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Mammalian skeletal muscle: Long-lasting contractures and potentiated tetani produced by conditioning with weak acid anions[1]

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Summary. Reversible contractures can be induced in slow mammalian muscles by manipulations that probably generate a long-lasting alkalinization of the muscle cell interior. Such contractures reach about ¼ of the tetanic force, P_0, and last 10 times longer than potassium contractures. While in contracture, the muscle fibers have high resting potentials so that they can be electrically stimulated. Tetanic force is then increased and added to that of the contracture so that total force may reach 2 P_0. This level of potentiation has not been reached by any previously-known method.

The procedure for producing slow contractures consists of a 2-step solution change. In the 1st step, thin muscle preparations (consisting of several anatomical bundles) dissected from rat or mouse soleus, rat lumbricalis or human intercostalis muscles, are bathed in a Na propionate solution (140 mM Na, 4 mM K, 2 mM Ca as gluconate, 2 mM Tris (tris(hydroxymethyl)aminomethane), 146 mM propionate, pH 7.2, 19-23 °C) for 10 to 20 min. In the 2nd step, a TrisCl solution (156 mM Tris, 4 mM K, 2 mM Ca, 147 mM Cl, pH 7.2) is applied. In the experiment illustrated in figure 1, force began to develop about 30 sec after the application of the TrisCl solution and reached a maximum at about 6 min (range 3-8 min in 10 tests). The peak force was 24% (range 15-60%) the force produced in a maximal potassium contracture provoked earlier on. Relaxation to ½ the peak force occurred in 23 min (range 18-30 min). Since neither solution produced slow contractures when applied alone and since the contracture appeared only when the sequence of application was Na propionate-TrisCl it seemed that exposure to Na propionate somehow sensitized the muscle to TrisCl.

The fact that slow contractures did not appear on application of TrisCl after a Ringer solution indicated that Na ions had no specific role in sensitizing the muscle. Indeed, about the same conditioning effects were produced with Na and Li, somewhat less with TEA (triethanolamine), about 40% less with K and about 70% less with Tris, all as propionate. The weaker conditioning effectiveness of K and of Tris will be explained later.

The conditioning effect of the propionate anion was quantitatively assessed by measuring the relation between the concentration of propionate and the amplitude of the slow contracture caused by TrisCl. The relation is shown in figure 2. It can be seen that 10 mM propionate was sufficient to produce a noticeable conditioning effect and that about 30 mM propionate was required for the force of the ensuing TrisCl-contracture to reach 50% maximum. The conditioning effect reached a maximum at about 150 mM propionate. In 3 out of 4 muscles, maximum conditioning was reached in less than 15 min. Conditioning was not improved by soaking the muscles in Na propionate for more than 20 min.

Reimmersion of a contracted muscle into a Na propionate-containing solution caused a prompt, complete, and reversible relaxation (fig. 3). Relaxation was incomplete when the concentration of propionate in the relaxing solution was less than 20 mM. The relation between the amount of relaxation and the concentration of propionate is given in figure 4. Relaxing activity was clearly present in the presence of as little as 2 mM propionate and about 50% relaxation was reached with 5 mM propionate. Fur-

ther experiments were made to clarify the state of the muscle during the conditioning and contracture periods.

Measurements with the microelectrode technique showed that the contracture was not associated with depolarization (fig. 5). More evidence against any role of membrane potential in slow contractures was obtained by soaking a Na propionate-conditioned muscle in 200 mM KCl for 15 min prior to the application of TrisCl (fig. 6). The slow contracture appeared in TrisCl although the muscle fibers were depolarized for several minutes following the replacement of KCl by TrisCl (for an explanation of the prolonged depolarization by KCl see Hodgkin and Horowicz[11]).

The good reversibility of the contracture during repeated Na propionate - TrisCl applications (fig. 3) indicated that the slow contracture was not reflecting a state similar to rigor. We obtained further evidence against this possibility by testing twitch and tetanic contractions in response to electrical stimulation of the muscle during the contracture. A Ringer solution (140 mM Na, 4 mM K, 2 mM Ca, 148 mM Cl, 1 mM Hepes buffer) was applied for that purpose when the contracture caused by TrisCl had reached its peak value. The replacement of Tris by Na caused a further increase in contracture force (fig. 7) and the muscle responded to electrical stimulation by twitches. When compared to the values before conditioning, the twitch force was elevated (by 74% on the average in 11 measurements) and the time between the stimulus and half-relaxation of twitch force was prolonged by 149%.

Beyond proving that the muscle was fully excitable during the slow contracture (provided Na ions were present) these experiments revealed an unexpected addition of the contracture force and the force produced by electrical stimulation. The force caused by tetanic stimulation alone was always larger after the Na propionate-TrisCl treatment than before it (fig. 7), so that the total force (slow contracture plus tetanus) exceeded the maximum tetanic force under the control conditions (P_0) by a factor of up to 2. Maximum forces of 1.1 P_0 have been produced in K contractures[12] or treatment with caffeine[23]. Potentiations of the size found here have not yet been reported to our knowledge.

Potassium and caffeine contractures were also potentiated while the muscle was in a slow contracture (fig. 8). The mechanical threshold and the curve relating K contracture force to the K concentration, $[K^+]$, were shifted to lower $[K^+]$, whereas the curve relating contracture repriming to $[K^+]$ was shifted in the

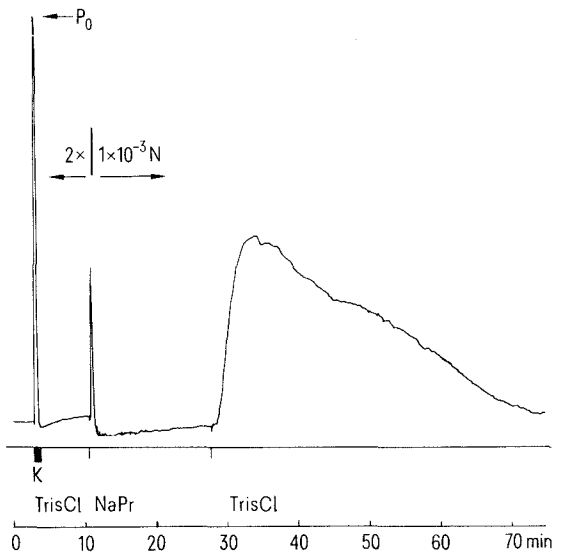

Figure 1. Mechanical responses produced by a fiber bundle (cross sectional area about 10^{-3} cm^2) dissected from a mouse soleus. The muscle was fastened to a teflon-coated metal rod dipping into a 20 ml vial which contained the desired solution. The other end was connected to a stiff strain gauge (Aksjeselskapet, Horton, Norway) to record force development. The muscle was initially bathed in a TrisCl solution. Maximal force development (P_0, about equal to maximum tetanic force) was estimated by applying a high K solution (150 mM K methane sulfonate, 2 mM $CaCl_2$, 1 mM Hepes buffer, applied at heavy bar). After a period of rest in TrisCl, Na propionate was applied (NaPr in the figure), whereby a smaller and phasic contracture was produced by a transient depolarization. After 17 min, TrisCl was applied again producing the slow contracture. Continuous record. Note change in force calibration before the application of Na propionate.

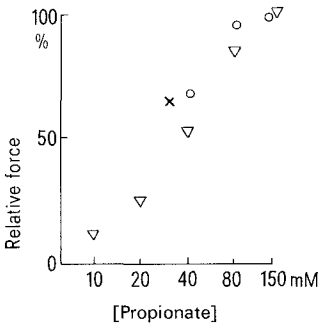

Figure 2. Maximum contracture force produced by TrisCl as a function of the propionate concentration of the conditioning solution. Three preparations. The results are expressed in percent of the contracture force produced when 150 mM propionate was applied during the conditioning period.

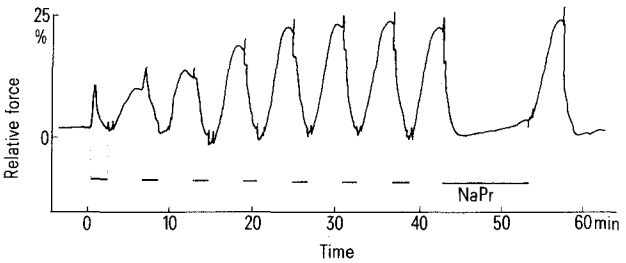

Figure 3. A series of slow contractures and relaxations provoked by alternating applications of TrisCl and Na propionate solutions. Contracture force is expressed in percent of P_0. The phasic contracture is due to transient depolarization.

opposite direction, each by about 0.25 log $[K^+]$ units (fig. 9). These results suggest that the intracellular Ca concentration was elevated during the contracture (for detailed literature see Lüttgau and Spiecker[17]). Prolonged twitches and potentiated K contractures were also found in frog ventricles after replacement of a high HCO_3, high CO_2 solution by a normal Ringer solution[16].

We assume that the processes of conditioning and contracture production are related to changes of the intracellular proton concentration, pH_i. Such changes, measured by intracellular pH microelectrodes, were found to occur in rat soleus muscles on application and withdrawal of 20 mM Na propionate[5]. When Na propionate was applied, pH_i decreased by about 0.2 pH units within 2 min, presumably because of entry of undissociated propionic acid. Thereafter the pH_i slowly returned toward the initial value. Upon reapplication of the Ringer solution there was a transient increase of the pH_i whose magnitude and time course were mirror images of the decrease caused by the initial application of Na propionate. The existence of proton pumps in muscle and nerve membranes has been postulated by others[2-4]. Changes in pH_i, similar to those measured by De Hemptinne and Marrannes[5], were predicted for CO_2 effects in the squid giant axon by computer simulation and confirmed by actual measurements[4]. The time course of the conditioning and contracture force development found in our experiments closely agrees with the time courses of pH_i changes in the work of others.

The pH_i overshoot caused by the withdrawal of propionate was probably enhanced by the simultaneous application of the weak (and presumably permeant[19]) Tris base. The resulting strong alkalinisation of the cell interior, possibly combined with a direct action of Tris[3], must have caused a sustained release of Ca^{2+} from the sarcoplasmic reticulum[6,20,21]. The resulting increase in $[Ca^{2+}]_i$, combined with an increased Ca-sensitivity of the regulatory proteins of the contractile apparatus[9] is the most likely cause of the slow contractures provoked by Tris chloride.

To test this idea, we used other anions instead of propionate during the conditioning phase. Anions of weak acids, like butyrate and benzoate, were as effective as propionate in conditioning the muscle for contractures, whereas anions of strong acids, like chloride, nitrate and methane sulfonate, were ineffective. Glutamate, which is a weak acid anion, was also ineffective, presumably because of the impermeance of its neutral, zwitterion form.

On the basis of our hypothesis we expected the anions of weak acids to be ineffective in producing contractures when applied together with Tris to a previously conditioned muscle. This was indeed the case. By contrast, the anions of 3 strong acids tested, chloride, methane sulfonate, and nitrate, and also glutamate, were effective in producing contractures irrespective of their permeance. The equal effectiveness of the

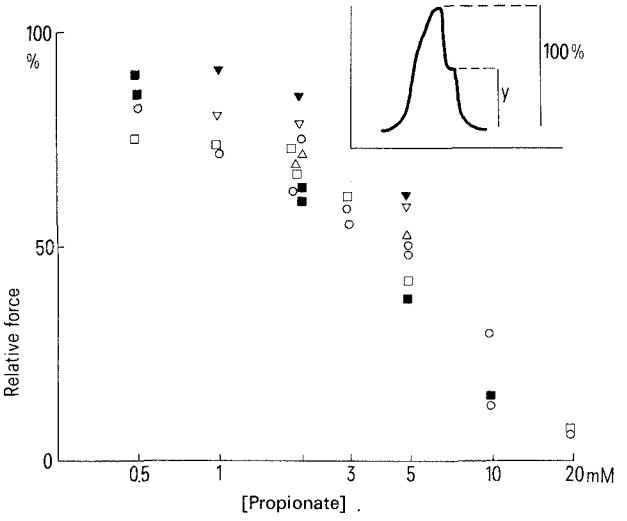

Figure 4. Contracture force produced by TrisCl as a function of the propionate concentration of the contracture solution. The force (y, see inset) is expressed as percentage of the contracture force produced in the absence of propionate in the contracture solution (maximum force in inset).

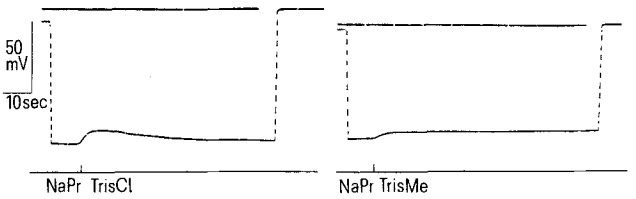

Figure 5. Membrane potential, measured in 2 soleus muscle fibers. The fibers were impaled by the microelectrode during superfusion with a Na propionate solution. Left: at the mark, a TrisCl solution was washed in, resulting in an altered junction potential. About 10 sec after the solution change the membrane began to hyperpolarize. The increased resting potential became evident upon withdrawing the microelectrode (upswing of trace). Right: an analogous experiment with Tris methane sulfonate (TrisMe) instead of Tris Cl. Note that there was no hyperpolarization in TrisMe. Upper line: 2nd sweep.

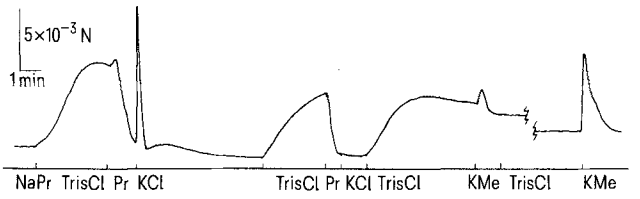

Figure 6. Slow contractures produced by TrisCl before (left) and after exposure to 200 mM KCl. Following the peak of the 1st slow contracture the muscle was relaxed by applying NaPr for 1 min. The 1st application of KCl produced a brief phasic contracture. When the same solution was applied for the 2nd time, no contracture resulted because the prolonged depolarization prevented contractile repriming from occurring. The response to TrisCl was unaffected by depolarization. The end of the record shows the gradual recovery of the responsiveness to depolarization by high K. 150 mM K methane sulfonate was used in this part of the experiment to avoid entry of KCl into the muscle fibers and the delayed repolarization caused by it.

different strong-acid anions argues against the relevance for contracture production of such properties of anions as lyotropic character, pK-value and length of the carbon chain.

We have also tested the effects of different cations on the contracture process. Tris was by far the most effective cation, Na, Li, TEA and choline were barely effective. The Ca concentration of the conditioning and contracture solution had no consistent influence on the slow contractures. These results and the finding that the effectiveness of Tris was greatly increased by raising the pH of the TrisCl solution support the idea that the contracture effect depends on the entry into the cells of the permeant Tris base.

In the following, some alternative explanations of the slow contractures will be discussed. The possibility may be considered that the exposure of the muscle to Na propionate results in an effect similar to chemical skinning. Disruptive effects of aliphatic anions on the membrane structure have been described[7,22]. If such effects were present in our experiments, one would expect the contractures to occur during the application of propionate or butyrate; in fact, contractures appeared upon withdrawal of these anions.

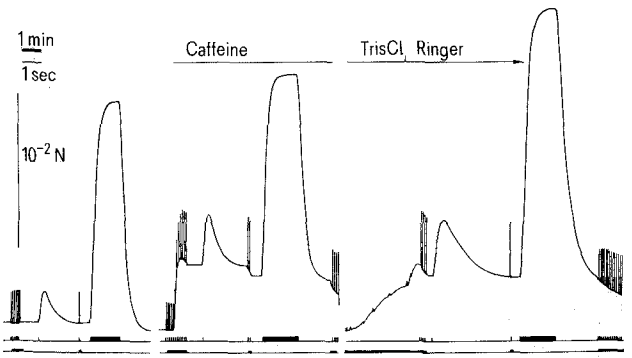

Figure 7. Twitches and tetani produced in a muscle under control conditions, in the presence of 10 mM caffeine, and during a slow contracture provoked by TrisCl (conditioning in Na propionate not shown). Supramaximal stimuli, 0.6 msec, 30 Hz. Note the difference in total force (contracture + tetanus) during the caffeine and TrisCl contractures.

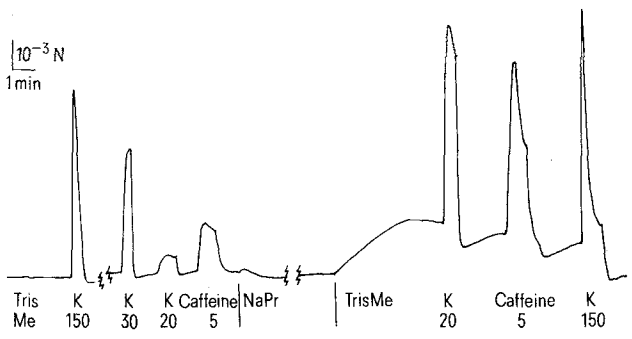

Figure 8. Mechanical responses of a soleus muscle to different concentrations of K and to 5 mM caffeine before (left) and after a period of conditioning by Na propionate.

Contractures may be provoked in skinned muscle fibers by soaking them first in a propionate or methane sulfonate solution and then replacing these anions by chloride[8,10]. Such contractures have been explained by a depolarization of the membranes of the sarcoplasmic reticulum (SR) or, alternatively, by the accompanying osmotic phenomena[18]. The contractures described here cannot have such causes because anions thought to be impermeant to the SR membranes, like propionate and methane sulfonate, differed in their effects both on conditioning and contracture production, whereas the effect on contracture of the permeant chloride equalled that of the impermeant methane sulfonate.

It is also improbable that slow contractures arise from a direct effect of propionate or Tris on the conformation of the contractile proteins because the intracellular concentrations of these agents do not reach the level required for the effect (500 mM for Tris[13]).

Although they are present in large amounts in the soleus muscles, mitochondria do not seem to play a role in the slow contractures because Ca release from mitochondria occurs in acid media[25], whereas contractures presumably occur because of internal alkalinization.

Our assumption that internal alkalinization results in an elevated $[Ca^{2+}]_i$ is based on measurements of Ca-shifts in SR isolated from mammalian muscle[6,20,21]. However, it was shown recently that mechanical responses may be produced in skinned fibers of barnacles, crabs and frogs by exposing them to high CO_2 at constant pH[14,15]. It was shown that the responses were caused by Ca release from a store within the bundles of fibrils, presumably the SR. The Ca release was explained by a fall in the intraluminal pH of the SR. In discussing the apparent discrepancy between these results and ours the following points

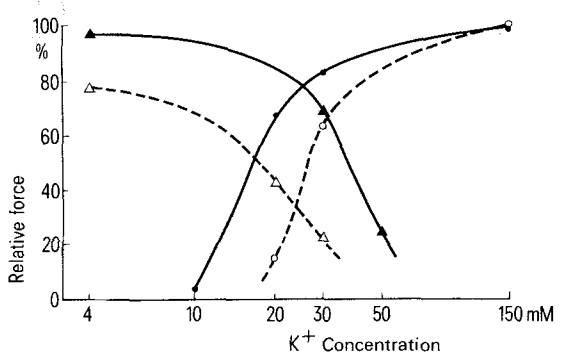

Figure 9. Maximum contracture force as function of the K concentration during the contracture period (circles) and during a 1-min period of repriming interposed between 2 exposures to 150 mM K methane sulfonate; the force of the response to the second application is expressed in percent of that to the first (triangles). Empty symbols: control values; full symbols: values obtained following a period of conditioning in Na propionate. Appropriate mixtures of Tris and K methane sulfonate solutions were used during the testing periods. Full symbols: slow contracture force was subtracted from the total force (for an explanation see fig. 8).

should be considered: 1. it has been shown that the pH dependence of the frog muscle SR capacity for Ca is a complicated function of $[Ca^{2+}]$[9]. A model based on these results is compatible with a Ca release from the SR upon acidification. Such Ca release might have occurred in our experiments upon application of Na propionate but no slow contracture resulted from it because, in contrast to the situation described by Lea and Ashley[15], the threshold $[Ca^{2+}]$ for the mechanical interaction of the contractile proteins was shifted by the fall in pH to a $[Ca^{2+}]$ value high enough to prevent force production; 2. an internal alkalinization (which was absent in the work of Lea and Ashley) would, according to the same model, produce a slow leak of Ca^{2+} due to the reduced Ca capacity of the SR. The resulting small elevation of $[Ca^{2+}]$ would favor force production because of the shift to lower $[Ca^{2+}]$ of the $[Ca^{2+}]$-contraction curve; 3. the properties of the SR and the pH dependence of the Ca sensitivity of the regulatory proteins may be different in frog and crustacean muscles on the one side and slow mammalian muscles on the other.

It is of interest that anions capable of conditioning mammalian muscles for slow contractures belong to the group whose permeance in frog muscle membrane is increased by lowering the pH, whereas the non-conditioning anions are less permeant at low than at normal pH[25]. The significance of this fact is not clear but it might be related to intracellular pH changes produce by the 1st group of anions.

The somewhat lower conditioning potency of benzoate may be explained by the higher permeability of the membrane to the benzoate anion (as compared to that of propionate[26] and a possibly lower permeability to the undissociated benzoic acid. The lesser efficiency of the conditioning in the presence of K^+ than of Na^+ may be due to the depolarization caused by high $[K^+]$. Depolarization may lessen the fall in pH_i which occurs on applying K propionate by eliminating the electrochemical gradient forcing H ions into the cell. A lesser activity of the H pump would thus result, and thus a lesser pH overshoot on withdrawing K propionate.

Slow contracture experiments may be used to study the reactions of muscles to agents which under normal conditions produce only sub-threshold phenomena. Another possible area of application may be the study of the actin-myosin interactions in the living muscle. The long duration of the contractures suggests that ATP consuption may be low under these conditions since a high metabolism should quickly lower the intracellular pH.

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