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MAXIMUM ACTIVATION OF THE CONTRACTILE MECHANISM IN FROG'S SKELETAL MUSCLE BY POTASSIUM DEPOLARIZATION

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Although there is little doubt that an action potential normally precedes and initiates a contraction of skeletal muscle, there is still some question as to which of the electrical events associated with the action potential is responsible for initiating the contraction. A considerable body of evidence indicates that depolarization of the muscle fibre surface is the responsible electrical event (Biedermann, 1896; Kuffler, 1946; Sandow, 1952; Sten-Knudsen, 1954, 1960), However, some authors have considered the longitudinal current which accompanies the action potential and flows through the myoplasm parallel to the cell surface as the essential event (Bay, Goodall & Szent-Gyorgyi, 1953).

Recently Csapo & Suzuki (1958) have proposed a composite hypothesis. While conceding that depolarization is essential, they propose that longitudinal currents are needed to produce a maximum activation of the contractile mechanism. They further suggest that the lack of longitudinal current could explain the well known observation (Gasser, 1930) that the maximum tension of a contracture produced by placing a frog's sartorius muscle in an isotonic KCl solution is always considerably less than the maximum tetanic tension of the same muscle.

However, there is another possible explanation for the difference in the maximum tensions obtained by the two procedures. Owing to the limited duration of the potassium-induced contracture and the dimensions of the sartorius muscle, it is possible that each muscle fibre is maximally activated when the muscle is placed in an isotonic KCl solution but that the fibres at the surface of the muscle are relaxing before those closer to the centre have begun to develop tension. This would result in a lowered maximum tension of the entire muscle during a potassium-induced contracture. The experiments presented below were designed to test the validity of this explanation.

METHODS

Either the sartorius or the extensor longus digiti IV muscle of the frog, *Rana pipiens*, was used in all experiments. The ext. long. dig. IV, toe muscle, was removed from the frog and dissected free from its sheath and surrounding muscles with the aid of a dissecting microscope. The sartorius was removed from the frog with a portion of the pelvic girdle containing the proximal attachment of the muscle.

The muscles were mounted vertically in a bath which was drained by means of an opening at the bottom and filled from above with a syringe. The lower end of the muscle was fixed near the bottom of the bath and the upper end was attached by means of a nylon thread to the end of a thin brass plate lever approximately 3 in. (76 mm) above the top of the bath. The resting tension on the muscle was adjusted to be just sufficient to maintain the muscle in a vertical position with fluid in the bath.

Electrical stimuli were applied to the muscles either by means of two platinum electrodes 3 mm apart near one end of the muscle, or by two electrodes, one placed at the bottom of the bath and the other at the top of the fluid in the bath. In either case the muscle was completely immersed in solution during stimulation. Supramaximal rectangular pulses, 1–5 msec in duration, were used and the frequency of stimulation was adjusted to obtain a maximum tetanic response of the muscle (60–120 pulses/sec).

The tension produced by the muscles was recorded by means of two strain gauges (SP-4 Type A-7, Baldwin-Lima-Hamilton Corp.) mounted on each side of the lever near its fulcrum. These strain gauges formed two series arms of a Wheatstone bridge. All recordings were made with a Grass model P 5 polygraph through a model 5 Pl low-level d.c. preamplifier.

Potential changes were recorded by placing a toe muscle in a two-compartment bath. The compartments were isolated by means of an 8 mm petroleum jelly gap surrounding the muscle (see Frank, 1958, Fig. 3). Both compartments contained approximately equal lengths of muscle. The potential difference between the lengths of muscle in the two compartments was altered by changing the ionic composition of the solution in one of the compartments.

In a few experiments the sartorius muscle was reduced in size by successive removal of muscle fibres from the surface and the response of the muscle to isotonic KCl and tetanic stimulation was tested at each stage. The dissections were performed with the aid of a dissecting microscope, care being taken to prevent or minimize damage to muscle fibres remaining in the bundle. An attempt was made to remove fibres so that the remaining bundle of fibres approximated the shape of a cylinder and thereafter to remove fibres uniformly from all surfaces of the bundle. Because of the irregular shape of the sartorius muscle, the weight was used to measure the size of the bundle of muscle fibres at the various stages.

The solutions used had the following composition (mM): NaCl, 111·8; KCl, 2·47; $CaCl_2$, 1·08; NaH_2PO_4, 0·087; $NaHCO_3$, 2·38; glucose, 11·1. The isotonic KCl solution contained 123 mM KCl only or 123 mM KCl + 1·08 mM $CaCl_2$. Solutions with various K^+ concentrations were made by adding appropriate amounts of the isotonic KCl solution to a solution of the above composition, except that the NaCl was replaced by an equimolar quantity of choline chloride. Sufficient D-tubocurarine chloride (Burroughs Wellcome) was added to all the solutions to make a final concentration of 10^{-4} g/ml. During some of the experiments a gas mixture of 0·5 % CO_2 + 99·5 % O_2 was bubbled through the solution. (Solution pH had changed from 7·4 to 7·2 after 30 min exposure to this gas mixture.) All experiments were conducted at room temperature, 20–24° C.

RESULTS

As previously reported (Gasser, 1930), it was observed that the maximum tension produced by placing a frog's sartorius muscle in an isotonic KCl solution was considerably less than the maximum tetanic contraction of the same muscle. This is illustrated by the upper two tracings in Fig. 1. However, when the same comparison was made with the ext. long. dig. IV muscle, the maximum tension of the potassium-induced contracture was usually 10–20 % greater than the maximum tetanic tension of the same muscle (Fig. 1, lower tracings). The most obvious difference in these two muscles is their size. The diameter of the toe muscle is generally less than 0·5 mm, whereas the cross-sectional dimensions of the sartorius are generally 2–4 mm, by 0·5–1·5 mm.

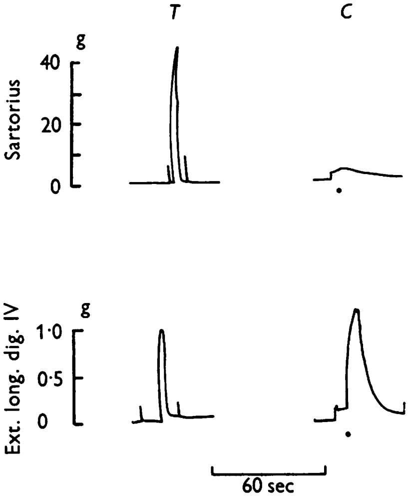

Fig. 1. Maximum tetanic contractions (*T*) and contractures (*C*) produced by isotonic KCl in two muscles of the frog. A single sartorius and single ext. long. dig. IV muscle were used to obtain each pair of responses. Each tetanus is preceded and followed by a twitch. The step at the start of each contracture is an artifact produced by changing the solution in the bath. The dot below each contracture record indicates the point at which the isotonic KCl was put in the bath.

The effects of altering the potassium concentration of the test solution on the potassium-induced contracture and depolarization in the frog's toe muscle are shown in Fig. 2. The depolarization and tension records were taken from separate experiments on two different muscles. As expected, both the degree of depolarization and the maximum tension of the contracture decreased as the potassium concentration of the test solution was reduced. However, as the potassium concentration was reduced the duration of the contracture was prolonged. It is well known that there is a threshold concentration for the potassium-induced contracture (Gasser, 1930). It is possible that the duration of the contracture in the toe muscle is determined both by the duration of the contracture in the individual muscle fibres and by the time needed for the potassium concentration surrounding muscle fibres at various depths in the muscle to reach threshold.

The effects of diffusion on the duration of the potassium-induced contracture can be more clearly demonstrated by reducing the size of a sartorius muscle by the successive removal of muscle fibres from the surface and testing the muscle with isotonic KCl at each stage. When this was done, it was found that the maximum tension of the contracture was only slightly reduced and that the duration of the contracture remained virtually unchanged until the diameter of the remaining bundle of fibres was reduced to about 0·5 mm. At this stage there was an abrupt decrease in both the maximum tension and the duration of the contracture. A further reduction in the size of the muscle fibre bundle reduced the maximum tension but not the duration of the contracture.

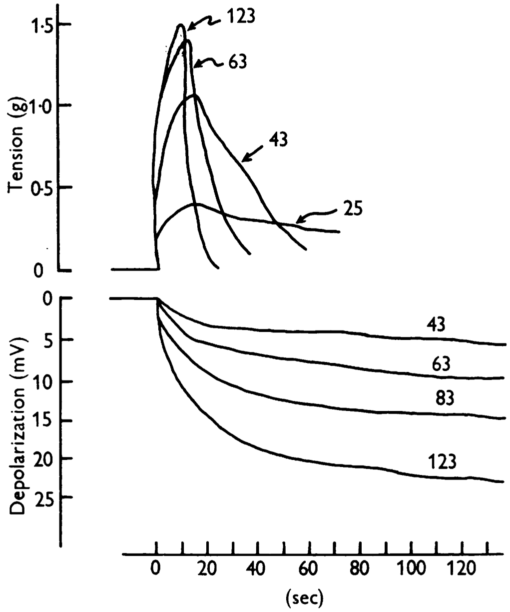

Fig. 2. Contractures and depolarizations of the ext. long. dig. IV muscle produced by placing the muscles in solutions of various potassium concentrations: numbers show mM concentrations. The tension and depolarization records were obtained in separate experiments on different muscles.

If, in this experiment, the maximum tensions of contracture and of tetanus are compared at each stage (Fig. 3), it can be seen that the difference between the two values decreases abruptly as the size of the remaining bundle of muscle fibres approaches that of the toe muscle. At the latter stage the maximum tension of the contracture is at least as great as that of the tetanus.

In the experiments illustrated in Fig. 3 it was assumed that the maximum tetanic tension was a measure of the maximum possible response of the

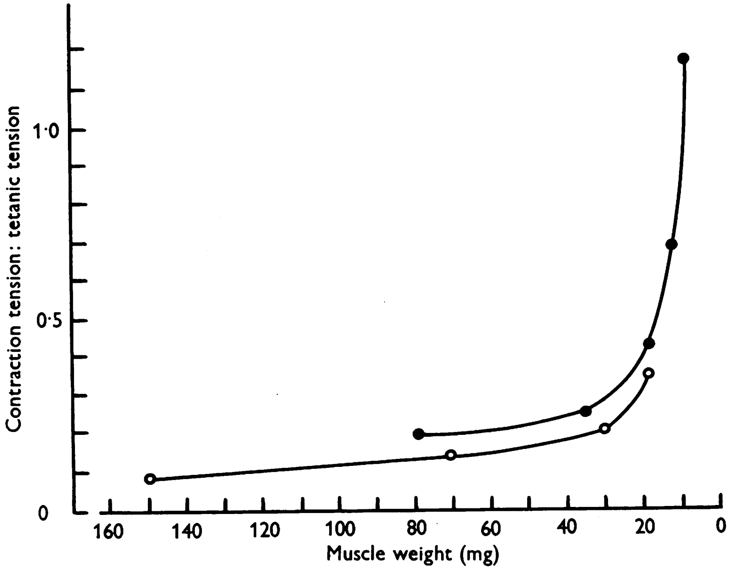

Fig. 3. Change in the ratio of the maximum tension of a potassium-induced contracture to the maximum tetanic tension in the sartorius, resulting from a reduction in the size of the muscle by removing fibres from the outer surface of the muscle in successive stages. ○ and ● represent results obtained from two separate experiments.

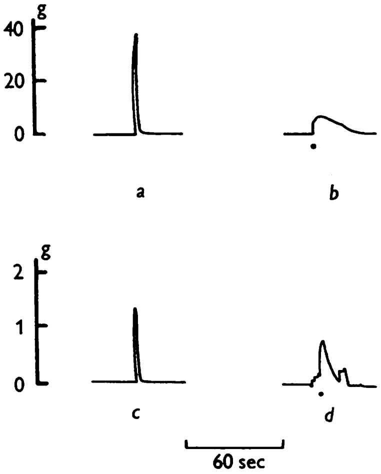

Fig. 4. Effects of reducing the cross-sectional area of a sartorius muscle on maximum tetanic contractions (*a* and *c*) and contractures produced by isotonic KCl (*b* and *d*). *a* and *b*, responses of intact muscle; *c* and *d*, responses following dissection. The dot below each contracture record indicates the point at which isotonic KCl was put in the bath. For details see text.

muscle under the prevailing conditions. However, if special pains are taken it is possible to get maximum tetanic tensions (in kg/cm^2) which are not significantly changed during the experiment. These special precautions include the use of oxygen, a minimal exposure to isotonic KCl, a rapid, careful dissection and a careful adjustment of the muscle length. In the experiment of Fig. 4, the wet weight of the whole muscle (Fig. 4*a* and *b*) was 51 mg, and after dissection (Fig. 4*c* and *d*) the remaining muscle weighed 2·5 mg. In both cases the length was about 40 mm. From these figures and the tensions recorded (Fig. 4*a* and *c*) it was calculated, by tension × length/weight, that the maximum tetanic tension of the intact muscle was 2·29 kg/cm^2 and after dissection it was 2·21 kg/cm^2. The calculated cross-sectional area of the dissected muscle was $0{\cdot}63 \times 10^{-3}$ cm^2. By direct measurement the dissected muscle was found to have a cross-sectional area of $0{\cdot}64 \times 10^{-3}$ cm^2 at its narrowest point and $1{\cdot}22 \times 10^{-3}$ cm^2 at its widest point. The contracture tension: tetanic tension ratio of the intact muscle was 0·21 and after dissection it was 0·58.

DISCUSSION

A considerable body of evidence dating from the double myograph studies described by Biedermann (1896) to the recent micro-electrode studies of Hagiwara & Watanabe (1955) shows that depolarizing a skeletal muscle will lead to a mechanical response, whereas longitudinal currents will not. The question considered here is whether depolarization *per se* is sufficient for maximum activation of the contractile mechanism of a skeletal muscle fibre. The only indication that it might not be sufficient is the difference in the maximum tensions of the potassium-induced contracture and the tetanus in skeletal muscles the size of the frog sartorius and larger (Csapo & Suzuki, 1958). However, the fibres of the toe muscle are apparently maximally activated by an isotonic KCl solution (Fig. 1; see also Frank, 1960), and it would seem reasonable to assume that the same holds true for the fibres of the sartorius muscle. Therefore the reduced size of the potassium-induced contracture in the sartorius muscle appears to be a diffusion phenomenon in which the fibres at the surface relax before the potassium concentration surrounding fibres at the centre of the muscle is large enough to produce a maximum activation of the latter fibres. In accord with this explanation, the difference in the two maximum tensions in the sartorius muscle is eliminated when the diffusion distance for potassium ions is sufficiently reduced (Fig. 3), and it also has been reported (Horowicz & Hodgkin, 1956) that the contracture tension of an isolated semitendinosus muscle fibre is about equal to the maximum tetanic tension.

The larger magnitude of the maximum tension of the potassium-induced contracture in the toe muscle was observed in seven out of nine experiments and in the other two experiments the differences in the maximum contracture tension and maximum tetanic tensions of the same muscle were small. It is therefore surprising that Csapo & Suzuki (1958) failed to make this observation. One possible explanation of the different observations in their work and in the work reported here is that they might have used toe muscles having larger cross-sectional dimensions than those used in the experiments reported here. Their use of larger muscles is indicated by the fact that the tensions produced by the toe muscles in their experiments are larger than those usually observed in the work reported here and they rejected toe muscles which developed maximum tensions less than 4 g. The cross-sectional areas of all the toe muscles used in the present investigation were between 0·04 and 0·14 mm^2. Assuming that the toe muscle is capable of developing a tetanic tension equivalent to the sartorius, e.g. 2·5 kg/cm^2 (Hajdu, 1951), the theoretical maximum tension for the largest toe muscle used here would be 3·5 g. They also report an experiment similar to the one illustrated in Fig. 3. However, from their results it appears that they did not sufficiently reduce the size of their muscle bundle and therefore they observed only the small change in the ratio shown in the lower part of the curve in Fig. 3.

As was mentioned above, the maximum tension of the potassium-induced contracture in the toe muscle was usually greater than the maximum tetanic tension of the same muscle. This difference was probably due to the presence of an appreciable number of slow muscle fibres (Kuffler & Vaughan Williams, 1953; Gray, 1958). Since these fibres do not have propagated action potentials it is likely that they would not be maximally activated by direct muscle stimulation in the presence of tubocurarine. Indeed, it was often possible to demonstrate a contracture lasting several minutes by placing a toe muscle in a solution containing acetylcholine. The tension of this maintained contracture was at most some 20 % of the maximum tetanic contraction and could account for the difference between the potassium-induced contracture tension and the tetanic tension in the same muscle. It is interesting that Gray (1958) found by anatomical observation that 6–20 % of the fibres in the toe muscle of the frog, *Rana temporaria*, were slow muscle fibres.

It has long been known that depolarization is not the only way in which the contractile mechanism of a skeletal muscle fibre can be activated (Gasser, 1930). There is also evidence that the degree of activation is not directly tied to the degree of depolarization. For example, in Fig. 2 above it is seen that contracture is of a limited duration despite the continued presence of a depolarization when the toe muscle is kept in a solution having an elevated potassium concentration. The lack of an inflexible relation between depolarization and tension development has been interpreted by Shanes (1958) as suggesting that some other process might be more directly responsible for activating the contractile mechanism. However, the above demonstration that a depolarization by itself is sufficient to cause a maximal activation of the contractile mechanism of a skeletal muscle, strongly supports the contention of previous workers that the depolarization associated with the action potential is the responsible electrical event.

SUMMARY

1. It was observed that the maximum tension of a contracture produced by placing the frog's extensor longus digiti IV muscle in an isotonic KCl solution was usually at least as large as the maximum tetanic tension of the same muscle. In the intact sartorius muscle the maximum tetanic tension is always greater.

2. When the sartorius muscle was reduced in stages by the successive removal of fibres from the outer surface of the muscle, it was found that the difference in the maximum tension of the potassium-induced contracture and the tetanus was reduced and eventually eliminated.

3. It is suggested that the smaller size of the maximum tension of the potassium-induced contracture in muscles as large as the frog sartorius and larger is due to a diffusion phenomenon in which the fibres at the surface of the muscle have begun to relax before the potassium concentration in the extracellular fluid surrounding fibres at the centre is large enough to initiate a mechanical response.

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