THE EFFECT OF CERTAIN INHIBITORS OF CARBOHYDRATE-PHOSPHORUS METABOLISM ON THE TONIC AND TETANIC REACTIONS OF ISOLATED VERTEBRATE MUSCLE FIBERS

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The problem of tonus occupies a prominent position in modern physiology. Its physiologic mechanisms have at present received detailed study; studies of the biochemical processes associated with tonic contraction, however, have been fairly contradictory.

We have succeeded in suggesting certain differences in the carbohydrate-phosphorus metabolism supplying energy to tonic and to tetanic reactions based on our experiments on the action of inhibitors of carbohydrate-phosphorus metabolism in frog muscles [4, 5].

For further elucidation of this question work has been done on the isolated muscle fibers of frog and rat. It is known that frog muscle has three main types of muscle fibers: tetanic, tonic and transitional [1, 2]. We have recently established that similar types of muscle fibers can be isolated in rat muscle also.*

The present report deals with 170 experiments on tetanic, tonic and transitional fibers.

EXPERIMENTAL METHOD

The method for isolation of single muscle fibers in frog, their maintenance and recording of their contractions have been described in the work of Serkov, Sorokin, Zhukov, Leushina and other authors. The procedure for isolation of muscle fibers from rat muscles is reported by P. Honcke [6] and in a report by the present author.*

Stimulation of the fiber was effected by single induction shocks with voltage of 6 v in the primary circuit (accumulator). After the control contractions were recorded the Ringer's solution in which the muscle fiber was kept was replaced by solution of one or another inhibitor and stimulation of the fiber was carried out every 3-5 minutes to observe the character of contraction.

The following substances were used as inhibitors: 1) 2, 4-dinitrophenol, which abolishes respiratory phosphorylation and adenosine triphosphate and creatine phosphate breakdown; 2) sodium fluoride which interrupts glycolysis at the stage of pyruvic acid formation, and 3) sodium monoiodoacetate which inactivates the phosphoglyceraldehyde and dehydrogenase. It is characteristic for the action of this substance that no lactic acid is formed during contraction of the muscle.

EXPERIMENTAL RESULTS

a) Effect of Dinitrophenol

Solutions from 0.0025 to 0.00015 M were used. The majority of experiments was performed on transitional fibers which showed, on adequate stimulation, a typical tetanic peak and tonic plateau in control

* In press.

contractions. On weaker stimulation only rapid tetanic contraction of the fiber could be observed. Under the influence of dinitrophenol the tonus-like plateau gradually disappeared and the contraction became tetanic, evidently as the result of reduced excitability, since on stronger stimulation it was again possible to demonstrate the tonic component. Then the tetanic peaks disappeared and the response of the fiber to stimulation of a given strength ceased, but slow tonic contraction, which persisted for a fairly long time, could be demonstrated in response to stronger stimulation. 20-30 minutes after the disappearance of the tetanic peaks an increase in the latent period of tonic contraction and slowing of the development of contraction were noted; the latter's amplitude was reduced. After 40-60 minutes responses to all strengths of stimulation were lost (Fig. 1).

Tonic fibers usually exhibit similar changes in contraction as those shown by the tonic part of transitional fibers.

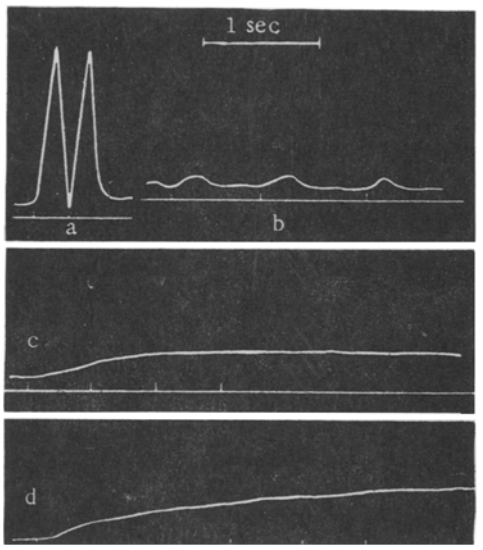

Fig. 1. Effect of 0.00125 M solution of 2,4-dinitrophenol on contraction of isolated transitional fiber of the ilio-tibial muscle in frog. a) Control contractions; b) contractions after 11 minutes of action of dinitrophenol (stimulation with induction current, distance between coils 25 cm); c) contractions after 31 minutes of dinitrophenol action; d) contractions after 41 minutes of dinitrophenol action (stimulation with induction current, distance between coils 8 cm).

Contractions of tonic fibers diminish and disappear under the influence of dinitrophenol with almost no change in character. The reaction of such fibers disappears much more rapidly than that of transitional and tonic fibers, but persists a little longer than that of the tetanic component in transitional fibers. With low concentrations of dinitrophenol (0.00015 M) tetanic muscle fibers maintain their contracting ability for a long time; the high-amplitude contraction observed in the control experiment decreases very rapidly (after 3-4 minutes); what remains is a small twitch of the fiber which may go on for hours.

Changes in rat muscle fiber contractions under the influence of dinitrophenol are similar to those described above. Rat transitional and tonic muscle fibers show particularly marked increase in the latent period of contraction under the action of dinitrophenol.

b) Effect of Sodium Fluoride

Sodium fluoride was used in concentrations from 0.01 to 0.03 M. Its action on frog transitional muscle fibers led to the rapid decrease and disappearance of tetanic peaks and development of tonic contraction in response to very strong stimulation, the contraction disappearing after a fairly rapid passage through the same phases as those seen under the influence of dinitrophenol.

Experiments with tonic fibers also showed gradual decrease and slowing of their contractions under the influence of sodium fluoride.

Contractions of tetanic fibers gradually diminished and disappeared. Sometimes it was possible to observe in the middle of an experiment that the response to stimulation began at the third-fourth impulse, the contraction being at first barely perceptible, then increasing gradually at the sixth-ninth impulse.

In rat transitional fibers the tetanic and tonic components disappear almost simultaneously under the influence of sodium fluoride. However, if the fiber is periodically immersed in pure Ringer-Locke solution for 0.5-1 minute after the disappearance of the tetanic component it is possible to achieve prolonged preservation of tonus-like contraction after cessation of tetanic peaks (Fig. 2).

Changes in the fast and slow fibers of rat under the influence of sodium fluoride were similar to those in the corresponding muscle fibers of frog.

c) Effect of Sodium Monoiodoacetate

Experiments were performed with 0.00003 M solutions of sodium monoiodoacetate.

As has been mentioned above, when a transitional fiber is stimulated by sufficiently strong current its contraction clearly shows a tonic background against which appear the tetanic peaks.

Under the influence of monoiodoacetate the described contraction of frog transitional fibers is so altered that first the tonic plateau becomes lower and disappears and then, very rapidly, disappearance of tetanic peaks occurs; these two phenomena sometimes take place simultaneously (Fig. 3).

Tonic fibers undergo the same changes in contraction under the influence of monoiodoacetate as those described for dinitrophenol and sodium fluoride.

Tetanic fibers show lower amplitude of contraction without change in its character under the influence of monoiodoacetate.

Monoiodoacetate elicits similar changes in rat muscle fibers. Development of irreversible contracture is characteristic for the action of monoiodoacetate.

E. Z. Moreva [3] suggests that the muscle's ability to function against the background of developing contracture elicited by monoiodoacetate may be explained by isolated muscle fibers being able to contract while they have not yet undergone contracture, whereas those fibers which had passed into a state of contracture cease to contract.

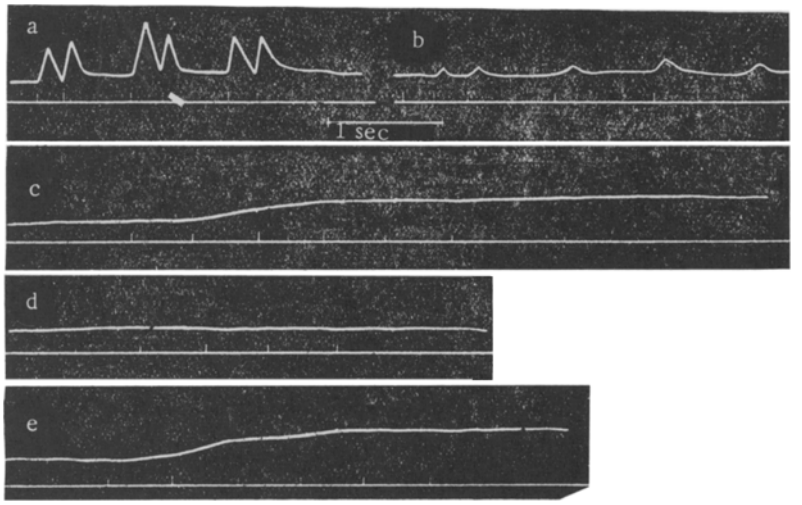

Fig. 2. Effect of 0.03 M solution of sodium fluoride on contractions of isolated transitional fiber of the heel muscle in rat. a) Control contractions (stimulation with inter-coil distance of 15 cm), b) contractions after 20 minutes of sodium fluoride solution action, c) contractions after 35 minutes of action of sodium fluoride solution, d) after 38 minutes of sodium fluoride action failure of the fiber to respond to stimulation, e) contractions of the fiber after being in Ringer-Locke solution for 1 minute (stimulation with induction current, distance between coils 10 cm).

Our data show that contractions can take place in the isolated muscle fiber even against the background of more or less pronounced contracture.

It is usually possible to restore the muscle fiber, frog or rat, from a state of complete cessation of contraction produced by one or other inhibitor to a state approaching the normal by immersing it for some time in Ringer-Locke solution. However, following the action of monoiodoacetate no restoration of muscle fiber contractility was ever observed.

The material presented suggests the following.

Dinitrophenol, which extinguishes respiratory phosphorylation, affects first of all tetanic contraction, while blocking of glycolysis, particularly blocking of lactic acid formation by monoiodoacetate, elicits first of all impairment of tonic reactions of muscle fibers.

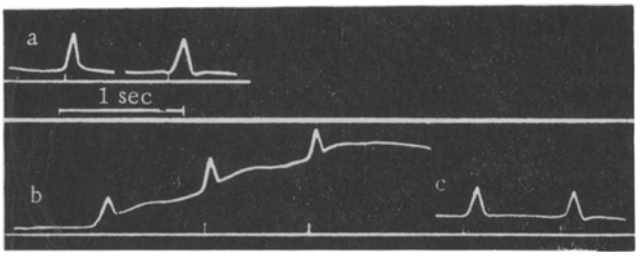

Fig. 3. Effect of 0.0003 M solution of sodium monoiodoacetate on contraction of isolated transitional fiber of ilio-tibial muscle in frog. a) Contractions on stimulation with induction current, distance between coils at 25 cm, b) contractions on stimulation with induction current, distance between coils 10 cm; c) contractions on similar stimulation after 50 minutes' exposure to monoiodoacetate.

Inhibition of metabolism by one or other inhibitor invariably leads to lowering of the amplitude of contractions without change in their character in the case of tetanic fibers, and a marked change in character as well as lowering of amplitude in the case of tonic fiber contractions: there is an increase in the latent period of contraction, an increase in the period of development and growth of contraction, slowing of post-contraction relaxation of the fiber, etc.

SUMMARY

Experiments on the influence of certain inhibitors of the carbohydrate-phosphorus metabolism on the contraction of various types of muscular fibers permitted the conclusion that the tonic and tetanic reactions change in a different way. Evidently, certain biochemical processes are especially important for tonus, while others for tetanus. Block by the inhibitors was reversible in character. No pronounced difference was found in the action of toxins on the muscle fibers of frogs and rats.

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