Potentiating effect of potassium on skeletal muscle twitch

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CHAPMAN, J. B. Potentiating effect of potassium on skeletal muscle twitch. Am. J. Physiol. 217(3): 898-902. 1969.—The electrophysiological, myothermic, and mechanical consequences of raising the external potassium ion (K+) concentration from 2.5 to 5 mM have been investigated in toad sartorius muscles and compared with the effects of nitrate ion. These ions (5 mM K+ and 111.1 mM NO3—) increased the isometric twitch tension by an average of 20%, and both agents prolonged the active state. In parallel with the mechanical effects, the activation heat was increased by averages of 18% and 16%, under the influence of 5 mM K+ and 111.1 mM NO3—, respectively. The elevation of the K+ concentration to 5 mM K+ caused an average depolarization of 10 mv and this was associated with a diminished amplitude and rate of rise of the action potential. The likely effects of the K+-induced depolarization and nitrate on the intracellular binding of calcium are discussed, and it is suggested that the availability of intracellular calcium for release during the action potential is increased by raising the K+ concentration to 5 mM.

active state; heat-tension curve; activation heat; potassium ion; nitrate ion

THE POTENTIATING EFFECT of potassium on the mechanical activity of the mammalian diaphragm has been described briefly by Goffart and Ritchie (9). However, the results do not contain sufficient detail to allow an analysis of the mechanism of potassium potentiation. In the present experiments the potentiating action of nitrate has been mimicked by a small elevation of the extracellular potassium concentration. It is suggested that the consequent reduction of membrane potential results in an increased availability of calcium for release from the triad during the action potential. A preliminary report of this work has been communicated previously (1).

METHOD

The experiments were performed during winter on 23 pairs of sartorii from the toad Bufo marinus. The muscle mass ranged between 200 and 550 mg, and the average resting length was 38 mm. The normal physiological saline solution contained (mM): NaCl, 111.1; KCl, 2.5; CaCl2, 1.08; NaH2PO4, 0.5; Na2HPO4, 2.5; glucose, 10. A small elevation of the external potassium concentration, [K+]o, at constant [K+]i, [Cl—]o product was introduced with a solution containing (mM): NaCl, 39.9; Na2SO4, 40.3; KCl, 5.0; CaCl2, 6.5; NaH2PO4, 0.5; Na2HPO4, 2.5; glucose, 10. The extra calcium was added to compensate for the calcium-sequestering effect of sulfate. This effect delayed the expected precipitation of calcium phosphates. Freshly made solutions were always used in each experiment, and under these circumstances there were never any signs of precipitation or turbidity. In some experiments the effects of 5 mM K+ were compared with the effects of nitrate. For this purpose a nitrate solution was prepared by replacing all of the sodium chloride of the normal saline solution with sodium nitrate. The solutions were aerated with 100% oxygen and the experimental temperatures ranged between 5 C and 8 C. Temperature was held within 0.001 C of the desired value by means of a water bath controlled by a Haake model NBS ultrathermostat coupled to a Haake model K11 refrigeration unit. Muscles were always equilibrated for at least 30 min after changing from one solution to another.

Electrical recording. Resting and action potentials were recorded with glass microelectrodes filled with 3M KCl coupled to a Pico metric high-impedance unity gain preamplifier. The potentials were amplified in the usual manner and photographed with a Cosor oscilloscope camera.

Mechanical recording. Isometric tension was recorded with a Sanborn FTA 100 mechanoelectric transducer coupled to a Sanborn 350-1100C carrier preamplifier whose output was fed into a Tektronix 502A oscilloscope. For the quick-release experiments the muscle pair was mounted vertically, secured at the pelvic end by screws driven into the acetabula of the pelvic bone. Each muscle was laid on either side of a thin, flat, multielectrode assembly for stimulating the muscle synchronously over its entire length. The tibial ends of the muscles were tied together and connected by two light stainless steel rods to the transducer via a lever which was held firmly by a stop prior to the release. The resting tension was 2-g weight in all experiments with the exception of those in which mechanical latencies during the twitch were compared in the different solutions. The stimulus was delivered to the multielectrode assembly from a Grass S8 stimulator using a stimulus isolation unit. The stimulus duration was 3 msec and the voltage was adjusted to give maximal mechanical response.

The theory underlying the quick-release method of measuring active-state decay has been described by Ritchie (19) and discussed by Jewell and Wilkie (15). In the present experiments a constant release distance of 0.5 mm was used throughout. This short release allows better estimation of active-state intensity at longer times (15). The second pulse available from the stimulator was used to drive a solenoid which withdrew the stop for the quick release at the desired...
interval following the stimulus. The intervals for quick release were used in the order 0, 300, 500, 200, 50, 400, 600, and 100 msec. The compliance of the transducer and thread ties was 5.8 μ per gram weight.

In some experiments the output from the Sanborn carrier preamplifier was differentiated using a Burr-Brown operational amplifier. The differentiator had a high-frequency cutoff at 50 Hz. Traces of the rate of change of force against time were photographed with the oscilloscopic camera.

**Heat recording.** The heat measurements were obtained with a thermopile of the type designed by Ricchiuti (18). The active region consisted of 148 silver-constantan junctions with an output of 5.67 mv per centigrade degree. The total length of the active and protective regions of the thermopile was 13 mm, and the thickness was 40 μ. Platinum electrodes were situated 0.25 mm from each end of the pile and were used for electrical stimulation. The muscle pairs were clamped at the pelvic end and arranged with one muscle on each side of the pile. The tibial ends of the muscles were tied together and connected to the force transducer by a long, light stainless steel rod. The signal from the thermopile was amplified with an Astrodata 120-μv amplifier whose output was filtered to give a frequency response from d-c to 20 Hz. The amplified signal was electrically corrected for the exponential heat loss of the muscle thermopile system. The y plates of the oscilloscope were connected to the inputs of a Brush recorder Mark 280 so that the amplified mechanical and myothermic signals were displayed oscillographically. Curves of heat versus tension were obtained by shortening the muscles down by steps of 2 mm from the resting length (l0) to the length at which twitch tension was abolished.

In both the quick-release and the myothermic experiments the experimental data were obtained by stimulating the muscles once every 90 sec.

**RESULTS**

**Electrophysiology.** Resting potentials were recorded from two muscles equilibrated first in normal solution and then in 5 mM K+ solution. Thirty impalements were made in each solution. The average resting potential was 64.4 ± 2.6 (sd) mv in normal solution and 54.2 ± 3.8 mv in 5 mM K+. The average difference of 10 mv compares with the value of 12 mv obtained by Hodgkin and Horowitz (13) in raising [K+]o from 2.5 to 5 mM for frog semitendinosus fibers.

Figure 1 shows action potentials recorded intracellularly from four cells in two muscles (A and B). The most consistent changes induced in the action potential by 5 mM K+ were a decrease in the rate of rise and a decrease in amplitude caused by the smaller resting potential. A less consistent observation was a slight prolongation of the repolarization (see Fig. 1B) that was, however, always within the limits accepted by Sandow for a type A potentiator (23). These potentiators increase the mechanical response of a twitch by lowering the mechanical threshold without producing any significant change in the shape of the action potential.

**Mechanical measurements.** Figure 2 shows active-state curves obtained using the quick release method (19) from one muscle pair at 7.4 °C in normal solution (crosses), 5 mM K+ (filled circles), and nitrate solution (open circles). The experimental procedure was to obtain curves in the following order: control; 5 mM K+; control; nitrate; control. The control curve was drawn as the average of the three curves obtained because there was a small but progressive shift to the left of the normal active-state curve as the preparation deteriorated. It can be seen that the potentiation of the normal twitch amplitude and the prolongation of the active state due to 5 mM K+ are only slightly less than the similar well-known effects due to nitrate (19). In 10 experiments 5 mM K+ increased the peak twitch tension by an average of 19.7 ± 8.6% (sd). In three of these experiments nitrate produced an average increase of 20.3 ± 7.6%. It was generally found that the peak tetanic tension was either unaffected by 5 mM K+ or slightly diminished. This observation is also comparable with the effect of nitrate on tetanic tension (19).
versus time (t) obtained from a muscle pair at 7.2°C in msec latency. However, both agents produced potentiation within a 25-detectable change before 23 msec following the stimulus. The mechanical latency was 13 msec under all conditions and neither 5 mM K+ nor nitrate produced any 5-g weight in order to increase the chance of detecting early potentiation caused by nitrate is detectable during latency weight (right vertical), and 20 msec (horizontal).

Sandow and Preiser (22) have shown that the twitch potentiation caused by nitrate is detectable during latency relaxation in frog sartorius muscle at 24°C and this early potentiation is held to be characteristic of the type A potentiators. However, in the present experiments it was not possible to demonstrate twitch potentiation any earlier than 20 msec after the stimulus with either nitrate or 5 mM K+.

Figure 3 shows records of isometric force (F) and dF/dt versus time (t) obtained from a muscle pair at 7.2°C in normal solution (dashed lines), 5 mM K+, and nitrate solution. The muscle pair was set up with a resting tension of 5-g weight in order to increase the chance of detecting early effects. The mechanical latency was 13 msec under all conditions and neither 5 mM K+ nor nitrate produced any detectable change before 23 msec following the stimulus. However, both agents produced potentiation within a 25-msec latency.

**Myothermic measurements.** It has been pointed out by Jewell and Wilkie (15) that there is no one particular measurement that can claim to be the only index of the active state of muscle. It is probably just as valid to take the intracellular free calcium level or the rate of a fundamental chemical reaction as an index of active state. Gibbs and associates (6) have shown that caffeine and nitrate ion cause an increase in activation heat in parallel with their potentiating effect on the mechanically measured active state. Now the activation heat, as defined by Hill (11), is that heat which is always released during a twitch independent of any shortening, work, or tension development that may occur. It appears early in the heat trace of a twitch and is thought to be related to the processes underlying the activation of the contractile elements (see discussion).

The simplest method of estimating the activation heat is to stimulate the muscle at various lengths and plot a heat-tension curve. This method was used throughout as it produced less deterioration than the other methods available (6). Figure 4 shows heat-tension curves obtained by plotting the total initial heat at a given length against the peak contractile force developed at that length (see inset). The sequence of applying the various solutions was the same as that for the experiment of Fig. 2, and the control curve (crosses) was drawn as the average of the controls obtained. The activation heat is given by the intercept of each curve with the ordinate and it can be seen that 5 mM K+ (closed circles) and nitrate (open circles) have both increased this quantity to similar extents. In nine experiments 5 mM K+ increased the activation heat by an average of 18.3 ± 5.0%.

In three of these experiments nitrate was also used and produced an average increase of 16.2 ± 4.2%. The scatter of the results makes the effects of these two agents essentially the same in quality and quantity.

A consistent anomalous feature shown in the curves of Fig. 4 is that, instead of remaining parallel, they cross as the muscles approach l0 so that their relationship to each other at high tensions is the reverse of that at zero tension. A tentative explanation may lie in the fact that the muscles were generally more than 3 times the length of the active region of the thermopile that recorded the heat produced from the pelvic third of the muscle. As the stimulus was delivered across this region, the upper electrode being the cathode, it is clear that the muscle was activated asymmetrically in reference to the thermopile. Hence the temporal distribution of the active state within the whole muscle would have been such that active elements in the tibial region of the muscle could perform work on less active elements in the region of the thermopile. Under such conditions the heat recorded would have contained the extra heat due to this degraded work. However, as the muscle is shortened, not only does the temporal distribution of muscle contraction become less asymmetric with respect to the thermopile, but also the muscle becomes less capable of developing tension. Therefore one would expect that the value obtained for activation heat would not be affected by this situation. Evidence in support of this argument is presented in Fig. 5, which shows heat-tension curves, A and B, obtained from one muscle using the experimental arrangements shown in insets A and B, respectively. The arrangement used for curve B is clearly more symmetric than that for curve A and is associated with greater contractile force.
The results of the present studies may also be related to the description of posttetanic potentiation in muscle (3, 20).
This type of potentiation may be due to accumulation of potassium in the transverse tubules following a train of stimuli (5).

The idea that the membrane potential across the transverse tubules controls the association and dissociation of the release may be too low to activate the contractile elements. Clinch (2) has suggested the possibility that the calcium loss from the region of the triad, the rate of calcium energy expended by the calcium pump of the sarcoplasmic reticulum. The rate of calcium uptake is raised 

10 concentrations on the activity of amphibian skeletal muscle. 

The idea that the membrane potential across the transverse tubular system of frog muscle. 

The relation between the late after potential and the increased metabolic rate in muscle subjected to stretch may be related to "looser binding of some substances involved in excitation-contraction coupling processes." This stretch response is facilitated by type A potentiators and 15 mM K⁺. However, as the Solandt effect in 15 mM K⁺ and the effect of 15 mM K⁺ on the stretch response differ from each other in the time course (2) and sensitivity to anoxia (12), one cannot explain each effect purely in terms of one common mechanism.

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