BIOMETRIC AND HISTO-STRUCTURAL FEATURES OF SKELETAL MUSCLE TISSUE AND SYNTHESIS OF MUSCLE PROTEINS DURING CORTISONE ADMINISTRATION

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Prolonged administration of cortisone causes a marked decrease in area of cross section of the muscle fibers and reduces the intensity of incorporation of methionine-H^3 into muscle proteins. A disturbance of differentiation of the myofibrillary apparatus of the muscle fibers is observed in rabbits on the 15th day of cortisone administration.

Reactive changes in muscles and, in particular, in somatic muscles, when the balance of the glucocorticoid hormones in the body is disturbed have not yet been adequately studied. Few investigations have been made and, as a rule, they have been confined to a description of individual histopathological phenomena [5, 7].

The object of the present investigation was to study certain reactive changes in skeletal muscle tissue induced by administration of cortisone and to compare them with the intensity of protein synthesis. The results could be interesting in connection with the clinical applications of corticosteroid therapy.

EXPERIMENTAL METHOD

The test object consisted of the tibialis anterior muscle of 16 sexually immature male rabbits weighing 1100-1200 g and 8 Wistar rats weighing 95-100 g. The experimental animals (8 rabbits and 4 rats) received cortisone by intramuscular injection (into the triceps brachii muscle) every day. The dose of cortisone was calculated on the basis of the daily therapeutic dose (0.1 g) employed in clinical practice, by the formula

$$a = a_0\left(\frac{P}{P_0}\right)^{\frac{2}{3}},$$

where a_0 is the dose for man, P_0 the weight of a man, and P the weight of the animal.

The control animals received injections of physiological saline. The animals were sacrificed at different times. The tibialis anterior muscle was weighed. Material was fixed in Carnoy's mixture, and longitudinal and transverse sections were cut and stained with Carazzi's hematoxylin and Heidenhain's iron-hematoxylin. The number of muscle nuclei per segment of myon measuring 0.2 mm was counted (in 300 muscle fibers from each muscle) in the longitudinal sections. The area of cross section of the muscle fibers was determined in the transverse sections of the muscle (100 fibers from each muscle). For this purpose, outlines of the transverse sections of the muscle fibers were drawn by means of a projection drawing apparatus, cut out, and weighed.

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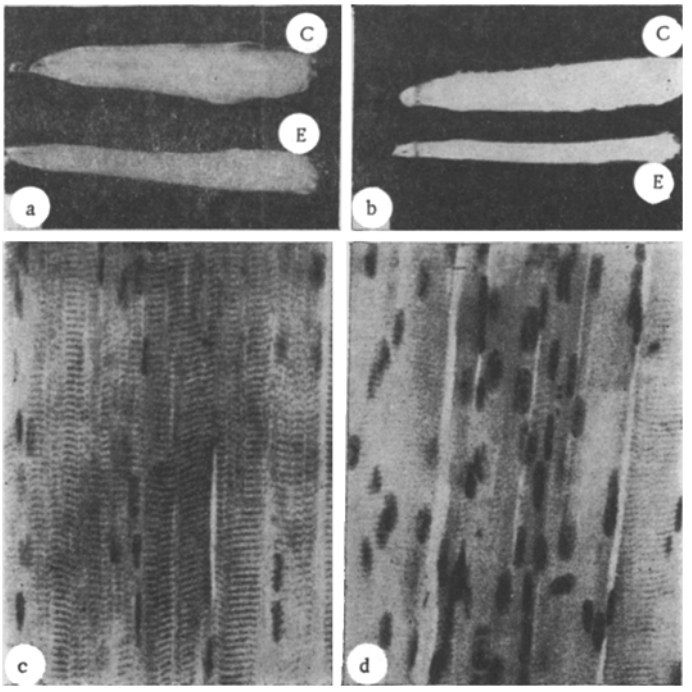

Fig. 1. Tibialis anterior muscle of control rabbits (C) and experimental rabbits (E) 7 days (a) and 15 days (b) after beginning of cortisone administration. Muscle fibers of tibialis anterior in control (c) and experimental (d) 15 days after beginning of cortisone administration. Carazzi's hematoxylin, 280×.

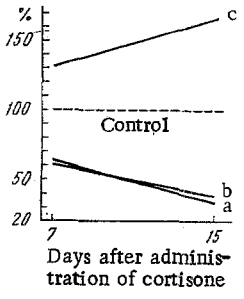

Fig. 2. Weight of tibialis anterior muscle (a), area of cross section of muscle fibers (b), and number of muscle nuclei (c) of experimental animals expressed as percentages of corresponding control values.

To study the effect of cortisone on the intensity of protein synthesis in muscle tissue, the experimental rats received an injection of methionine-H^3 in a dose of 3.5 μCi/g (specific activity of methionine-H^3 90 mCi/g). The rats were decapitated on the 10th day after the beginning of cortisone administration and 16 h after the injection of methionine-H^3. Transverse sections through the tibialis anterior muscle were coated with type "R" emulsion. The autoradiographs were exposed for 40 days. The intensity of incorporation of methionine-H^3 was determined by counting the number of tracks in the section of each individual fiber. From the numbers obtained by counting the mean number of tracks per 100 μ^2 area of muscle fiber was calculated. The numerical results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

In the experimental rabbits the tibialis anterior muscle on the 7th and, in particular, on the 15th day of the experiment differed significantly in its appearance to the eye from muscles of the control animals (Fig. 1a, b). The decrease in weight of the muscle was clearly related to the decrease in mean area of cross sections of the muscle fibers and the increase in number of muscle nuclei and the times of injection of the hormone (Fig. 2).

The distribution of the muscle fibers by classes, depending on their area of cross section, showed that in the rabbits receiving cortisone for 7 and, in particular, for 15 days thin muscle fibers were visibly predominant in the tibialis anterior muscle (compared with the control) and muscle fibers with a large area of cross section, characteristic of the tibialis anterior muscle of the control rabbits, were absent.

Examination of the structure of the muscle fibers 7 days after the beginning of cortisone administration revealed no significant differences in the myofibrillary apparatus compared with the muscle tissue of the control animals. On the 15th day, changes in the structure of the muscle fibers were regularly found.

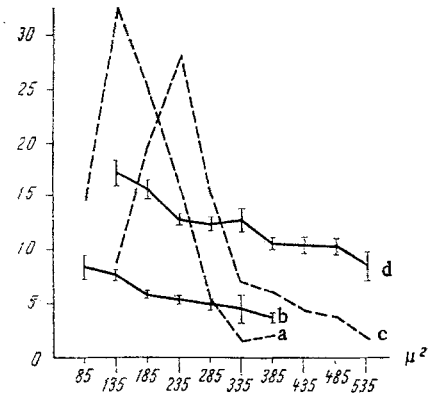

Fig. 3. Number of muscle fibers and number of tracks above cytoplasm of fibers as functions of their area of cross section: a, c) number of fibers in experimental and control series, respectively; b, d) number of tracks in experimental and control series, respectively. Ordinate, number of fibers or tracks; abscissa, classes of muscle fibers based on mean area of cross section.

In some muscle fibers, for instance, partial or complete dedifferentiation of the myofibrillary apparatus was observed in localized areas or over a wide extent of the fiber. In some dedifferentiated areas circular clusters of muscle nuclei could sometimes be seen. In individual very narrow fibers, a series of contiguous muscle nuclei occupied a central position. In some rabbits areas of Zenker's necrosis were visible in individual muscle fibers, and were accompanied by evidence of reparative regeneration: deposits of basophilic cytoplasm with round nuclei and thin myosyncytia.

The focal character of the changes in the muscle fibers was conspicuous. Side by side with muscle fibers which were virtually indistinguishable from those of the control animals, a group of fibers with substantial changes could be seen (Fig. 1c, d).

Administration of cortisone to the rats for 10 days, just as in the case of the rabbits, led to a decrease in weight of the tibialis anterior muscle and a decrease in mean area of cross section of the muscle fibers, although the number of muscle nuclei in them was substantially unchanged compared with the control. No appreciable structural changes likewise were observed in the myofibrillary apparatus of the muscle fibers. As the graph (Fig. 3a, c) shows, in rats just as in rabbits, the number of muscle fibers with a small area of cross section was increased.

Autoradiographic investigation of the intensity of incorporation of methionine-H^3 into the skeletal muscle of the rats on the 10th day after the beginning of cortisone administration revealed a statistically significant decrease in incorporation of the radioactive label (Fig. 3b, d), confirming the previously observed antianabolic effect of the hormone [2-4], although the possibility of a catabolic action of cortisone on the muscle tissue evidently cannot be ruled out [6].

It is interesting to analyze the curves of distribution of the muscle fibers by the specific density of distribution of the tracks in the control and experimental series as functions of their area of cross section (Fig. 3b, d). The greater the area of cross section of the muscle fiber, the less intensive the incorporation of methionine-H^3. It may be supposed that myons with a large area of cross section are what are known as white tetanic muscle fibers, and in that case they must differ in their level of protein synthesis from the narrow "red" tonic muscle fibers. The fact will be noted that under the influence of cortisone the intensity of incorporation of methionine-H^3 was reduced equally in both the wide and the narrow muscle fibers.

Comparison of the results of the autoradiographic investigation with those of the biometric study of the areas of cross section of the muscle fibers demonstrated the marked degree of inhibition of muscle protein synthesis. The possibility cannot be ruled out that in this case synthesis of structural muscle proteins may be inhibited. Changes in differentiation of the myofibrillary apparatus in some muscle fibers of the rabbits, expressed as disturbance of the clearly defined structure of the disks or as their total disappearance, can be assumed to be the result of the absence of synthesis of particular contractile proteins. There is evidently a definite connection between the disturbance of differentiation of the myofibrillary apparatus of the muscle fibers and the increased reproduction of muscle nuclei observed under these conditions during cortisone administration.

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