau & Spiecker, 1979). The effects of di- and tri-valent cations on the excitation-contraction coupling of potassium contractures have been suggested to be exerted by changes in the surface membrane potential (D'Arrigo, 1973; Dorrscheidt-Kafer, 1981). Other workers have shown that potassium contractures are modified by changes in the resting membrane potential (Frankenhaeuser & Lannergren, 1967) and in temperature (Caputo, 1972a; Foulks & Morishita, 1980).

There is a paucity of studies concerning potassium contractures and the processes of activation and inactivation which govern tension development in mammalian skeletal muscle. This study was designed to investigate potassium contractures in rat fast and slow twitch skeletal muscle, and to investigate the effects of external divalent cation concentration and temperature on these potassium contractures. The effect of tetracaine, a local anaesthetic thought to block the release of calcium from isolated sarcoplasmic reticulum (Bianchi, 1975), on potassium contractures was also investigated.

Methods

The soleus (10–12 mg) and extensor digitorus longus (e.d.l.) muscles (5–7 mg) of female rats were used in this study. Muscles were mounted in a physiological salt solution contained in a water jacketed muscle bath and equilibrated for 50 min to 1 h before the start of experiments. 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. Cobalt pre-

cipitated out of this salt solution as a bicarbonate salt and so a modified salt solution containing 2 mmol l^{-1} of NaHEPES in place of $NaHCO_3$ and KH_2PO_4 was used in some experiments. Solutions containing 10^{-9} M Ca were prepared by omitting $CaCl_2$ and adding 1 mmol l^{-1} EGTA. Solutions of high potassium were prepared using K_2SO_4 in order to maintain the (K) × (Cl) product constant and sucrose was added to keep solutions isotonic with the ordinary salt solution. Chemicals used were of general reagent or in the case of 10^{-9} M Ca solutions, A.R. grade chemicals only were used.

Solutions were maintained at a constant temperature by pumping water through the water jacket of the muscle bath. The final pH of solutions was 7.00 ± 0.05 and they were oxygenated continuously with 95% O_2 plus 5% CO_2 or in the case of the modified salt solution with 100% O_2.

Muscles were maintained under a resting tension of 1 g. Changes in tension were followed by a Statham UF1 strain gauge transducer, amplified by a Washington FC135 coupler and recorded on a Servoscribe 2S pen recorder. Such muscle was exposed to only one test with potassium.

Values in the text refer to the mean ± s.e. mean of n determinations. Differences in mean values were determined using Student's t test. P values < 0.05 were considered to be significant.

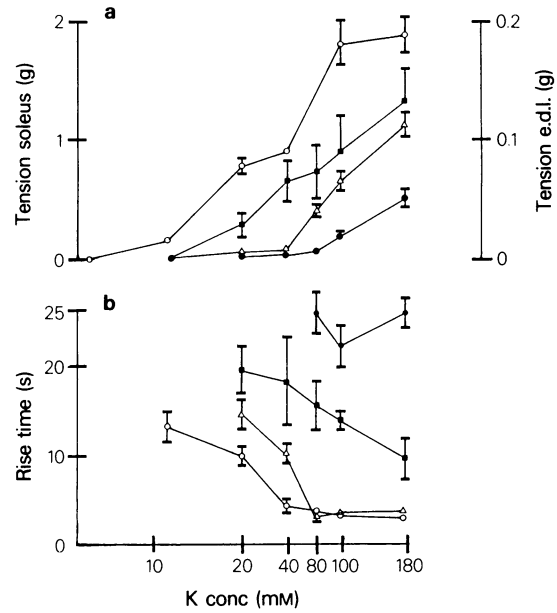

Figure 1 Details of potassium contractures in the soleus at 4(●), 22(△) and 37 °C(○) and in the e.d.l. muscle (■) at 37 °C. (a) Peak tension, (b) time to peak tension. Each point represents the mean value ($n > 4$) and vertical lines show s.e.means.

Results

Application of elevated concentrations of potassium to rat skeletal muscle evoked contractures which rose rapidly to a peak and then decayed more slowly. The potassium activation curve of the rat soleus at 4, 22 and 37 °C and of the rat e.d.l. at 37 °C only are shown in Figure 1a. In the soleus, the contracture threshold was lowest (10–20 mM) at 37 °C and highest (40–60 mM) at 4 °C. Contracture tension was directly related to temperature, for example the 180 mM K contracture tension at 22 and 4 °C was only 60 and 27%, respectively, of that at 37 °C.

At 37 °C, the application of 180 mM K to strips of soleus muscle, one half to one fifth the size of whole muscles, produced contractures similar or larger in amplitude than those of intact muscles. The time courses of contractures in split muscles were similar to those of intact muscles.

The times to peak and half decay of tension were up to seven times longer at 4 °C than at 22 or 37 °C (Figure 1b and Table 1). As the K concentration was increased to 80 mM, rise time and half decay time decreased. Above 80 mM K, these values varied little compared to control values.

In the e.d.l., the contracture threshold was higher (20–40 mM) and the amplitude of contractures was up to fourteen times smaller than in the soleus. In addition, the times to peak tension and half decay of tension were at least twice as long in the e.d.l. as in the soleus (Figure 1).

Table 1 The 50% decay time of potassium contractures of the soleus muscle in control solution and in the presence of 1 mM tetracaine

	Potassium concentration (mM)				
	40	*60*	*80*	*100*	*180*
Control 37 °C	22.0 ± 1.3	17.7 ± 3.2	20.2 ± 2.2	22.0 ± 2.5	15.5 ± 2.5
Control 4 °C	–	–	–	90.0 ± 8.0	39.8 ± 2.8
Tetracaine 1 mM 37 °C	–	27.3 ± 2.6	30.4 ± 2.9	32.8 ± 3.6	29.7 ± 3.1

The values shown are the means ± s.e.means of at least 4 determinations.

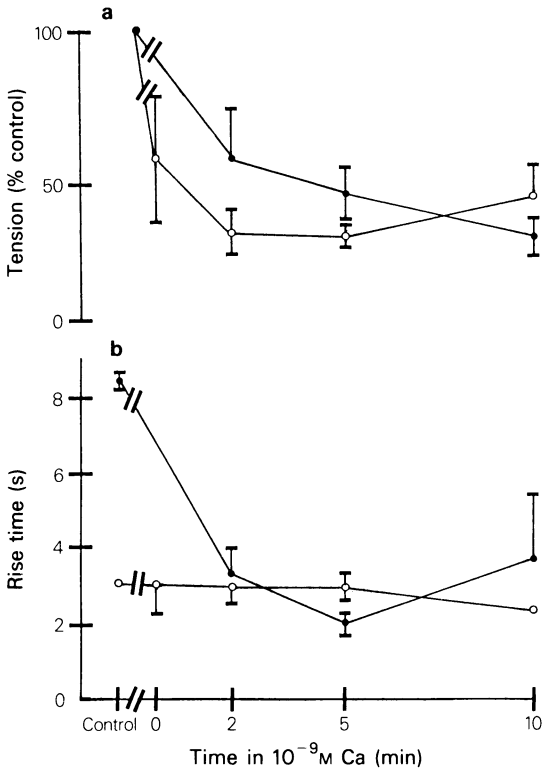

Figure 2 Amplitude and time course of a test 180 mM potassium contracture in the soleus (○) and e.d.l. (●) muscles as a function of time in 10^{-9} M calcium before the addition of potassium. In (a) tension is expressed as a percentage of the test contracture in normal calcium containing salt solution (b) shows the time to peak tension. Each point represents the mean value ($n > 4$) and vertical lines show s.e.means.

(a) *Effect of calcium*

The changes in the amplitude and time course of the 180 mM K contracture of the soleus and e.d.l. muscles produced by reducing calcium to 10^{-9} M at 37 °C are shown in Figure 2. In the soleus, the reduction of Ca to 10^{-9} M concomitant with the addition of 180 mM K reduced the amplitude of the contracture by 42%. Longer periods of equilibration in 10^{-9} M Ca before the addition of 180 mM K produced further reductions in the amplitude of the contracture and after 1 h pre-equilibration in 10^{-9} M Ca the contracture was abolished. The time to peak tension (Figure 2b) was not affected by equilibration periods in 10^{-9} M Ca up to 5 min but was significantly reduced by 24% ($P < 0.05$) after the 10 min equilibration period. Equilibration of the soleus for 10 min in 10^{-9} M Ca at 22 or 4 °C did not significantly alter the amplitude of the 180 mM K contracture. However, the times to peak and half decay of tension were significantly reduced (33–50%) at both 22 and 4 °C.

In the e.d.l. at 37 °C, the 180 mM K contracture was also reduced by all equilibration periods in 10^{-9} M Ca (Figure 2).

(b) *Effect of cobalt*

The addition of 10 mM cobalt to normal Ca containing salt solution caused the amplitude of the 180 mM K -induced contracture of the soleus muscle to be significantly reduced (Table 2). This reduction ranged from 79% at 37 °C to 83% at 4 °C. While the times to peak tension were significantly increased ($P < 0.05$) compared to control times at 22 and 37°C, the rise time of contractures at 4°C were similar in the presence and in the absence of cobalt. Cobalt did not significantly alter the half decay times of contractures at any temperature. In the e.d.l. at 37 °C, cobalt had

Table 2 Effect of a ten minute exposure to 10 mM cobalt on the 180 mM K contracture of rat soleus (Sol) and E.d.l. muscles

Muscle and Temperature	*Tension* (g)	*Rise time* (s)	*50% decay time* (s)
Sol control at 37 C	1.8±0.16	3.0±0.09	15.4±1.62
Sol + cobalt	0.3 ±0.13*	8.1±1.24	14.6±1.39
Sol control at 22 C	1.1±0.11	3.6±0.35	6.3±0.84
Sol + cobalt	0.2±0.08*	5.1±0.33*	8.5±2.18
Sol control at 4 C	0.5±0.08	25.9±1.84	39.7±2.58
Sol + cobalt	0.0±0.03*	24.2±2.25	36.5±5.56
E.d.l. control at 37 °C	0.4±0.05	>60	-
E.d.l. + cobalt	0.0±0.02	>60	-

The values shown are the means ± s.e.means of at least 4 determinations. The asterisk indicates values significantly different to control, $P < 0.01$.

greater effects on the 180 mM K -induced contracture than in the soleus. The amplitude of the contracture was reduced to 12% of control and the time to peak tension was increased more than seven fold by cobalt, both changes being significantly different from control ($P<0.05$).

(c) *Inactivation of K contractures*

Previous workers have described how the amplitude of a test potassium contracture depends upon the previous history of the muscle (Frankenhaeuser & Lannergren, 1967; Caputo, 1972a,b). Pre-incubation of a muscle in conditioning solutions containing elevated concentrations of potassium were shown to reduce the amplitude of a test contracture and this process was termed inactivation. The potassium inactivation curves of the soleus at 13, 22 and 37 °C obtained by exposing muscles to K conditioning solutions for 1 min are shown in Figure 3a. In the soleus, the potassium inactivation curve at 13 °C was to the left of that at 37 °C which in turn was to the left of that at 22 °C. With conditioning solutions of potassium up to 40 mM, the amplitude of a 180 mM K test contracture was increased. The greatest potentiation occurred with 10 mM K at 13 and 37 °C and with 30 mM K at 22 °C. Higher concentrations of potassium reduced the amplitude of the test contracture and the test contracture was completely abolished by pre-exposure to 100 mM K at all three temperatures.

The time to attain peak tension was significantly ($P<0.05$) increased compared to control values by K conditioning solutions above 10 mM at 13 and 22°C and above 40 mM at 37°C. At 22°C, conditioning K solutions up to 30 mM increased ($P<0.05$) the half decay time of contractures by up to 900% and with higher K conditioning solutions, the half decay time was reduced below control. At 37 °C, the half decay time of contractures was significantly increased compared to control times ($P<0.05$) by the 10 and 30 mM K conditioning solutions.

The time-dependence of inactivation of the 180 mM K test contracture using 30, 40 or 60 mM K as the conditioning solution at 22 °C was investigated. As the period of exposure to the conditioning solution increased, the amplitude of the test contracture

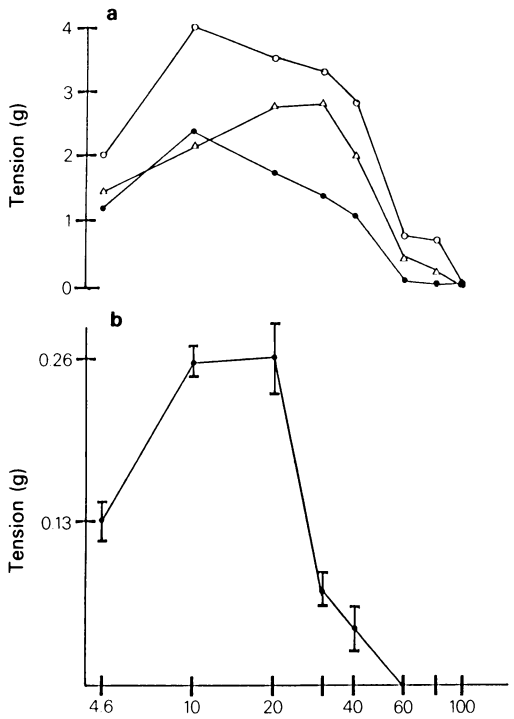

Figure 3 Details of inactivation of a test 180 mM potassium contracture in (a) soleus at 13(○), 22(△) or 37 °C(●) and (b) e.d.l. at 37 °C. Each point represents the mean value ($n>4$) and vertical lines show s.e.means.

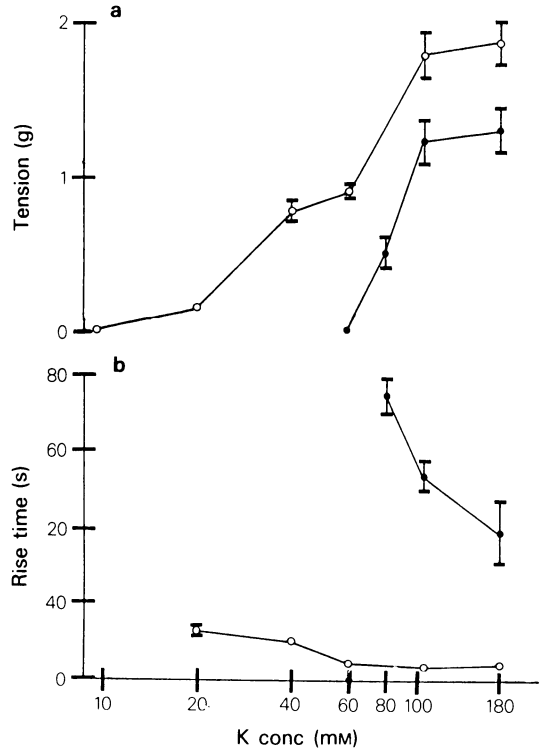

Figure 4 Effect of 1 mM tetracaine on (a) amplitude (b) time to peak tension of potassium contractures at 37 C in rat soleus. Control (○) and with tetracaine (●). Each point represents the mean value ($n>3$) and vertical lines show s.e.means.

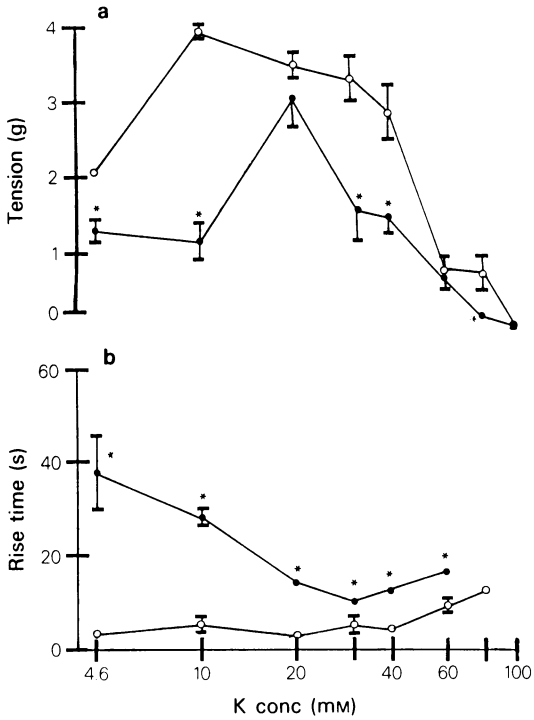

Figure 5 Effect of 1 mM tetracaine on inactivation parameters of rat soleus at 37 °C (a) amplitude, (b) time to peak tension. Control (○) and with tetracaine (●). Each point represents the mean value ($n > 3$) and vertical lines show s.e.means. Asterisk indicates value significantly different from control value ($P < 0.05$).

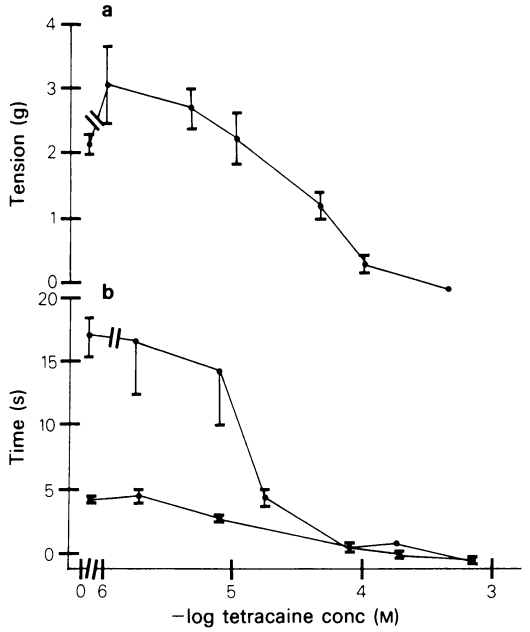

Figure 6 Changes in the (a) amplitude (b) time to peak and half-decay time of tension of the 180 mM potassium contracture of rat soleus produced by exposure of the muscle to different concentrations of tetracaine. Each point represents the mean value ($n > 3$) and vertical lines show s.e.means.

after an initial potentiation, decreased. The test contracture was abolished 2, 10 and 20 min after exposure to K conditioning solutions of 60, 40 and 30 mM, respectively. Increasing the exposure time to all conditioning solutions prolonged the time to peak tension but decreased the half decay time of the test contracture.

In the e.d.l. at 37 °C, pre-incubation in 10 and 20 mM K conditioning solutions for 1 min potentiated the amplitude of the test contracture by 98 and 99% respectively (Figure 3b). Higher concentrations of potassium reduced the amplitude of the test contracture. With 60 mM K as the conditioning solution, the test contracture was completely abolished. Both the time to peak tension and half decay time were progressively decreased as the K concentration of the conditioning solution was increased.

(d) *Effect of tetracaine*

Figure 4 and Table 1 show the effects of 1 mM tetracaine on potassium contractures in the soleus at 37 °C. The potassium contracture threshold was raised from 10–20 mM to 60–80 mM and the potassium activation curve was shifted to the right of control by 1 mM tetracaine.

The effects of tetracaine on the potassium inactivation curve of the soleus at 37 °C are shown in Figure 5. Tetracaine reduced the amplitude of the test contracture after exposure to all conditioning solutions, but did not reduce the K concentration required to abolish the test contracture. Peak enhancement of the test contracture now occurred with a higher concentration of potassium in the conditioning solution. The time to peak tension of the test contracture after exposure to all conditioning solutions was significantly ($P < 0.05$) increased compared to control values by tetracaine. The half decay time of the tension was greater than control values in the presence of tetracaine, with conditioning solutions containing up to 20 mM K. However, with higher concentrations of K in the conditioning solutions, tetracaine had little effect on the half decay time.

Figure 6 shows the effects of a 30 min equilibration period in tetracaine concentrations between 10^{-6} and 5×10^{-4} M on the 180 mM K contracture at 37 °C. The amplitude of the 180 mM K contracture was

increased up to 50% above control values by 10^{-6}–10^{-5} M tetracaine. Higher concentrations of tetracaine depressed the contracture and the 180 mM K contracture was abolished by pre-equilibration in 5×10^{-5} M tetracaine. Both the time to peak tension and the half decay time of the contracture were reduced by all concentrations of tetracaine and these reductions were directly related to the concentration of tetracaine used.

Discussion

This study has clearly demonstrated that the processes of activation and inactivation which control the tension development of potassium contractures are affected by the level of divalent cations in the bathing medium, the resting membrane potential and the temperature. The reduction of external Ca to 10^{-9} M reduced the amplitude and time course of potassium contractures, while the addition of cobalt reduced the amplitude but prolonged the time course of contractures. Previous workers have found that the amplitude and time course of potassium contractures in single fibre and whole muscle preparations are reduced and the contracture threshold increased by the reduction of Ca to 10^{-6} M (Frank, 1960; Luttgau, 1963) or 10^{-9} M (Frank, 1980). The amplitude of the potassium contracture was found to be unaffected if an appropriate concentration of magnesium or other divalent cation was added to Ca deficient media (Frank, 1962; Luttgau & Oetliker, 1968). When higher concentrations of magnesium (e.g. 5 mM) or other cations were added to either normal or Ca deficient media, the potassium contracture was reduced in amplitude and its time course prolonged (Cuputo, 1972b, Chiarandini, & Stefani 1973; Cota & Stefani, 1981; 1982).

The effects of divalent cations on potassium contractures described here and in previous studies can be explained in terms of changes in activation and inactivation. Divalent cations in the extracellular bathing medium have been suggested to control these processes by binding or screening charged groups on the surface of the sarcolemma (D'Arrigo 1973; Dorrscheidt-Kafer, 1981). As the external cationic composition is reduced, an apparent depolarization is induced. This causes a predominant shift on the inactivation curve closer to the resting membrane potential. On the other hand, the addition of the membrane impermeant cation, cobalt (McDonald *et al.*, 1981), increases the screening of surface charges and moves the activation and inactivation curves away from the resting potential.

In this study, exposure of a muscle for 1 min to conditioning solutions containing low concentrations of potassium potentiated the amplitude of a test contracture. Also, a given conditioning solution could either potentiate or reduce the amplitude of a test contracture depending on the period of exposure. The effect of potassium conditioning solutions on a test contracture have generally been reported as depressing its amplitude (Hodgkin & Horowicz, 1960; Luttgau & Oetliker, 1968; Caputo, 1972a). However, Frankenhaeuser & Lannergren (1967) noted that the test contracture was potentiated by exposure of a fibre to 20 mM potassium for 30 s but was depressed by longer exposure periods. This suggests that the level of inactivation existing in a muscle is determined not only by the resting potential but also the time for which the muscle has been at that potential.

Temperature affected both activation and inactivation. The potasium contracture threshold was increased, contractures were prolonged and the potassium activation curve moved to more negative potentials as the temperature was reduced from 37 to 4 C. Previous results of studies on the temperature dependency of potassium contractures are apparently contradictory. In frog single fibre or whole muscle preparations, the potassium activation curve was shifted to more negative potentials when the temperature was reduced from 18–20 °C (Gonzales-Seratos, 1965; Foulks & Morishita, 1980). Dulhunty (1981) showed that the potassium activation curve of mouse soleus was similar at 22 and 37 °C. Caputo (1972a) found that in frog single fibres, the potassium activation curve was shifted to more positive potentials by a decrease in temperature from 18 to 3 °C. However, this author reduced temperature to 3 °C only 30 s before the addition of potassium and a steady-state situation may not have existed.

Increasing or decreasing temperature from 22 °C moved the inactivation curve to more negative potentials but did not alter the potential at which inactivation became complete. The time course of the test contracture and the lesser effects of 10^{-9} M Ca on contractures at temperatures below 37 °C indicate that the rate at which inactivation proceeds is directly related to temperature. Inactivation was suggested by Luttgau & Spiecker (1979) to involve metabolic processes. However, the longer time course of contractures in the e.d.l. than in the soleus, together with the inhibitory effects of tetracaine on potassium contractures seen here do not corroborate this suggestion. Neither is it supported by the finding that at 4 °C, tetracaine accelerates the decay time of the tension of potassium contractures in frog single fibres (Caputo 1976). The process of inactivation may be mediated at the sarcolemma by a mechanism similar to that suggested by Caputo & Fernadez De Bolanos (1979).

In this study, tetracaine potentiated or depressed the amplitude of the potassium contracture and shor-

tened its time course depending upon the concentration used. Previous studies using relatively high concentrations of tetracaine (0.5–2 mM) showed a reduction in the amplitude of the potassium contracture and it was suggested that this was due to tetracaine blocking the release of calcium from the sarcoplasmic reticulum (Bianchi, 1975; Caputo, 1976; Almers & Best, 1976). Tetracaine was also shown to modify the kinetics of potassium channels (Almers, 1976). Thus, the effects of tetracaine on the potassium contracture could be due to actions exerted at two sites, one of which is easily accessible and the other more deeply located within the muscle fibre.

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