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Volume and Twitch Tension Changes in Single Muscle Fibers in Hypertonic Solutions

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ABSTRACT Single muscle fibers were exposed to solutions made hypertonic (approximately 460 milliosmols/kg water) by addition of either NaCl, glycerol, urea, acetamide, ethylene glycol, or propylene glycol. The changes in either the fiber twitch tension or the volume were measured. In the case of NaCl both fiber volume and twitch tension fall rapidly to 64 and 27% of the respective initial value. These two values were maintained for the duration of the exposure. In the case of the other substances, the fiber volume and twitch tension also decreased but in these cases the effect was transient and the fibers recovered their initial volume and twitch tension. The rate of recovery in the different hypertonic media increased in the order: glycerol < urea < ethylene glycol < propylene glycol < acetamide. In the cases of the last three substances, the initial twitch value was recovered in less than 5 min and even surpassed. However, on returning to normal Ringer the fibers' ability to twitch or to develop potassium contractures was lost. The return of the fibers to normal Ringer after exposure to these hypertonic solutions causes a transient swelling of the fibers. However, when fibers were swelled by exposure to hypotonic media, they did not lose their ability to twitch on return to the normal Ringer.

It is well-known (1–5) that hypertonic solutions, prepared by adding proper amounts of NaCl or sucrose to the normal Ringer solution, selectively affect the twitches and potassium contractures of frog muscle fibers, without impairing the conduction of normal action potentials or development of contractures induced by caffeine. The effect of hypertonic solutions has been interpreted (3–5) in terms of the structural changes that occur at the level of the sarcoplasmic reticulum and the transverse tubule system (6–7), and in terms of the changes in ionic strength that occur when the fibers shrink (8–10, and personal communication by Dr. Grundfest).

In the course of previous work (3), a few unreported experiments were

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carried out using as osmotic agents some nonelectrolyte substances, with molecular radii smaller than that of sucrose. It was found that in this case the results obtained were at least qualitatively similar to those obtained by Yamaguchi et al. (11), who used glycerol hypertonic solutions. In fact, it was found that upon exposure to such hypertonic media, the fiber twitches diminished transiently, and then recovered, in some cases even surpassing their original value. It was thought to be of interest to find out whether these changes in the twitching ability were accompanied by changes in the fiber volume. In fact, transient volume changes would be expected in the case of hypertonic solutions prepared with solutes to which the fiber membrane is permeable. However, Yamaguchi et al. (11) found that the weight loss which occurred when whole sartorius muscles were placed in glycerol hypertonic solutions, was not transient, at least during the exposure period in their experiment. Recently, Krolenko and Adamjan (12) have shown that the expected volume changes do in fact occur when single muscle fibers are exposed to solutions made hypertonic by addition of different penetrating substances.

Howell and Jenden (13) have reported that frog toe muscles after having been exposed to a Ringer solution to which 400 mM glycerol had been added, upon return to the normal Ringer solution, lose their ability to twitch, and that at this time drastic changes are observed in the structure of the transverse tubule system. This observation has been confirmed by Eisenberg and Eisenberg (14). These interesting observations have been followed by that of Eisenberg and Gage (15) who found that the fiber membrane capacity diminished after the same treatment.

In the course of the present work, the older observations have been repeated and extended in order to clarify whether fiber volume changes are associated with these effects. It was also thought of interest to study the effect of hypotonic solutions on the contractile properties of muscle fibers, to test whether there is any effect different from that of hypertonic solutions. Beside NaCl, the solutes used as osmotic agents in this work were glycerol, propylene glycol, ethylene glycol, urea, acetamide, and sucrose. Single muscle fibers were used throughout these experiments in order to better follow the time courses of these effects by diminishing the diffusion delays. A partial report of this work has been presented at the XXIV International Congress of Physiological Sciences, Washington, D. C., 1968.

MATERIALS AND METHODS

Single muscle fibers dissected from the semitendinosus muscle of the frog *Rana pipiens* were used. The dissection procedure, the experimental chamber, and the setup for registering tension, were the same as described previously (3). In the tension experiments, the fibers were stimulated at a frequency of 0.5 shock per sec.

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For measuring the volume changes caused by the experimental solutions, a special chamber was employed, which allowed rapid change of the solutions, and which could be mounted on the mechanical stage of a Nikon microscope. The fiber was fixed at both extremities on steel hooks, one of which was fixed and the other of which could be moved in order to stretch the fiber beyond slack length. The fiber was laid in a groove running longitudinally in the bottom of the chamber, and was covered by a glass coverslide over its whole length. A small segment of the fiber lay on a small plastic pedestal which was smeared with vaseline to prevent movement during the solution change. This portion of the fiber was photographed before, during, and at different times after the solution change. A Zeiss water immersion objective was used. The fiber diameters were determined from the photographic enlargements.

Special care was taken to measure the diameter in the same fiber region in each of the photographs of the experimental series. Blinks (16) has shown that the calculation of the fiber volume, assuming a circular cross-section, is a cause of error since muscle fibers have an elliptical rather than a circular cross-section. However, since in the present experiments it was necessary to make measurements immediately after the solution changes, the method described by Blinks (16) could not be used. Moreover since I was interested in measuring only the rate of change of the fiber volume and not the volume itself, it has been assumed that the value of the radius squared is proportional to the fiber volume.

Action potentials were recorded intracellularly by means of glass microelectrodes filled with KCl 3 M. A Bioelectric NF1 amplifier (Bioelectric Instruments, Yonkers, N. Y.) with neutralized input capacity connected to an oscilloscope was used.

The normal Ringer solution had the following composition in mM: NaCl 115; KCl 2.5; $CaCl_2$ 1.8; Na_2HPO_4, 2.15, and NaH_2PO_4 0.85. The osmolality of this solution was measured with a freezing point osmometer and was found to be 230 milliosmols/kg of water. The hypotonic solution contained 40 mM of NaCl instead of 115 and its osmolality was 80 milliosmols/kg of water. The hypertonic solutions were prepared by adding 230 mM of the nonelectrolyte substances, or 115 mM of NaCl to the normal Ringer solution. In these cases the osmolalities of these solutions were approximately double those of normal Ringer solution. All the experiments were carried out between 20° and 22°C.

RESULTS

Fig. 1 shows the effect on the volume and the twitch tension of two different single fibers of a solution made hypertonic by adding NaCl. The upper graph shows the shrinking of the fiber caused by this solution. In this and the next experiments the volume change during the fiber shrinking or swelling is considered to be proportional to the change in the value of the radius squared; these values are expressed in per cent of the initial value. In this case it may be observed that the value of r^2 falls to 61% of the initial value in less than 30 sec. This new value is maintained for the entire time of exposure. The equilibrium value for three fibers was found to be 64 ± 2% of the normal. It is interesting to notice that this value is quite similar to that obtained by Blinks (16) who

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measured the change in fiber cross-section. Therefore, in the range of osmolalities used here, the assumption that r^2 is proportional to fiber volume seems justified. The lower portion of the figure shows the change in twitch tension for another fiber after exposure to the same hypertonic solution. In this

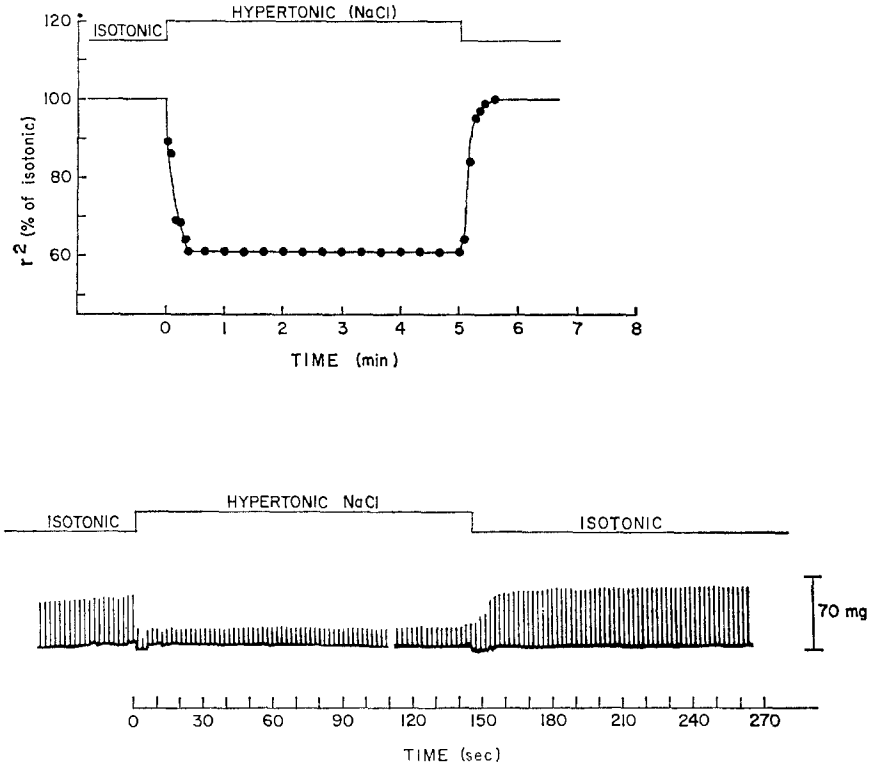

FIGURE 1. The graph in the upper portion of this figure shows an experiment in which a single fiber was exposed to a hypertonic medium prepared by addition of NaCl. The shrinking of the fiber in this solution is expressed in terms of the value of the radius squared, in per cent of the value measured in the normal Ringer. The oscilloscope record in the lower portion of the figure shows the effect of the same hypertonic medium on the isometric twitch tension of a single fiber. The fiber was stimulated at a frequency of 0.5 per sec. Fiber diameter 75 μ. Notice the difference between the time scales of the two experiments.

case the tension fell to 36% of the initial tension. For seven fibers the mean was found to be 27 $\pm$ 2% of the original value.

From these experiments it appears that after exposure to a hypertonic medium prepared by adding NaCl, both fiber volume and twitch tension values fall rapidly and the new values are maintained for the duration of the exposure. Recovery was observed only after removing the excess NaCl. In these experiments the maximum exposure time was 10 min.

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Figs. 2–6 show the results obtained in experiments similar to those of Fig. 1, but in which the hypertonic solutions used were prepared by adding 230 mM of glycerol, urea, ethylene glycol, propylene glycol, or acetamide to the normal medium. From the figures it appears that the fibers shrank rapidly after exposure to the respective hypertonic solution; however, this effect was transient, and the fibers returned slowly toward the original volume. The

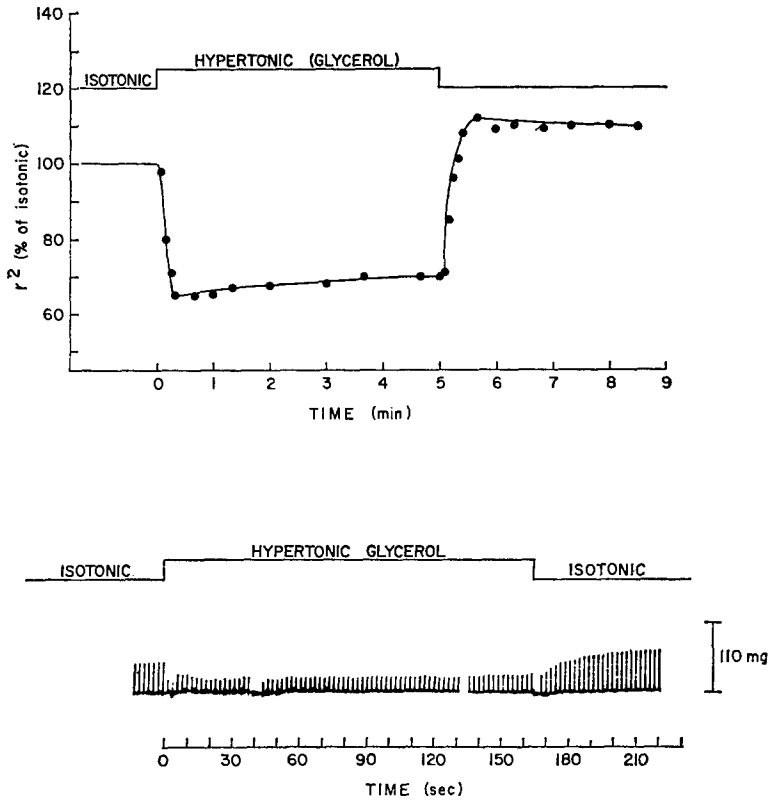

FIGURE 2. Effect of the glycerol hypertonic medium on the volume (upper graph) and twitch tension (lower record) of two different single fibers. The diameter of the second fiber was 70 μ.

same phenomenon was observed in the case of the twitch tension. The rate of recovery in the different hypertonic media, both of the original volume and the original twitch tension, increased following the order glycerol < urea < ethylene glycol < propylene glycol < acetamide.

On return to the normal solution the fiber volume transiently increased by varying amounts, according to the solute and then returned slowly toward the original value. These transient volume changes, observed when the fibers were exposed to the respective hypertonic media and then returned to the

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