Selective Inhibition of Potassium Contracture in Presence of Intact Twitch

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ABSTRACT If a sartorius muscle of frog is pretreated for 30 min. with a hyposmotic Ringer's (called "hyposmotic potassium chloride") in which all sodium chloride is replaced by 23 mM potassium chloride, and then is immersed in normal Ringer's for about 40 min., a twitch of normal size can be elicited although the potassium contracture is almost completely abolished. During the time that this selective inhibition of potassium contracture occurs, the action potential and both the size and the speed of depolarization due to potassium are almost the same as in a control muscle that is pretreated for 30 min. with 23 mM potassium chloride under *isosmotic* conditions and that can give a normal potassium contracture. If a muscle is pretreated not with hyposmotic potassium chloride but with a hyposmotic Ringer's in which the concentration of sodium chloride is reduced to 22 mM, the evoked potassium contracture is inhibited, but to a much smaller degree. The partial replacement of chloride by nitrate in hyposmotic potassium chloride has no influence on the degree of the selective inhibition. If after the pretreatment with hyposmotic potassium chloride the muscle is left in normal Ringer's for about 3 hours, there is considerable recovery of ability to undergo contracture. The mechanism of the selective inhibition is briefly discussed.

INTRODUCTION

It is an old and well established view that twitch and contracture, though the contractile processes in both eventually take place in the same element (Gasser, 1930), may be influenced independently. In support of this view, there are many examples of the fact that a powerful contracture can be still produced under conditions in which the twitch is abolished (*e.g.*, Csapo and Suzuki, 1958; Sten-Knudsen, 1960). It is, however, not yet known, at least to our knowledge, that the opposite can occur also, that is to say, that contracture can be inhibited under conditions in which a normal twitch and a normal action potential appear. In the course of a study on the relationship between caffeine contracture and hyposmotic pretreatment (Fujino and

Fujino, 1963), such an observation has been obtained. The present paper deals with this observation and will show that the potassium contracture is almost wholly inhibited by pretreating the muscle with a hyposmotic potassium Ringer's fluid, whereas this same pretreatment exerts no significant influence on either the size of the twitch or the membrane and action potentials.

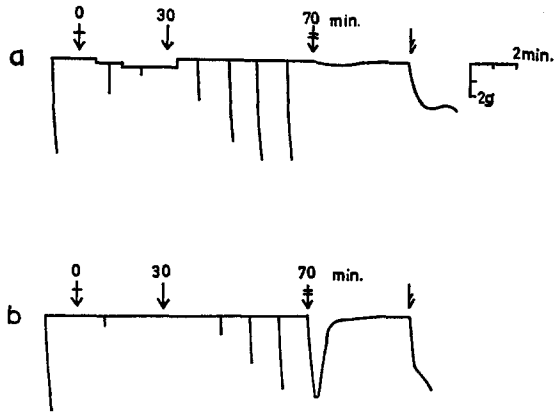

FIGURE 1. The effect of pretreatment with the hyposmotic potassium chloride Ringer's fluid on twitch and potassium contracture. *a* and *b*, whole sartorius muscles from the same frog; the numerals without units indicate time in minutes; up to the sign, ‡ , muscles were immersed in normal Ringer's; from ‡ to ↓, pretreatment for 30 min. with hyposmotic potassium chloride in (*a*) and with isosmotic Na-K in (*b*); ↓, exchange of the bathing fluids for normal Ringer's; ‡, exchange of normal Ringer's for potassium Ringer's in order to test potassium contracture; ↾, addition of 7 mM caffeine. The downward, slightly curved deflections are the traces of the isometric contractions due to single electric shocks. As soon as the contraction due to the last electric shock was completed, the bathing fluid, namely, normal Ringer's, was exchanged for potassium Ringer's immediately. For further explanation, see text.

METHODS

The experiments were done at about 20°C on the isolated sartorius muscle (about 40 mm of femur length) of *Rana temporaria*.

Tension recordings were made on a slowly moving smoked drum with an isometric lever under 1 gm load. The muscle was suspended in a cylindrical bath of about 20 ml content. The lower end of the preparation was fixed with a hook at the bottom of the bath and the upper end was connected to the isometric lever.

The smoked drum was driven only for short periods embracing the times of electrical and of chemical stimulations (periods of *ca.* 30 sec. and *ca.* 2 min., respectively) and before and after the exchange of bathing fluids. In the figures, for this reason, slow changes of tension are represented by steps even though they took place uninterruptedly.

Electrical potentials were recorded between an external Ag–AgCl–Ringer electrode

which was always kept in the external solution and a micropipette of the Ling-Gerard type filled with 3 M KCl (Ling and Gerard, 1949). The electrode system used was: Ag-AgCl | 3 M KCl microelectrode | muscle fiber | Ringer's fluid | Ag-AgCl. Microelectrodes of resistances of 10 to 20 megohms were selected for the experiments. In

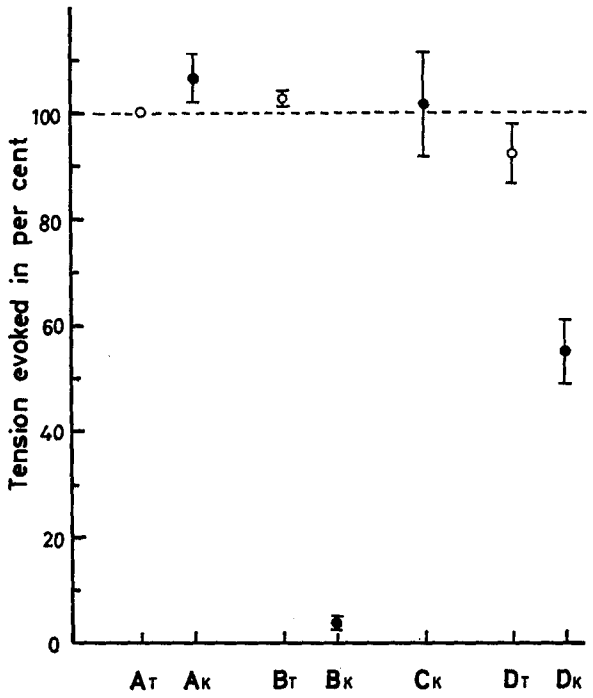

FIGURE 2. The effect of various pretreatments on the size of the twitch and of the potassium contracture. This figure is a summary of the results obtained from experiments like those of Fig. 1. The values shown above are concerned with the last twitch and the potassium contracture that were evoked at the end of the experiments; *i.e.*, 70 min. after the beginning of the pretreatments (*cf.* Fig. 1). Frog sartorius muscles were immersed in normal Ringer's for 40 min. after the completion of 30 min. pretreatment with hyposmotic potassium chloride (B), with isosmotic Na-K (C), and with hyposmotic sodium chloride (D). In A, however, muscles were immersed only in normal Ringer's for the same period of time as the above (*i.e.* 70 min.) without any treatment. Small T and K beside the letters, A–D, indicate twitch and potassium contracture, respectively. In the ordinate, the twitch size of the muscle without any pretreatment was taken as 100. Each point represents the mean values based on ten to twenty determinations, and vertical bars indicate standard error of the mean. For further explanation, see Fig. 1 and the text.

these experiments, the muscles were mounted horizontally in a Ringer bath with a volume of about 40 ml.

The muscles were stimulated with an electronic stimulator through Ag-AgCl wire electrodes in the case of potential measurements or through nickel-chromium wire electrodes in the case of tension recordings. In the latter case, stimulation was carried

out every 10 min. after the beginning of the pretreatments, which will be described below. Supramaximal single pulses were used always.

Normal Ringer's contained 110 mM NaCl, 2.7 mM KCl, 2.5 mM $CaCl_2$, and was buffered to pH 7.3 with $NaHCO_3$. In the potassium sulfate Ringer's, which was used for the purpose of evoking potassium contractures, the NaCl of normal Ringer's was totally replaced by 95 mM K_2SO_4 (called "potassium Ringer's"). The various modified Ringers', of which components will be given in the section on results, were also buffered to pH 7.3 with $NaHCO_3$.

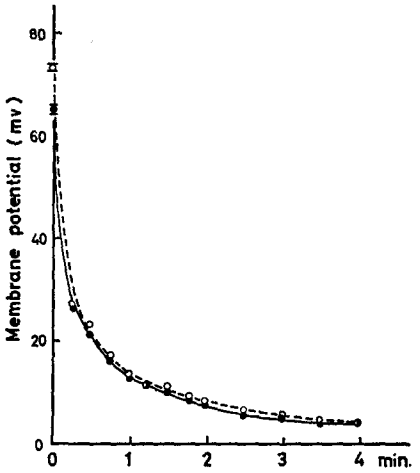

FIGURE 3 *a*. Time course of the depolarization of frog sartorius muscle pretreated with hyposmotic potassium chloride. In this figure, the time course of the high potassium depolarization, which started at the point, ‡, of Fig. 1, was traced by means of intracellular microelectrodes. As soon as the membrane potentials in normal Ringer's, shown at 0 min. in the above figure, were determined, potassium Ringer's was applied; the impalements were then initiated immediately and made at random on the surface of the muscle, one fiber after another as quickly as possible. Before the application of potassium Ringer's, muscles were immersed in normal Ringer's for 40 min. after the completion of the 30 min. pretreatment with hyposmotic potassium chloride (filled circles) and with isosmotic Na-K (open circles). For each point the mean value of 10 to 20 impalements in different fibers was taken. The standard error of the mean is indicated in the above figure by vertical bars only for the points at 0 min.; the error is very small also for the other points. For further explanation, see Fig. 1 and the text.

RESULTS

The Effect of Pretreatment with a Hyposmotic Potassium Ringer's Fluid on the Twitch and on the Potassium Contracture

As is seen in Fig. 1 *a*, if a sartorius muscle is immersed in a hyposmotic Ringer's (called "hyposmotic potassium chloride"), in which all the sodium chloride of normal Ringer's is replaced by only 23 mM potassium chloride, the size of the twitch declines gradually and a contracture with a small tension develops

slowly. When normal Ringer's is restored 30 min. after the immersion into hyposmotic potassium chloride, the contracture disappears more or less rapidly and the twitch recovers gradually; twitch size is almost completely normal 40 min. after the restoration of normal Ringer's. This phenomenon as such is not especially peculiar. It is, however, noteworthy that at this

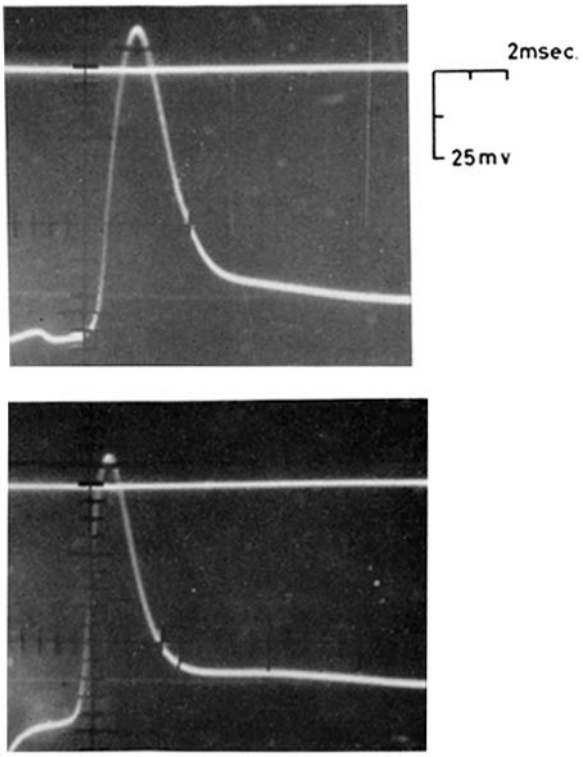

FIGURE 3 *b*. Action potential of frog sartorius muscle pretreated with hyposmotic potassium chloride. The observation was made just before the time point, ‡, of Fig. 1. Until the observation, muscle was immersed in normal Ringer's for 40 min. after the completion of the 30 min. pretreatment with isosmotic Na-K (upper photograph) and with hyposmotic potassium chloride (lower photograph). For further explanation, see Fig. 1 and the text.

time of the recovery of the twitch, the muscle is almost lacking in ability to produce a potassium contracture, as is shown in Figs. 1 *a* and 2 *B*.

In Fig. 1 *a*, the slow development of tension due to the hyposmotic pretreatment and the somewhat more rapid decrease of the tension in the restored normal Ringer's are represented by steps, in spite of the fact that they took place smoothly; for, as mentioned in the section on Methods, the smoked drum was driven only for short periods of time. Fig. 1 *a* shows, thus, that the

slow development of tension took place during the first and the second 10 min. in the 30 min. pretreatment, the tension increased no more during the third 10 min., and the developed tension disappeared during the first 10 min. after the restoration of normal Ringer's. Fig. 1 *a* demonstrates furthermore that the third and last electrical stimulation during the hyposmotic pretreatment could not evoke a contraction, as is understood from the smooth horizontal trace without any fluctuation just before the restoration of normal Ringer's.

As is shown in Figs. 1 *b* and 2 *C*, if the muscle is pretreated with 23 mM potassium chloride not under the hyposmotic but under an isosmotic condition, in which the concentration of sodium chloride in normal Ringer's is reduced only to 90 mM instead of to zero (called "isosmotic Na-K"), and then is immersed into normal Ringer's, the twitch recovers somewhat more slowly than in the case of the aforementioned hyposmotic pretreatment, but otherwise similarly. However, the potassium contracture evoked 40 min. after the restoration of normal Ringer's occurs as vigorously as in the case without any pretreatment, which is shown in Fig. 2 *A*.

From these results, it is indicated that if 23 mM potassium chloride is applied to the muscle for 30 min. under the hyposmotic condition, the ability to develop a potassium contracture is selectively abolished 40 min. after the restoration of normal Ringer's.

In Fig. 1, it is also shown that, in a muscle pretreated with hyposmotic potassium chloride for 30 min., a contracture induced by 7 mM caffeine immediately after the completion of the potassium contracture occurs in a somewhat inhibited form compared with that in a muscle pretreated with isosmotic Na-K, but still almost as vigorously.

The Electrical Behavior of the Muscle Pretreated with the Hyposmotic Potassium Ringer's Fluid

The results of the previous experiment might suggest that in a muscle that has been pretreated with hyposmotic potassium chloride and then immersed into normal Ringer's, the degree and/or the speed of the depolarization caused by the application of a high concentration of potassium decrease and thus the potassium contracture diminishes. Fig. 3 *a* shows, however, that both the degree and the speed of the depolarization in the muscle pretreated with hyposmotic potassium chloride are almost the same as in the control muscle that is pretreated with isosmotic Na-K and that can give a vigorous potassium contracture. According to Kuffler (1946) and Huxley (1959), a membrane potential change is the essential factor for the appearance of some contractures, and in potassium contracture, especially, this view is supported by the fact that the contracture can be inhibited by anodal current flow (Kuffler, 1946; Fleckenstein, Hille, and Adam, 1951). On the

basis of this concept, the present observations indicate that the pretreatment with hyposmotic potassium chloride does not exert any remarkable influence on the depolarization but inhibits the action of a process linking the depolarization of the membrane with the contractile machinery.

The lower photograph of Fig. 3 *b* shows an action potential obtained from a muscle pretreated for 30 min. with hyposmotic potassium chloride and then immersed for 40 min. in normal Ringer's. The potential is modified only slightly by this pretreatment, as is seen from comparison with the control (the upper photograph of Fig. 3 *b*), in which the muscle has been pretreated for 30 min. with isosmotic Na-K instead of hyposmotic potassium chloride. Experiments of this kind have shown that the response of the mem-

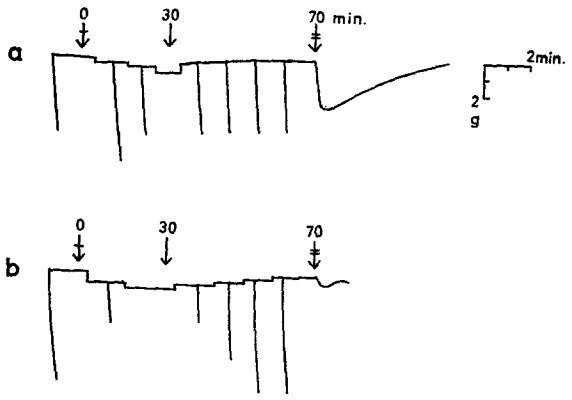

FIGURE 4. Ions effective in the selective inhibition of potassium contracture. From ‡ to ↓, muscles were pretreated for 30 min. with hyposmotic sodium chloride (*a*) and with a hyposmotic Ringer (*b*) in which one-half of the concentration of potassium chloride, *i.e.* 11.5 mM, in hyposmotic potassium chloride is replaced by 11.5 mM potassium nitrate. For further explanation, see Fig. 1 and the text.

brane to a single electric shock is not tetanic but single, indicating that the recovered contraction, which is of normal twitch size in such hyposmotically pretreated muscles, is due to a single excitation and consists solely of one twitch. From these observations, it can be deduced that the pretreatment in question exerts its inhibitory influence not on the linkage between action potential and contraction but only on the linkage between depolarization and contracture.

Ions Effective in the Selective Inhibition of Potassium Contracture

As is seen in Fig. 4 *a*, if a sartorius muscle is immersed in a hyposmotic Ringer's in which the concentration of sodium chloride is reduced to 22 mM (called "hyposmotic sodium chloride"), a contracture with a small tension develops slowly and the twitch does not occur in response to a test shock at 30 min.

When normal Ringer's is restored 30 min. after the immersion into hyposmotic sodium chloride, the twitch recovers and the slow contracture disappears within 40 min. after the restoration. These features are essentially the same as in the case of treatment with hyposmotic potassium chloride. The potassium contracture evoked 40 min. after the restoration of normal Ringer's,

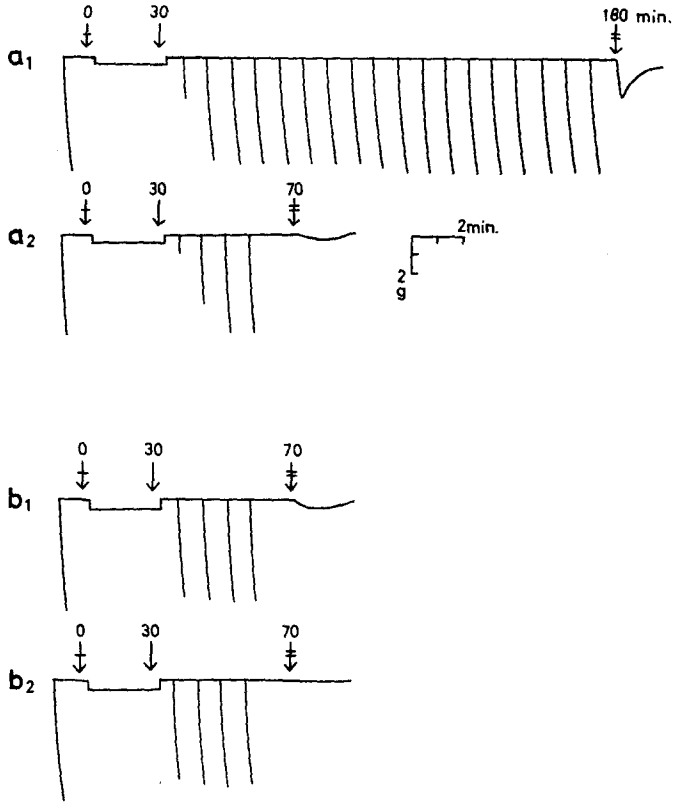

FIGURE 5. Recovery from the inability to produce the potassium contracture. In all cases in this figure, muscles were pretreated with hyposmotic potassium chloride for 30 min. from ‡ to ↓ and were not stimulated electrically during this time. In the cases, a_1, a_2, and b_2, normal Ringer's was restored at ↓, and in the case of b_1, a Ringer's that contained calcium chloride at three times the normal concentration was given at ↓. a_1 and a_2, b_1 and b_2, were paired muscles. For further explanation, see Fig. 1 and the text.

however, is half the normal size, *i.e.* it is relatively large compared with that in the case of hyposmotic potassium chloride (see Fig. 2 *D*), indicating that the inhibitory action of sodium on the potassium contracture is weaker than that of potassium.

Even if a solution in which one-half the concentration of potassium chloride, *i.e.* 11.5 mM, in hyposmotic potassium chloride is replaced by 11.5 mM of

potassium nitrate, is used for the pretreatment, the general features are the same as in the case of hyposmotic potassium chloride, as is shown in Fig. 4 *b*; namely, the muscle that has been pretreated for 30 min. with the hyposmotic solution containing nitrate and then immersed for 40 min. in normal Ringer's, gives a twitch of normal size and is almost lacking in ability to produce a potassium contracture. This result indicates that the anion has so far no essential effect on the production of the selective inhibition of potassium contracture.

If, however, all the potassium chloride of hyposmotic potassium chloride was replaced by potassium nitrate or potassium bromide of the same concentration, *i.e.* 23 mM, there was often, during the pretreatment, a gradual but marked development of tension, which was not usually removed by the immersion of the muscle in normal Ringer's; the contractility after restoration of normal Ringer's was irreversibly low. Therefore, it is necessary that the potassium in the solution used for the hyposmotic pretreatment be accompanied by chloride as the counter ion, in order to obtain the typical selective inhibition of potassium contracture in the presence of a twitch of normal size.

Recovery from the Inability to Produce Potassium Contracture

If, after the completion of the pretreatment with hyposmotic potassium chloride, the muscle is left without any further treatment for a long time, *e.g.* 2 to 3 hrs., in normal Ringer's, a moderately large potassium contracture can be produced (Fig. 5 *a*). If the muscle is left overnight in normal Ringer's, the ability to produce a potassium contracture recovers nearly completely. These results indicate that the inability to produce potassium contracture is not irreversible but only temporary.

Though an attempt was made to determine whether the cause for the selective inhibition of potassium contracture was related to a lack of calcium at some important site, the results so far obtained have not been conclusive; *e.g.*, if, after the pretreatment, the muscle is immersed into an isosmotic Ringer's that contains calcium chloride at 3 times the normal concentration, the potassium contracture evoked 40 min. after the immersion is somewhat greater than in the case of the normal concentration of calcium (Fig. 5 *b*), but still small.

DISCUSSION

The main result of the present experiments is that the ability of frog sartorius muscle to undergo potassium contracture is nearly completely abolished by the specified pretreatment with hyposmotic potassium solution due to an inhibition of a process linking the membrane depolarization with the con-

tracture, whereas the muscle is at the same time capable of generating a vigorous twitch of normal size and a nearly normal action potential.

It is widely accepted that the spike is a membrane potential change (a depolarization) and that the membrane potential change is the essential factor in activation of the contractile machinery, regardless of whether contraction or potassium contracture is considered (Kuffler, 1946; Huxley, 1959). The experiments presented here, however, seem to indicate that this general concept of an intimate linkage between membrane potential change and contractile machinery is at least invalid under certain circumstances: After the action of a hyposmotic potassium solution, the potassium contracture is selectively abolished though a nearly normal depolarization occurs.

An explanation of this divergence between the general concept and the conclusion from the present experiments can scarcely be given in the present state of our knowledge. Since the information about the mechanism of the linkage between excitation and contraction is still extremely small, it is too early to propose a model of a process even more complicated than the initiation of a normal twitch. The present authors might argue that the velocity of the depolarization may be of decisive influence, because the rapid potential change, *i.e.* the action potential, remains effective, whereas the slow depolarization due to the increase of external potassium concentration is ineffective. How this difference in the time course of the depolarization influences the process that activates the contractile machinery is still not understood.

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