Biochimica et Biophysica Acta, 682 (1982) 238–244
Elsevier Biomedical Press

BBA 41188

CALCIUM EFFLUX DURING THE COLD-INDUCED CONTRACTION OF MAMMALIAN STRIATED MUSCLE FIBRES

R.E. JEACOCKE

Agricultural Research Council, Muscle Biology Division, Meat Research Institute, Langford, Bristol (U.K.)

(Received July 16th, 1982)

Key words: Ca^{2+} efflux; Ca^{2+} concentration; Muscle contraction; Sarcoplasm; (Bovine muscle)

The efflux of ^{45}Ca from mammalian slow twitch muscle fibres has been studied to provide a measure of the concentration of free Ca^{2+} in the sarcoplasm. The kinetically complex early phases of washout of the isotope are succeeded by a prolonged slower phase which exhibits first-order kinetics. This later phase is accelerated by caffeine, by preventing oxidative phosphorylation and also during an isometric contraction, whether this contraction is produced by lowering the temperature or by electrical stimulation. The local anaesthetic tetracaine abolishes the contraction caused by cold and in this case the rate constant for efflux is progressively lowered as the temperature is reduced (Q_{10} value of 2.3). The removal of external Na^+ and Ca^{2+} reduces the efflux rate constant. Caffeine, sodium removal and the inhibition of oxidative phosphorylation, all potentiate the cold contraction and the associated extra ^{45}Ca efflux. Ca removal causes the cold contraction to become phasic. It appears that caffeine, sodium removal, the inhibition of oxidative phosphorylation and a decrease in temperature to below 10°C are all treatments which, like electrical stimulation, increase the sarcoplasmic free calcium concentration to varying degrees.

Introduction

Certain mammalian striated muscles contract when cooled to temperatures below 10°C [1–7] and in the meat industry there is concern over the resultant decline in the quality of the meat which is produced. It seems probable that, in common with other types of muscle contraction, this contracture is initiated by an increase in the concentration of Ca^{2+} free in the sarcoplasm as Ca^{2+} is released from the sarcoplasmic reticulum [8–11]. It is possible, however, that low temperatures cause a contraction by other means, such as an increase in the Ca^{2+} affinity of the contractile apparatus. It was the purpose of this investigation to test the elevated Ca^{2+} hypothesis by making measurements of the rate of loss of ^{45}Ca from mammalian muscle fibres before, during and after a cold contraction. Direct measurement of intracellular free Ca^{2+} is now possible using either electrometric or optical techniques (for a review see Ref. 12), but such measurements pose very substantial difficulties. The problems of direct measurement dictated the use of an indirect measure in these preliminary studies.

Various authors have shown that the rate of efflux of ^{45}Ca, from a muscle previously loaded with this isotope, is accelerated when the muscle contracts (see, for example, Refs. 13–20). It is reasonable to suppose that this accelerated rate of loss of the isotope reflects the increase in concentration of free Ca^{2+} in the cytoplasm of the muscle cell during contraction. The detailed interpretation, however, of the kinetics and amplitude of the enhanced efflux with respect to the kinetics

Abbreviation: $pCa = -\log_{10}$(calcium ion concentration).

and amplitude of the change in cytoplasmic free Ca^{2+} remains uncertain. As a consequence, these efflux measurements can provide only a qualitative guide to the changes in sarcoplasmic Ca^{2+} concentration and it is necessary to bear in mind that various factors other than a change in intracellular Ca^{2+} concentration may cause changes in the rate of loss of the isotope. To gain some understanding of these complicating factors, this study also investigated the way a variety of factors affect the rate of loss of the isotope.

Materials and Methods

Bundles of muscle fibres were carefully dissected from the musculus sternomandibularis of beef animals immediately after the slaughter of the animal in the conventional manner. The sternomandibularis muscle is of the red slow twitch type and cold contracts quite strongly [3–7]. The dissection was performed in normal saline solution (see below) at 10°C and the preparation was finally reduced to cylindrical bundles of fibres about 0.3 mm diameter and 12 mm long. To the ends of these bundles were tied fine silk threads and, after transfer of the preparation to a small perspex trough, these threads were used to stretch the bundles between two micromanipulators to a sarcomere length of 2.5 μm. One micromanipulator carried a force transducer (Kulite Semiconductor) to measure the isometric force generated by the preparation. The total compliance of the measuring system was 200 $\mu m \cdot N^{-1}$. The cut ends of the fibres were sealed with a coat of vaseline/liquid paraffin mixture applied to both ends of the bundle. After 10 min the trough containing normal saline was replaced by a trough containing ^{45}Ca-labelled saline at 10°C in which the preparation was bathed for 3 h. This period was insufficient to enable all muscle Ca to reach isotopic equilibrium. Subsequently the fibres were washed, with magnetic stirring, in four successive baths of 20 ml normal saline solution at 10°C. Each bath lasted 10 min. The preparation was then placed in the experimental chamber (see below) and the isotope wash-out measurements were begun by collecting aliquots of the effluent from the central section (effluent rate 0.3 ml/min) whilst the preparation was continually irrigated with normal saline solution. The aliquots of effluent were mixed with 20 ml of Instagel (Packard) and the radioactivity was measured by liquid scintillation counting in a scintillation spectrometer (Packard Tri-Carb). At the end of the experiment the muscle fibres were dissolved in 100 vol. (30%, w/v) H_2O_2 and counted (with quench correction) by liquid scintillation as above. In this way, the radioactivity remaining in the preparation was determined. The efflux results were expressed either as semilogarithmic plots against time, of the count rate (cpm.) in that amount of effluent collected per unit time (per min) or as a first-order rate constant (min^{-1}) calculated as:

$$\frac{\text{radioactivity lost per min}}{\text{mean radioactivity remaining in the preparation during the collecting period}}$$

The normal saline solution which was employed to bathe the preparation had the following composition: 130 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM glucose, 3 mM sodium phosphate, pH 7.1. D-Tubocuracrine chloride, 20 μg/ml, was also present. When Na^+ was to be eliminated, NaCl was replaced by LiCl or choline chloride and when Ca^{2+} was to be absent, the 1.5 mM $CaCl_2$ was simply omitted. When potassium-containing saline was used, 100 mM K_2SO_4 replaced the NaCl and KCl. In all these experiments, air-equilibrated solutions were used. When the saline solution contained ^{45}Ca (approx. 100 μCi/ml) the isotope was added as $^{45}CaCl_2$ (Radiochemical Centre, Amersham) partly to replace the 1.5 mM $CaCl_2$. Other chemicals were AR grade.

The perspex experimental trough for the efflux measurements was similar to that used by Curtis [15] and consisted of a narrow central working section, 10 mm long, of 50 μl working volume, interconnecting two larger capacity end chambers. It was designed to separate, and collect, effluent which had irrigated the central section of the fibre preparation and to reject that which had irrigated the two ends. In this way ^{45}Ca, which was lost from the damaged ends of the fibres and from the attachments, was not collected. Direct electrical stimulation (0.7 ms, supramaximal (7 V) pulses) was provided through axial platinum wire elec-

trodes embedded in the sides of the central chamber.

The temperature of the fluid entering the trough (at 1.5 ml per min) was regulated by a proportionally controlled heater coil wound around a stainless-steel tube (through which the fluid flowed) and operating against a Peltier-effect refrigerator (Cambion Thermionic Products) which worked at constant power. The temperature of the fluid in the central working section of the trough was measured continuously with a fine (0.1 mm diameter) copper-constantan thermocouple junction.

Sarcomere length was measured by optical diffraction at 633 nm with a 2 mW helium/neon laser (Scientifica & Cook type SL-H/2).

Results and Discussion

The time course of loss of ^{45}Ca from a loaded preparation is indicated in Fig. 1. The measurements of the rate of loss start at 40 min after the series of four discontinuous washes had been completed. As has been observed with frog muscles in the past (see, e.g. Refs. 15, 17 and 19) the initial rate of loss is kinetically complex. Later, after about 100 min at 10°C, the kinetics become first order. There was little evidence, in these experiments, of a subsequent very slow component as had been observed in frog muscle by Caputo and Bolanos [19].

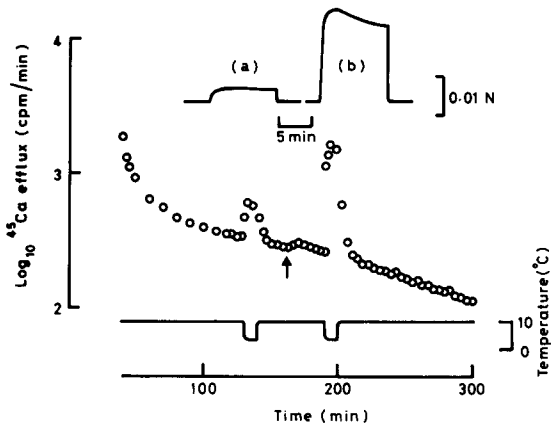

Fig. 1. The time course of ^{45}Ca efflux from musculus sternomandibularis fibres. Results are expressed as radioactivity in the effluent collected in unit time, against the mean wash-out time for that aliquot. The bath temperature was lowered from 10 to 3°C for two 10-min periods as indicated. At the arrow, normal saline solution was replaced by saline solution containing 0.8 mM caffeine. The tension changes in response to cooling are indicated (on an expanded time scale) in the insets.

The effect of decreasing the temperature of the preparation from 10 to 3°C for 10 min is to cause a contraction which is well maintained and approximately to double the rate of loss of the isotope. On rewarming to 10°C, the preparation relaxes and the efflux rate declines. The addition of caffeine (0.8 mM) causes a slight increase in the efflux rate constant as in frog muscle [14,20] but at this temperature, no contraction is produced. In the presence of caffeine, lowering the temperature to 3°C causes a much bigger contraction and an increase of about 6-fold in the efflux rate constant. Rewarming causes relaxation and a slow reduction in the efflux rate constant is observed. The large and rather similar contractures produced in frog muscle by cooling in the presence of low concentrations of caffeine have been studied by Sakai and his colleagues [21,22] and by Lüttgau and Oetliker [23] and it seems likely that they arise because caffeine potentiates Ca^{2+} release from the sarcoplasmic reticulum [10,11].

In a series of similar experiments, summarised in Fig. 2, the concentration of caffeine was altered in order to vary the isometric force developed at 3°C. The rate constant for efflux at 10°C was found to be rather variable in different preparations ($0.0022 + 0.0008$ min^{-1}, mean ± S.D., 18 observations) and so the observed rate constants are expressed relative to those measured in the same preparation at 3°C in the absence of caffeine. The forces developed were normalised in a similar way (see Fig. 2). The isometric force developed at 3°C increases with caffeine concentration (see inset to Fig. 2) and the relationship between efflux rate constant and force, plotted in Fig. 2, is non-linear and appears to saturate at high forces.

In other experiments, the magnitude of the force generated at 3°C was varied by other means. The local anaesthetic tetracaine (0.5 mM) abolishes the contraction normally observed at 3°C just as it inhibits other forms of contraction [20,23] whilst respiratory inhibition (with cyanide) or the uncoupling of oxidative phosphorylation (with 2,4-dinitrophenol) both cause increases in the force developed at 3°C. The latter effects had previously

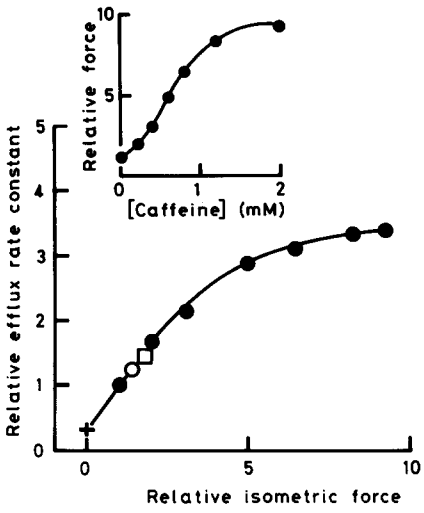

Fig. 2. The relationship (●) between the rate constant for ^{45}Ca efflux (relative to that observed in the same preparation upon cooling to 3°C in the absence of caffeine) and the relative isometric force developed after 4 min of the cooling procedure. The cross (+) represents the relative rate constant at 3°C in the presence of 0.5 mM tetracaine. Experiments were similar to those of Fig. 1 but the caffeine concentration was changed in order to vary the force (see the inset which depicts relative force measured as above against caffeine concentration). In the experiment (○) the increased isometric force was produced by the addition of 1 mM NaCN 10 min previously and in (□) the addition of 50 μM 2,4-dinitrophenol 30 min before, caused the augmented response.

been observed by Buege and Marsh [5]. It is clear from Fig. 2 that varying the force developed in these other ways varies the efflux rate constant by an amount similar to the administration of caffeine. In addition, and in common with low concentrations of caffeine, cyanide and dinitrophenol also cause slight increases in the rate of ^{45}Ca efflux from the resting preparation at 10°C (data not shown). The inset in Fig. 2 indicates that 2 mM caffeine is almost maximally effective in augmenting the tension produced by cooling. This concentration, however, causes only a small increase in steady force at 10°C (the force increment is about 8% of that produced subsequently by cooling). The maximal tension produced by cooling in the presence of caffeine is somewhat smaller than that produced in an electrically stimulated fused tetanus at 20°C.

The relationship between contractile force and p*Ca* is S-shaped with a steep dependence upon free Ca^{2+} concentration around p*Ca* 6 [11] whilst the relationship obtained here between ^{45}Ca efflux rate and tension is apparently hyperbolic. An appreciable Ca^{2+} efflux remains, however, at 3°C even when no tension is developed, and this residual efflux may be made to alter, for example, by altering the tetracaine concentration (data not shown). More than one process may contribute to Ca^{2+} efflux [23] and these processes probably have very different affinities for Ca^{2+}, so that efflux might be expected to increase over a wide range of p*Ca*.

Contraction elicited by electrical stimulation also produces an increased rate of loss of ^{45}Ca in this preparation, as has been observed in other muscles. At 10°C the contractile response to electrical stimulation is rather poor but at 20°C a tetanic contraction is obtained in response to stimulation at 25 Hz. A tetanic contraction of 2 min duration causes the rate constant for ^{45}Ca efflux to rise about 6-fold and then to decay to the original pre-stimulus level over the succeeding 10 min (data not shown). The efflux at 3°C is increased by a similar factor by a maximal cold contracture. Contractile activation by electrical stimulation and by cooling thus appear to have qualitatively similar effects on the ^{45}Ca efflux.

Since tetracaine abolishes the cold contraction, it seems likely that in its presence the temperature depence of the ^{45}Ca efflux process itself may be examined. Fig. 3 illustrates the inhibitory effect of a low concentration of tetracaine upon the ^{45}Ca efflux rate constant and upon the contraction induced by cooling. The slight decline in tension produced on cooling the preparation in this case probably reflects the anomalous rubber-like thermoelastic behaviour of the resting muscle [2] which is unmasked by suppressing the cold contracture. The tetracaine treatment of the muscle causes only a small decrease in efflux rate constant itself, but when the preparation is cooled to 3°C the normal cold contraction is abolished and now the rate constant drops considerably, to rise rapidly once more when the fibres are rewarmed.

In Fig. 4 the efflux rate constant (relative to that observed at 10°C in the particular preparation) is plotted against temperature for various tetracine-treated preparations. The Q_{10} value of

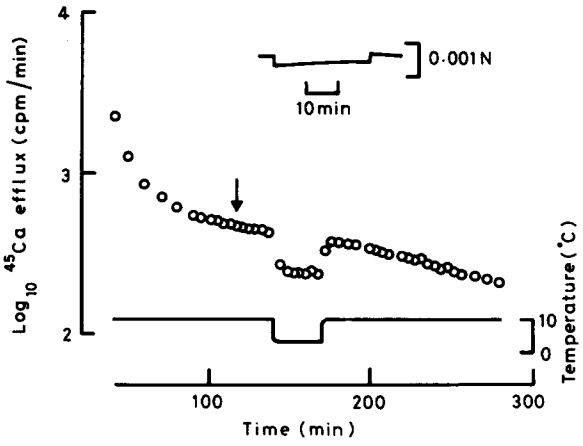

Fig. 3. The effect of cooling upon the rate of ^{45}Ca efflux and the tension in the presence of tetracaine. At the arrow, 0.5 mM tetracaine-containing saline replaced the normal saline solution. The bath was cooled from 10 to 3°C for a period of 30 min as indicated and the resultant change in tension is recorded in the inset (on an expanded time scale).

the efflux process in the absence of a cold contracture is 2.3 over the temperature range studied, and the energy of activation of the process, derived from an Arrhenius plot of the data, is 63 kJ · mol^{-1}. A temperature dependence equivalent to a Q_{10} of rather greater than 2 was also observed by Ashley et al. [25] in barnacle muscle fibres and by Reeves and Sutko [26] in cardiac sarcolemma membrane vesicles but a lower value (Q_{10} of 1.35) which may have been complicated by cold contracture (see Ref. 21) was obtained by Reuter and Seitz [27] in experiments with mammalian cardiac muscle. If due allowance is made for the temperature dependence of efflux observed in the present study, then the increase in calcium efflux associated with a cold contracture is rather greater than the increment actually observed upon cooling.

In frog muscle, Curtis [28] did not observe any increase in the rate of ^{45}Ca efflux during a potassium contracture at 1°C, although such an increase was evident at higher temperatures. This may have been related to the very high temperature coefficient of the efflux observed in his experiments.

In an attempt to characterise the efflux process further, the influence of the ionic composition of the bathing medium, upon the efflux rate constant, was also studied. The removal of either Ca^{2+} or Na^+ (at 10°C) results in a drop in efflux and when

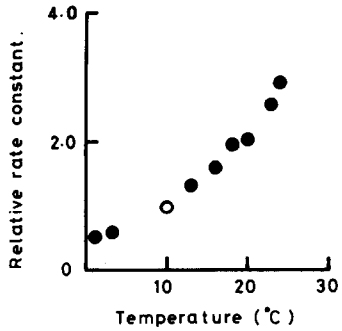

Fig. 4. The temperature dependence of the efflux rate constant (relative to that observed at 10°C in the same preparation in the presence of tetracaine (0.5 mM). The results were obtained in experiments similar to that of Fig. 3 but in which the bath temperature was varied.

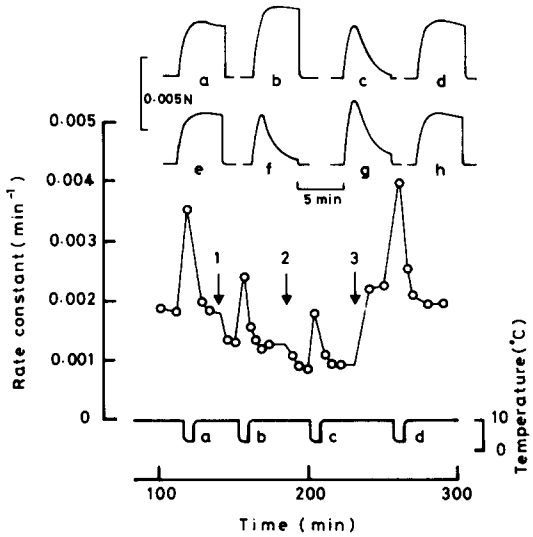

Fig. 5. The effect of external Ca^{2+} and Na^+ removal upon ^{45}Ca efflux and cold contractures. At the first arrow the normal saline solution was replaced by zero-Na^+ saline, and at the second arrow this was replaced by zero-Ca^{2+}, zero-Na^+ saline. At the third arrow normal saline solution was restored. The temperature was 10°C except where four cold contractures (a–d), each of 5 min duration, were elicited by reducing the temperature to 3°C. In zero-Na^+ salines, LiCl replaced NaCl. The isometric tension responses during the periods at 3°C (a–d) are recorded in the inset above the main figure. Insets e–h record the isometric tension responses in another, similar, experiment in which, however, zero-Ca^{2+} saline was applied at the first arrow followed by zero-Ca^{2+}, zero-Na^+ saline at the second arrow.

both ions are removed a further drop results (see Fig. 5). The original higher rate is re-established rapidly when perfusion with normal saline is resumed. Choline$^+$ and Li^+ behave similarly as Na^+ replacements (data not shown); similar results were obtained by Caputo and Bolanos [19] in frog muscle.

A substantial part (60%) of the observed ^{45}Ca efflux is apparently Ca^{2+} and Na^+ dependent; behaviour consistent with the catalysis of part of the efflux across the cell membrane by a Ca^{2+}-Na^+ exchange diffusion [29] process or Ca^{2+}/Na^+ antiporter in the terminology of Mitchell [30]. A similar process has now been observed in a variety of nerve and muscle preparations (for reviews see Refs. 31 and 32) and by driving Ca^{2+} out of the cell against its electrochemical potential gradient at the expense of the transmembrane Na^+ electrochemical potential difference, it probably accounts for part of the energy-dependent Ca^{2+} efflux which prevents a persistent increase in the intracellular Ca^{2+} content from occurring. If all the free energy necessary to drive Ca^{2+} out of the cell is derived from the transmembrane electrochemical potential difference of Na^+, then the antiporter must be electrogenic, catalysing the exchange of three or more Na^+ per Ca^{2+} (Refs. 26, 33 and 34, and for reviews, see Refs. 35 and 36).

An ATP-dependent, Ca^{2+}- and Na^+-independent, component of the Ca^{2+} efflux is also present [24] which may reflect a separate Ca^{2+}-translocating ATPase or an effect of ATP upon the Ca^{2+}-Na^+ exchange as has been observed by several authors (see, for example, Refs. 24 and 37). This ATP-dependent component may account for that portion of the Ca^{2+} efflux which remains when external Ca^{2+} and Na^+ are removed.

Fig. 5 records the results of Na^+ and Ca^{2+} withdrawal in an experiment in which the preparation was also cooled periodically to 3°C. The resultant cold contractures are indicated in the inset to Fig. 5 and it is evident that the amplitude of the contracture is increased somewhat by sodium removal and that the subsequent removal of Ca^{2+} reduces the amplitude and curtails the duration of the cold contracture. In other experiments, the effect of calcium removal was as marked in the presence of Na^+ as in its absence (inset Fig. 5, f, g). This behaviour is reminiscent of the curtailing effects of Ca^{2+} withdrawal upon a K^+ contracture in frog muscle (see, for example, Ref. 39). The enhancing effect of the cold contracture upon the rate constant for ^{45}Ca efflux does, however, persist even in the absence of added Ca^{2+} and Na^+.

Muscle contraction can be induced by caffeine in a manner which is independent of changes in membrane potential [9,10,23]. The contraction induced by cooling a rat soleus muscle is still observed in a potassium-depolarised preparation [2] and the cold contracture and the associated extra ^{45}Ca efflux exhibited by these preparations were also largely unaffected by prolonged depolarisation (results not shown). Since, in the depolarised preparation, contractile activation by cooling and the associated extra ^{45}Ca efflux, proceed in a manner which is, superficially at least, similar to those events seen in a normal, electrically excitable preparation, processes dependent on membrane potential are unlikely to play a major part in this type of contractile activation.

The rate of ATP-dependent Ca^{2+} accumulation by the sarcoplasmic reticulum has a high temperature coefficient (see, for example, Refs. 40 and 41), whilst the separate release process mediating Ca^{2+} loss down its electrochemical potential gradient probably exhibits a much smaller temperature dependence [41]. In this way, the steady-state Ca^{2+} distribution across the sarcoplasmic reticulum membrane in relaxed, unstimulated muscle, which reflects the balance between the rates of accumulation and of loss, is expected to exhibit a temperature dependence such that, as the temperature falls, sarcoplasmic free calcium concentration rises. It has been suggested (e.g., see Refs. 2–4 and 42) that this is the origin of the cold contracture in mammalian skeletal muscle and certainly it is noteworthy that the phenomenon is generally only apparent in red slow twitch muscles [3] which have a rather poorly developed sarcoplasmic reticulum [3,43]. Various observations in this paper, however, indicate that this is probably an insufficient explanation of the phenomenon. The modulation of the mechanical response by caffeine, tetracaine and reductions in external calcium, treatments all of which appear to affect particularly the release process [10,11,23,39,44], seems to indicate that low temperatures actually augment Ca^{2+} release and that the inhibitory influence normally exerted upon

Ca^{2+} release from the sarcoplasmic reticulum in resting muscle may be partially removed by a sufficient drop in temperature just as it may be transiently withdrawn, in more normal circumstances, by membrane depolarisation.

When oxidative phosphorylation is abolished by respiratory inhibition or by the administration of classical uncoupling agents the cold contracture is potentiated. It seems possible that these treatments, when applied to the resting muscle cell, cause a slight elevation of the sarcoplasmic free Ca^{2+} concentration, sufficient to augment ^{45}Ca loss but insufficient, alone, to activate contraction although able to augment further Ca^{2+} release, perhaps by the Ca^{2+}-induced Ca^{2+}-release mechanism [10,11]. The contractile potentiation produced by Na^+ removal may have a similar cause and it is reminiscent of the effect of this treatment upon the contraction of cardiac muscle (see, for example, Refs. 26, 32, 34 and 35).

In conclusion, it appears that the cold contraction is initiated by a rise in sarcoplasmic free Ca^{2+} concentration due to Ca^{2+} release from the sarcoplasmic reticulum, that this release process and the consequent contraction may be inhibited by local anaesthetics, as in other cases and that caffeine, withdrawal of Na^+ and the inhibition of oxidative phosphorylation all potentiate calcium release and contraction at low temperatures.

References

1 Locker, R.H. and Hagyard, C.J. (1963), J. Sci. Food Agric. 14, 787–793
2 Hill, D.K. (1972) J. Physiol. 221, 161–171
3 Bendall, J.R. (1973) in The Structure and Function of Muscle (Bourne, G.H., ed.), Vol. 2, pp. 132–309, Academic Press, New York
4 Davey, C.L. and Gilbert, K.V. (1974) J. Food Technol. 9, 51–58
5 Buege, D.R. and Marsh, B.B. (1975) Biochem. Biophys. Res. Commun. 65, 478–482
6 Jeacocke, R.E. (1977) J. Sci. Food Agric. 28, 551–556
7 Cornforth, D.P., Pearson, A.M. and Merkel, R.A. (1980) Meat Sci. 4, 103–121
8 Ebashi, S. and Endo, M. (1968) Prog. Biophys. Mol. Biol. 18, 123–183
9 Constantin, L.L. (1975) Prog. Biophys Mol. Biol. 29, 197–224
10 Ebashi, S. (1976) Annu. Rev. Physiol. 38, 293–213
11 Endo, M. (1977) Physiol. Rev. 57, 71–108
12 Ashley, C.C. and Campbell, A.K. (1979) Detection and Measurement of Free Ca^{2+} in Cells, Elsevier/North-Holland, Amsterdam
13 Shanes, A.M. and Bianchi, C.P. (1960) J. Gen. Physiol. 43, 481–493
14 Bianchi, C.P. (1961) J. Gen. Physiol. 44, 845–858
15 Curtis, B.A. (1970) J. Gen. Physiol. 55, 243–253
16 Ashley, C.C., Caldwell, P.C. and Lowe, A.G. (1972) J. Physiol. 223, 735–755
17 Kirby, A.C., Lindley, B.D. and Picken, J.R. (1975) J. Physiol. 253, 37–52
18 Frank, J.S. and Winegrad, S. (1976) Am. J. Physiol. 231, 555–559
19 Caputo, C. and Bolanos, P. (1978) J. Membrane Biol. 41, 1–14
20 Feinstein, M.B. (1963) J. Gen. Physiol. 47, 151–172
21 Conway, D. and Sakai, T. (1960) Proc. Natl. Acad. Sci. U.S.A. 46, 897–903
22 Sakai, T. (1974) Jikei Med. J. 21, 47–88
23 Lüttgau, J.C. and Oetliker, H. (1968) J. Physiol. 194, 51–74
24 Dipolo, R. and Beaugé, L. (1979) Nature 278, 271–273
25 Ashley, C.C., Ellory, J.C. and Hainaut, K. (1974) J. Physiol. 242, 255–272
26 Reeves, J.P. and Sutko, J.L. (1980) Science 208, 1461–1464
27 Reuter, H. and Seitz, N. (1968) J. Physiol. 195, 451–470
28 Curtis, B.A. (1970) Fed. Proc. 29, 846
29 Ussing, H.H. (1947) Nature 160, 262–263
30 Mitchell, P. (1967) Adv. Enzymol. 29, 33–87
31 Baker, P.F. (1972) Prog. Biophys. Mol. Biol. 24, 177–223
32 Sulakhe, P.V. and St. Louis, P.J. (1980) Prog. Biophys. Mol. Biol. 35, 135–195
33 Philipson, K.D. and Nishimoto, A.Y. (1980) J. Biol. Chem. 255, 6880–6882
34 Chapman, R.A. and Tunstall, J. (1980) J. Physiol. 305, 109–123
35 Chapman, R.A. (1979) Prog. Biophys. Mol. Biol. 35, 1–52
36 Mullins, L.J. (1979) Am. J. Physiol. 236, C103–C110
37 Baker, P.F. and McNaughton, P.A. (1976) J. Physiol. 259, 103–144
38 Dipolo, R. and Caputo, C. (1977) Biochim. Biophys. Acta 470, 389–394
39 Caputo, C. (1976) J. Physiol. 255, 191–207
40 Inesi, G., Millman, M. and Eletr, S. (1973) J. Mol. Biol. 81, 483–504
41 Madeira, V.M.C., Antunes-Madeira, M.C. and Carvalho, A.P. (1974) Biochem. Biophys. Res. Commun. 58, 897–904
42 Newbold, R.P. and Tume, R.K. (1977) Aust. J. Biol. Sci. 30, 519–526
43 Briggs, F.N., Poland, J.L. and Solaro, R.J. (1977) J. Physiol. 266, 587–594
44 Caputo, C. (1978) Annu. Rev. Biophys. Bioeng. 7, 63–83