Sodium Withdrawal Contractures in Rat Slow Twitch Skeletal Muscle

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Abstract. Contractile responses due to alterations in $[Na^+]_o$ have been investigated in fast (iliacus) and slow (soleus) twitch muscles of the rat. On exposure to a Na-free solution, the soleus in contrast to the iliacus cells, generated contractile responses without depolarizing the surface membrane. Following glycerol treatment, the twitch and a part of the Na-withdrawal contracture were abolished. The amplitude of the remaining contracture was between 5 and 50 % of the original response and the time to peak was 0.4 to 2 times longer. In intact and detubulated preparations, the amplitude of the zero-Na contracture was modified by changes in $[Ca^{2+}]_o$ and a linear relationship was found if the reciprocal of tension was plotted against $1/\sqrt{[Ca^{2+}]_o}$. In intact and detubulated fibres, a steep dependence of the Na-withdrawal contracture on $[Na^+]_o$ was found and $[Na^+]_o$ which induced the half maximal response at each $[Ca^{2+}]_o$ was the same, the responses were inhibited by Mg-ions in a competitive way. It is assumed that the activator Ca is triggered at the tubular and sarcolemmal membrane level by lowering $[Na^+]_o$ and that a calcium-induced calcium release mechanism at the S.R. level may also be involved.

Key words: Na-withdrawal contractures — Slow twitch muscles — Mammalian skeletal muscle

Introduction

Our knowledge of the coupling between the electrical and mechanical events in skeletal muscle has largely been based on the use of drugs that modify the regulation of contraction and/or on studies of the relationship between mechanical activity and membrane potential. Graded changes in membrane potential were achieved, either by increasing the potassium concentration in the bathing fluid (Hodgkin and Horowicz 1960; Lorkovic 1971) or by using the voltage clamp technique (Heistracher and Hunt 1969; Caillé et al. 1978). Recently, a comparative study, using both these methods, has shown a similar dependence of the

contraction on the membrane potential in mammalian fast and slow twitch skeletal muscles (Léoty and Léauté 1982).

The great sensitivity of excitable cells to the ionic environment suggests that the effect of a given ion on contraction can only be studied when the effects due to changes in others ions are known or eliminated. Compared to the normal physiological medium, in depolarizing solutions the increase in $[K^+]_o$ is generally compensated by an equivalent reduction in $[Na^+]_o$. The chloride concentration is also modified because the K-contracture developed by frog (Hodgkin and Horowicz 1960) and mammalian skeletal muscles (Léoty and Léauté 1982) is more reproducible if in high $[K^+]_o$ solutions the [K] . [Cl] product is kept constant.

The substitution of $[Cl^-]_o$ by other anions has been shown to potentiate K-contractures (Hutter and Noble 1960; Sandow 1964), but generally the effect of the reduction in $[Na^+]_o$ is thought to be without effect on the contractile responses. This contrasts to what is found in frog slow muscle, heart and smooth muscles, where a reduction in $[Na^+]_o$ induces a contracture (Lüttgau and Niedergerke 1958; Schaechtelin 1961; Sholz 1969; Rüegg 1971; Chapman 1974). In mammalian twitch muscles, Léoty and Léauté (1982) noticed that the relationship between contracture tension and membrane potential $[K^+]_o$ becomes slightly modified by lowering $[Na^+]_o$. These results may mean that, at least in mammalian muscle, an effect of reducing $[Na^+]_o$ on the link between excitation and contraction is present. Thus we have analysed changes in the contractile responses in fast and slow mammalian twitch muscles due to alterations in $[Na^+]_o$.

Materials and Methods

Experiments were performed on muscle cells isolated from the iliacus or soleus muscles of the rat (*Rattus norvegicus*, 400—500 g). The isolated muscle was placed in a dissecting chamber containing mammalian Ringer solution at room temperature. Under the microscope, bundles of a few fibres were isolated and large cells were selected and dissected in their whole length. In some experiments, short cut segments of cells or short cut bundles of fibres were used and dissected as reported previously (Léoty and Léauté 1982). The preparations were transferred to the experimental dish on a cover slide and mounted in the way described by Léoty and Léauté (1982). The two ends of the muscle were snared by fine platinum wire loops, one fixed in the experimental dish and the other to the tip of an isomeric force transducer (Endevco 8107). During the period between challenges with experimental solutions the isolated muscle was stimulated electrically by current pulses of 0.5 ms duration applied between two electrodes on each side of the channel. The analysis of the characteristics of the twitch were used to control whether the preparation remained in a good condition. Contracture experiments were carried out in the absence of electrical stimulation. The membrane potential was also measured using conventional 3M-KCl filled glass microelectrodes (resistance 10—20 MΩ). The microelectrode was connected to an electrometer input negative capacitance amplifier.

The normal physiological solution contained (in mmol/l): NaCl 140; KCl 6; $MgCl_2$ 1; $CaCl_2$ 3; Tris-HCl 8; and pH was adjusted to 7.4 with Tris-Aminomethane. The Na-free solution was prepared by replacing sodium chloride by an osmotically equivalent amount of choline chloride (with α-bungarotoxin 2.5×10^{-6} mol/l or 10^{-5} mol/l D-tubocurarine also added) or of Tris chloride or lithium

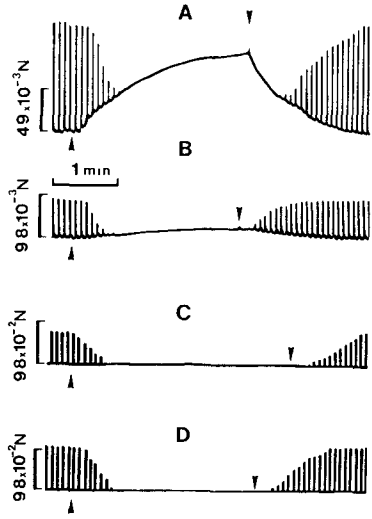

Fig. 1. Effect of exposing different isolated soleus (*A*, *B*) and iliacus (*C*, *D*) muscle fibres to Na-free Ringer. In *B* and *C* choline chloride, in *A* and *D* Tris-chloride solutions were used. The space between the two arrows corresponds to the duration of each challenge. 18°C.

chloride. Experiments were performed at room temperature (18—21°C). The stock solutions of caffeine had a final concentration of 40 mmol/l in Ringer solution; tetracaine (0.5 mol/l tetracaine HCl in aqueous solution), procaine (1 mol/l procaine HCl), lidocaine (1 mol/l lidocaine HCl), acetylcholine (10^{-3} mol/l acetylcholine chloride solution) were all added to the various physiological solution to obtain final concentrations required.

In some experiments isolated fibres were subjected to glycerol treatment according to the method described by Eisenberg et al. (1971). Student's *t* test and linear regression were made using a programmable calculator HP 41C. Collected data were expressed as means ± S.E. and number of experiments (n) was given.

Results

1. *Contractures due to changes in ionic composition of the perfusing solution*

Isolated iliacus or soleus fibres responded to electrical stimulation as already described by Duval and Léoty (1978). The exposure of isolated fibres to a solution free of Na ions resulted in a rapid decline of twitches which failed altogether in about 30 to 45 sec (Fig. 1*C*, *D*). In iliacus fibres there was no change in the resting tension (Fig. 1*C*, *D*), while soleus muscle fibres generated a contracture (Fig. 1*A*, *B*). This response, which reached a maximum in about 2 minutes, was generally stronger in Tris-chloride than in choline-chloride (Fig. 1*A*, *B*). The maximum amplitude of the contracture ranged between 20 to 80 % of the twitch response

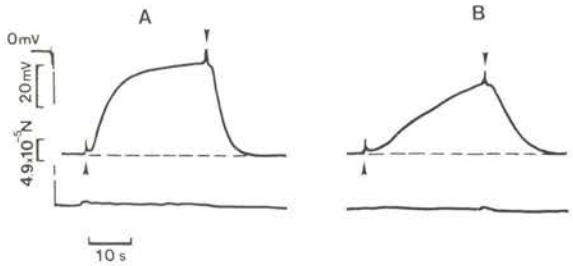

Fig. 2. Simultaneous recording of changes in tension (upper traces) and the membrane potential (lower traces) in the same isolated soleus fibre. Contractures were induced by Na-free choline-chloride solution (*A*), or by reducing $[Ca^{2+}]_o$ from 3 to 0 mmol/l (*B*). 18°C.

and a complete relaxation was obtained on return to normal mammalian Ringer. In soleus fibres, the twitch amplitude progressively returned to its original value as relaxation occurred. The effect of Na-free solution on the resting tension was observed in the presence or absence of electrical stimulation at all temperatures tested (17° to 37°C) and appeared to be typical of soleus muscle cells. Fig. 2*A* shows the rapid occurrence of the membrane potential (−72 mV) as a microelectrode penetrates a cell. The values found are similar to those reported by Duval and Léoty (1980) (-70 ± 1.1 mV). During an exposure to a Na-free solution only reduced changes in membrane potential were recorded (Fig. 2*A*). Generally, a small hyperpolarization (4—6 mV) was observed which disappeared in 2 to 3 minutes on return to Ringer solution. Details of the accompanying contractile responses were not analysed in these experiments because a slow perfusing flow rate was used (lower than 5 ml/min) to help the microelectrode to remain in the cell even during the contracture. Another feature of the soleus muscle, in the presence of normal $[Na^+]_o$ was the development of a contracture at reduced $[Ca^{2+}]_o$ (Fig. 2*B*). The presence of such a contractile response should be taken into account when the effect of $[Ca^{2+}]_o$ on Na-withdrawal contracture were to be studied.

The change of the perfusing fluid from normal to one free of Na ions lead to the development of a contracture insensitive to Mn^{2+} and La^{3+}, reaching a peak and then showing a phase of relaxation followed by a second rise in tension that subsequently relaxed (Fig. 4*A*). The presence of a second component was somewhat variable and it depended on the compounds substituting for NaCl. In LiCl the slowly developing response was larger than the initial response and it failed to reach a plateau even after 3 min of perfusion. On return to Ringer solution an 'off response' was observed. When NaCl was replaced by Tris HCl the two phases of the contractile response were often difficult to distinguish. When choline chloride was used instead of NaCl the two contractile responses could be more often recognised. Consequently, the subsequent results were obtained using

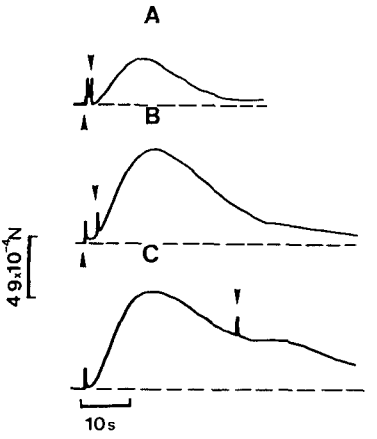

Fig. 3. Effect of different exposure time to Na-free Ringer on the contractile response developed by an isolated soleus fibre. *A*, 1 s; *B*, 2.5 s; *C*, 30 s.

choline chloride substitution which yielded a great stability in the responses, and the two components of the contracture could therefore be more easily analysed. However, as an acetylcholine-like effect may be present it was of interest to control whether the contractures recorded were solely due to Na-withdrawal. The application of α-bungarotoxin, at a concentration of 10^{-6} mol/l which blocks the acetylcholine contracture (10^{-4} mol/l), produced no change in the characteristics of the contractures evoked by Na-free fluid. Furthermore the study of contractures induced by adding acetylcholine to normal Ringer has shown a fast decrease in the sensitivity of soleus muscle to this substance, and after four to five applications of large acetylcholine concentrations (10^{-3} mol/l), no further acetylcholine contracture could be elicited; yet the Na-contractures remained. This method was generally used because bungarotoxin appeared to interfere with the repriming of the Na-withdrawal contracture. Small reduction in $[Na^+]_o$ (e. g. 30 mmol/l) initiated full contracture but the additon of a similar concentration of choline-chloride to normal Ringer (up to 40 mmol/l) failed to produce changes in resting tension. The effect of changing $[Ca^{2+}]_o$ on the Na-lack contracture were inverse as compared to those found with Ach-contractures, where the reduction in $[Ca^{2+}]_o$ was associated with an increase in the amplitude of the contracture (Léoty and Noireaud unpublished results; Gordon 1976). The innervation of the soleus muscular fibres occurs at the central part of the fibre and low $[Na^+]_o$ experiments carried out on fragments of muscular cells taken close to the tendon (Léoty and Léauté 1982) are the same as those made on whole fibres, i.e. Na-contractures persist while acetylcholine contractures are progressively blocked by a series of acetylcholine applications.

2. *Particularities of Na-withdrawal contractures — 'triggering' effect of Na-withdrawal*

The contractures evoked by the application of a K-rich fluid or by caffeine to soleus muscle are rapidly reversed on return to normal Ringer (Léoty and Léauté 1982). This is not the case for Na-lack contractures as illustrated in Fig. 3. A short exposure of 1 s to Na-free a solution initiated contracture (*A*) which developed, reached a maximum and then fully relaxed. Longer application (30 s, *C*) produced a response which was virtually identical to the contracture evoked by a 2.5 s exposure to Na-free fluid (*B*) except for the response seen on return to Ringer. These results suggest that, once initiated, the processes which lead to the generation of the Na-lack contracture cannot be reversed by the return of Na ions. In Fig. 3*A* and *B* the initial phase of contracture is present alone while in *C* a second response can be detected even when the fibre is perfused in Ringer containing Na ions. This observation may explain why, in some of the records, the return to normal Ringer was not always associated with a fast return to the resting tension.

— *The two components of the contracture*

In some preparations, there was a good temporal separation between the initial phase response and the later more prolonged response evoked by Na-free perfusion. However, in an attempt to analyse better each component of the contracture it would be useful to separate the two responses experimentally. The exposure to fluid made hypertonic with glycerol is known to disrupt the excitation-contraction coupling of the twitch response in skeletal muscle on return to normal physiological fluid (Eisenberg et al. 1971). Fig. 4 compares the contracture evoked by Na-free fluid before (*A*) and after (*B*) exposure to glycerol. The fast component of the contracture is abolished by glycerolation. In other experiments, a close correspondence between the failure of the twitch response and the initial phase of the Na-withdrawal contracture was seen, so that in preparations where the twitch partially recovered (not illustrated) following glycerolation, the initial phase of the Na-free contracture also showed some recovery. In preparations where the twitch response was fully inhibited by glycerolation (e.g. Fig. 4), the features of the contractile response to Na-free fluid could be compared to those obtained prior to the exposure to glycerol. The amplitude of the remaining slow contractures ranged between 5 and 50 % and the time to peak was by 0.4 to 2.0 times that of the original contracture. Assuming that the slow response was unaffected by glycerolation, then at the time to peak of the initial response the slow component would be contributing to the total tension in the intact muscle by 2 to 25 %. In most experiments, clear fast and slow contractures were seen and the fast response was

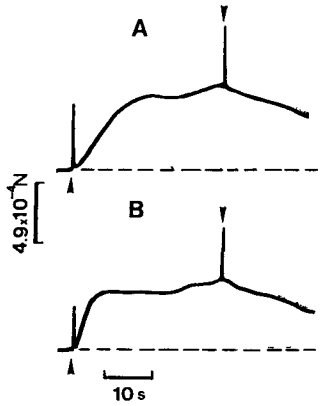

Fig. 4. Zero-Na-contractures recorded in an intact soleus muscle fibre (*A*) and following glycerol-treatment (*B*). 20°C. Note changes in the amplitude and in the time course of the responses.

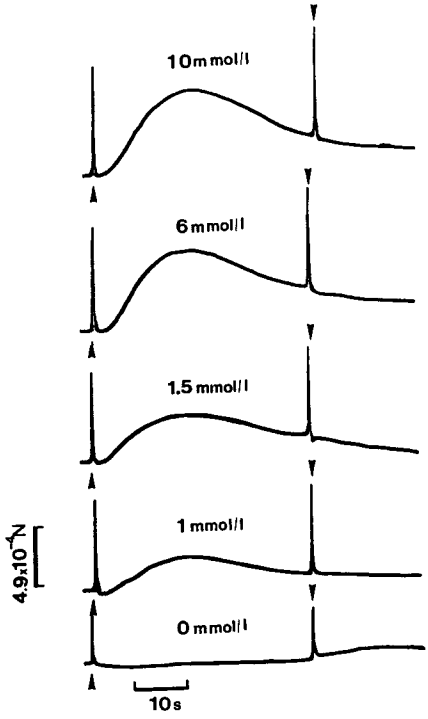

Fig. 5. Contractures evoked in an isolated skeletal soleus cell by exposure to Na-free-Ringer. Control responses are shown (upper trace); other contractures were evoked after different exposure time to Ringer solution following zero-Na challenge. 18°C.

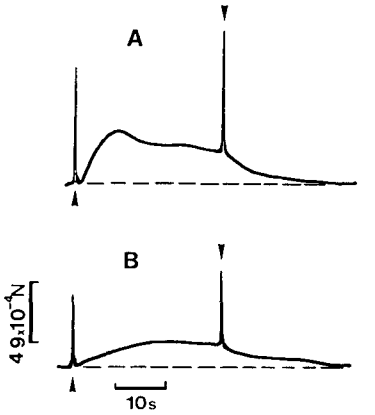

Fig. 6. Zero-Na-contractures induced in a soleus fibre at two different temperatures (*A*) 24°C; (*B*) 34°C.

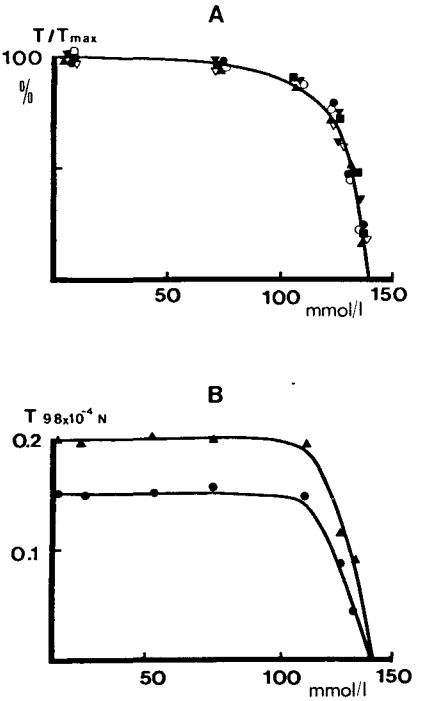

Fig. 7. *A* The strength of zero-Na-contractures (ordinate) dependence on $[Na^+]_o$ (abcissa). The responses in 3 mmol/l (full symbols) and in 1 mmol/l $[Ca^{2+}]_o$ (empty symbols) were plotted as percentual values of the zero-Na-contracture. Intact soleus fibres, temperature range 18—21°C. *B*: Dependence of the strength of the zero-Na-contracture (ordinate) on $[Na^+]_o$ (abscissa) Na--withdrawal contractures were evoked in 3 mmol/l (▲) or 1 mmol/l $[Ca^{2+}]_o$ (●). Detubulated soleus fibre, temperature 30°C.

abolished by glycerol treatment; in others, where a separation was less clear, glycerolation strongly reduced contractile tension to leave a contracture with a relatively unchanged time to peak, even when the twitches were totally abolished.

— *Repriming of the contracture*

The characteristics of the fast component may be analysed either by subtracting the contracture of the detubulated muscle from the responses recorded in normal cell, or in a preparation in which the slow component was small. In Fig. 5 such a preparation in shown; here, the zero-Na-contracture reached a maximum in 15s and relaxed in a roughly exponential way (time constant 15 to 17s) even when a perfusion with Na-free solution was maintained. Following a challenge by a Na-free fluid, a second application of the same medium after 2 min in normal Ringer induced a reduced contractile response, which reached a peak in 12.5s. The full recovery of the Na-contracture was achieved after 10 min in normal solution at room temperature. The time to peak tension was shorter after 2; 3; and 5 min of recovery with the time constant of the spontaneous relaxation remaining unchanged. A full repriming was always present in choline solution

— *Effect of temperature*

Fig. 6 shows the response obtained in the same muscle at two different temperatures. Both the fast and slow contractures were present at both temperatures and showed similar amplitudes but different time courses (time to peak $Q_{10}^{-1} = 2.5$).

3. ***The effect of*** $[Na^+]_o$ ***on Na-contracture***

Fig. 7*A* shows the relationship between the amplitude of Na-removal contractures and $[Na^+]_o$ concentration where a steep dependence of the contracture on $[Na^+]_o$ is seen without a clear threshold for the response. A reduction in $[Na^+]_o$ by 35 mmol/l (i.e. 105 mmol/l $[Na^+]_o$) evoked 90 % of the maximum response; at 70 mmol/l $[Na^+]_o$, the response had almost its full amplitude. In a series of experiments similar to those illustrated in Fig. 8, $[Ca^{2+}]_o$ was reduced and the relationship between $[Na^+]_o$ and tension determined. The reduction of $[Ca^{2+}]_o$ resulted in an eventual reduction of the strength of the Na-withdrawal contracture at all $[Na^+]_o$ (Fig. 7*B*) but $[Na^+]_o$ which yielded half maximal response at each $[Ca^{2+}]_o$ remained the same. If zero-Na-contractures are expressed as a percentage of the maximum response at each $[Ca^{2+}]_o$, then all the curves become superimposed (Fig. 7*A*).

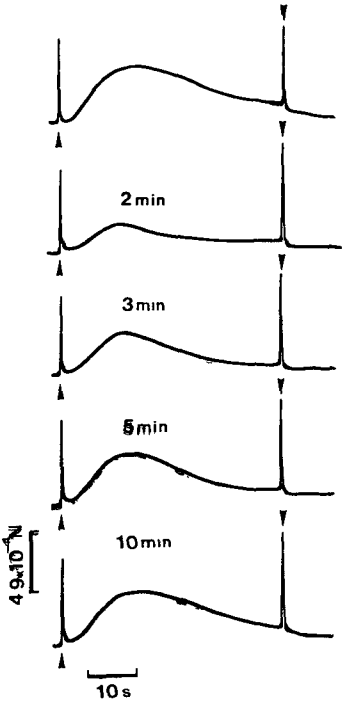

Fig. 8. Na-withdrawal contractures induced in an intact soleus cell at different calcium concentrations. $[Ca^{2+}]_o$ (indicated at the curves) was identical in the Ringer and Na-free-medium. Records from the experiment illustrated in Fig. 5 obtained under steady state conditons.

4. *The effect of* $[Ca^{2+}]_o$ *on zero-Na-contractures*

Effects of $[Ca^{2+}]_o$ change on Na-withdrawal contracture were tested both in normal and detubulated fibres. Following a perfusion in normal $[Ca^{2+}]_o$ a reduction in $[Ca^{2+}]_o$ to 0.1 or 1.5 mmol/l for 10 min with subsequent application of Na-free fluid had no effect on the contracture. By contrast, if $[Ca^{2+}]_o$ was raised, the amplitude of the zero-Na-contracture increased. This was also seen in detubulated fibres. These results suggest that the pool of activator Ca can be filled but not emptied from the extracellular fluid. This notion was initially tested by equilibrating the preparation in 6 mmol/l $[Ca^{2+}]_o$ Ringer, when the contracture was only affected by an increase in $[Ca^{2+}]_o$. However, if $[Ca^{2+}]_o$ was reduced before eliciting a zero-Na-contracture, the first exposure to Na-free fluid evoked an unmodified contracture; thereafter, changed responses were evoked during the second and subsequent challenges. Figs. 8 and 9 illustrate steady responses recorded at different calcium concentrations in

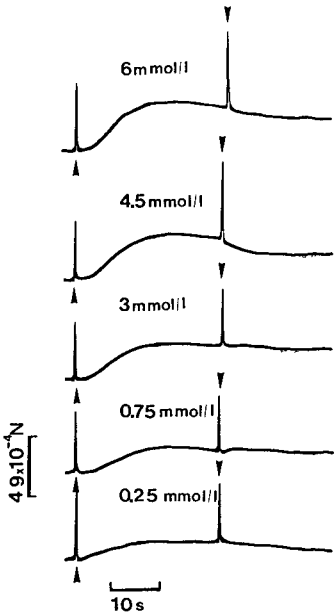

Fig. 9. Na-withdrawal contractures induced in a detubulated soleus fibre at different calcium concentrations. The slow contractures were obtained under steady state conditions. $[Ca^{2+}]_o$ is indicated. 23°C.

intact and detubulated preparations. Both the time to peak and the relaxation time constant were modified insignificantly by changes in $[Ca^{2+}]_o$. Fig. 10*A*, *B* shows the relationship between the amplitude of Na-withdrawal contractures and $[Ca^{2+}]_o$ obtained in an intact and a glycerolated preparation. The analysis of the double reciprocal plot of Na-free contractures and $[Ca^{2+}]_o$ gives regression coefficients generally below 0.8 (not illustrated); however, if the reciprocal of the tension is plotted against $1/\sqrt{[Ca^{2+}]_o}$ (Fig. 10*C*, *D*), the linearity is improved ($r = 0.98$). The intercepts with the Ca axis may correspond to an apparent half-saturation coefficient which, in the case of the illustrated experiment, would be 36.2 mmol/l for the intact muscle response and only 4.6 mmol/l for the response after glycerolation. However, in other similar experiments, the apparent K_m showed considerable variability, ranging between 1.7 and 44 mmol/l for intact muscles and between 4.6 and 27.7 mmol/l for detubulated muscles (i.e. the differences were insignificant).

5. *The effect of* $[Mg^{2+}]_o$ *on zero-Na-contracture*

Fig. 11*A* shows records from the same muscle and illustrates changes in Na

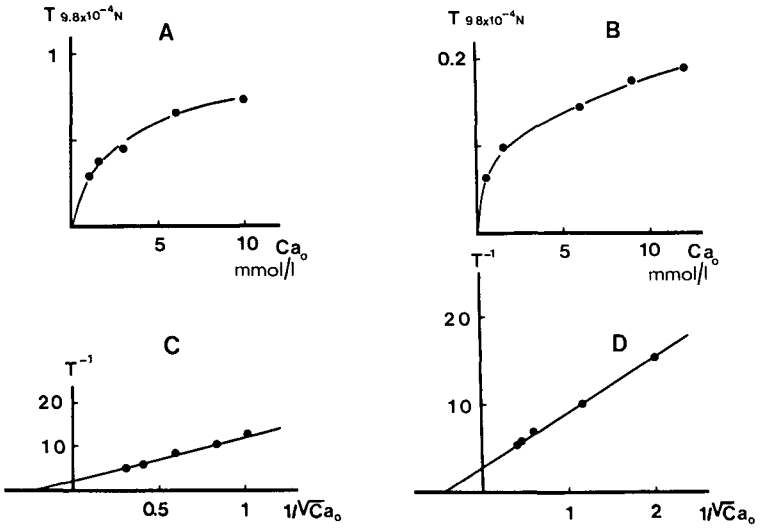

Fig. 10. The effect of $[Ca^{2+}]_o$ (abscissa) on the amplitude of the Na-free contracture (ordinate) in an intact muscle (*a*) and in a glycerol treated fibre (*B*). Results obtained from experiments illustrated in Figs. 8 and 9. The lines were drawn by eye. *C* and *D* concern intact (*A*) and detubulated (*B*) muscles, respectively. The reciprocal of the Na contracture (*A*) or of the slow contracture (*B*) is plotted against the reciprocal of $\sqrt{[Ca^{2+}]_o}$. The continuous lines selected are regression lines with a correlation coefficient ≥ 0.98.

contractures induced by changing $[Mg^{2+}]_o$. The time to peak of the contracture and the relaxation phase of the fast responses were modified insignificantly; in contrast, the contracture amplitude was reduced by increasing $[Mg^{2+}]_o$. The effects of $[Mg^{2+})_o$ were fully reversible as tested in the presence of different $[Ca^{2+}]_o$. Fig. 11*B* was constructed in the same way as Fig. 10*C, D*, and this plot would suggest that the strength of the zero-Na-contracture is inhibited by Mg ions in a competitive way. This effect of Mg^{2+} resembles that produced by this ion on acetylcholine contractures in frog muscle (Gordon 1976).

6. *The Na-withdrawal contracture and increase in* $[Ca^{2+}]_i$

The origin of the Na-withdrawal contractures in skeletal muscle would seem to be different from the origin of those obtained in cardiac muscle (Chapman 1979). In contrast to cardiac muscle, the results reported above suggest that the contracture on reducing $[Na^+]_o$ depends on the previous filling of the Ca-stores. The response also resembles the calcium-induced calcium release mechanism described in skinned skeletal and heart muscle cells (Endo et al. 1970; Fabiato and Fabiato

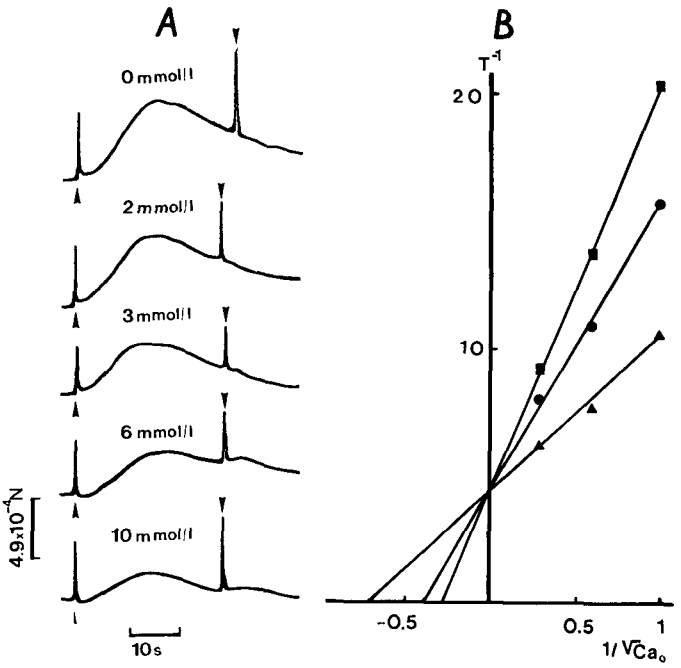

Fig. 11. *A*: Na-withdrawal contractures induced in an intact soleus fibre at different $[Mg^{2+}]_o$. The concentrations used are indicated at each record. The contractures were obtained in 3 mol/l $[Ca^{2+}]_o$. 20°C. *B*: The reciprocal of the Na-withdrawal contractures is plotted against the reciprocal of $\sqrt{[Ca^{2+}]_o}$ in the presence of different $[Ca^{2+}]_o$ and for 6 mol/l (■); 3 mol/l (●) and 2 mol/l (▲) $[Mg^{2+}]_o$, respectively.

1975). Consequently, it was of interest to test in slow muscle fibres some of the substances which have been shown to modify the releasing process in skinned cells.

Effect of caffeine

The effect of different caffeine concentrations (0.05—5 mmol/l) on zero-Na-contracture was tested. The contracture was depressed by caffeine concentrations exceeding 0.1 mmol/l; this may have been a result of the presence of a caffeine contracture which, after a partial relaxation, stabilized at a tension level higher than the resting one. By contrast, at caffeine concentrations below 0.1 mmol/l, Na-withdrawal contractures were modified insignificantly.

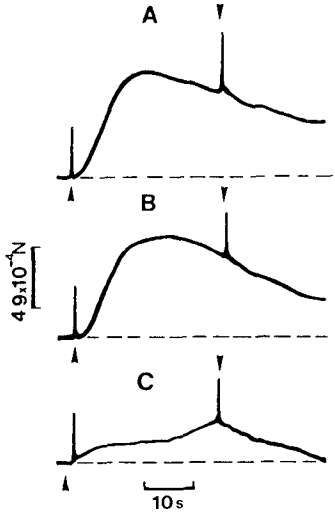

Fig. 12. Na-withdrawal contracture recorded in soleus fibre in normal Ringer (*A*) *B* a contracture which developed after 6 min exposure to lidocaine (10^{-4} mol/l) and after 6 min exposure to tetracaine (10^{-4} mol/l) (*C*) 20°C

Local anesthetics

In skinned muscle, tetracaine and lidocaine have been found to act differently on the calcium induced release mechanism (Endo et al. 1981). However a depressing effect of tetracaine was observed in soleus muscle (Fig. 12*C*) while lidocaine failed to produce changes in the contracture (Fig. 12*B*)

Discussion

Our experiments have revealed differences in the responses of fast and slow rat skeletal muscles. The soleus cells, unlike those of the iliacus, developed contractures when Na ions in the perfusing medium were replaced by choline, Tris or lithium ions. Similar results were also obtained in guinea pig muscle (unpublished data). A comparison of the results obtained with different substitutes for NaCl has lead to choline-chloride being preferentially used. With LiCl, the time course of the contracture was slower than with choline-chloride; on return to normal Ringer, an off contracture developed and a reduced fast component of the contracture was found showing a slow repriming rate. With Tris-chloride, the contracture only rarely showed two components. The use of choline-chloride in physiological medium has often been subject to criticism: a direct effect on cell membrane

similar to the action of acetylcholine and an intracellular effect should be taken into account especially if, as in frog skeletal muscle, choline chloride penetrates into the cells (Renkin 1961). However, control experiments suggest that the effects described here are due to the removal of Na ions, being little, if at all, complicated by cholinergic effects of the choline ion. At first sight, the responses of isolated soleus fibres of the rat to perfusion with Na-poor fluids would seem to resemble contractures seen in cardiac muscle (Lüttgau and Niedergerke 1958; Chapman 1974; Sholz 1969), and reponses found in frog slow muscle (Schaechtelin 1961). However there are a number of important differences: the Na-withdrawal contractures in the soleus muscle are inhibited by Mg^{2+} and tetracaine, and they continue to develop when the original $[Na^+]_o$ is re-established soon after the contracture begins to develop (Fig. 3).

In the soleus muscle, zero-Na-contractures generally show two components, a fast one which reaches a peak in 9 to 15s, and a slow contracture with a time to peak 0.4 to 2 times longer. The presence of a mixed contracture has complicated the analysis of the response but glycerol treatment resulting in disruption of the T-tubules blocked the fast component. Of course, the T-tubules may have not been fully disconnected (Franzini-Armstrong et al. 1973); however, if the absence of a twitch response is used as a criterion for successful de-tubulation, it follows that the integrity of this structure is required for a fast component of the Na-withdrawal contracture to develop. The persistence of part of the low Na-contracture after glycerolation suggests that the signal which initiates the contractile response is not exclusively located at the T-system level. Furthermore, the amplitude ratio of the whole contracture to that in glycerolated fibres, is approximately 2, i.e. similar to that of the membrane surface area to the T-system surface in the soleus muscle (Eisenberg and Kuda 1976). The low Na-contractures in intact and glycerolated fibres show very similar properties. It is therefore conceivable that the two components result from the effect of Na-withdrawal on the surface membrane and the T-tubules, respectively. The variability in responses timing may then be due to changes in access or clearence time in various preparations.

A number of observations have suggested that Na-withdrawal contractures are not initiated by a direct influx of Ca^{2+} from the bathing fluid. The repriming of the contracture, as well as the lack of an immediate effect of a reduced $[Ca^{2+}]_o$ and the direct effect of increased $[Ca^{2+}]_o$ all suggest that Ca is released from a store. This store can readily be refilled from but not emptied into the extracellular fluid. The ability of the Na-withdrawal contracture to continue to develop after being initiated by Na-withdrawal even when Na ions are returned to the bathing fluid suggests that the release of activator Ca is triggered in some way by the reduction in $[Na^+]_o$. The effects of caffeine and tetracaine on the strength of the Na-contractures suggest the release of Ca from the S.R. to be responsible for the initiation of the response. If the consequence of Na-deprivation that results in Ca^{2+} release into the

sarcoplasm lasted longer, the two phases of the contracture could be explained via the surface membrane. If S.R. is the source of activator Ca, diffusion within the sarcoplasm may be an important limiting feature

The amplitudes of the fast and slow contractures appear to be related to $[Ca^{2+}]_o$ by a rectangular hyperbola and the value of K_m and the maximum responses calculated from the analysis on reciprocal co-ordinate suggest that the two components may be of different origin. However, the variability of the detailed results obtained from fast and slow contractures on different muscle types may result form variations in the development of membrane structures or in the activity of the exchange mechanism present at membrane levels.

A competitive action of Ca^{2+} and Mg^{2+} was found without any variations in the membrane potential of the cells. The level of this competition remains obscur, the trigger as well as the releasing sites could be good candidates.

A calcium-induced calcium release mechanism at the S.R. level has been found in skeletal muscle (Endo et al. 1970); such a mechanism might be involved in the initiation of Na-withdrawal contractures and may therefore be of some importance in the initiation of the twitch. The trigger signal is unlikely to be associated with a change in the membrane potential and if an Na/Ca exchange of the sort found in other tissues (see Mullins 1981) is present in soleus muscle fibres, an increased Ca influx into the cell may account for this step. Cosmos and Harris (1961) have noted an increased Ca flux in frog muscle fibres in Na^+-free fluid. However, the failure of Ca^{2+}-free fluid to inhibit the response would not be entirely consistent with this notion; the release of membrane-bound Ca^{2+} may be initiated by a reduction in $[Na^+]_o$.

Our experiments have revealed differences in the contractile behaviour of mammalian fast and slow twitch fibres which, upon further investigation, may help to distinguish between muscle types.

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