INTERACTION BETWEEN NERVE AND MUSCLE DURING REINNERVATION OF MUSCLE IN RATS

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Atrophy of the rat gastrocnemius muscle resulting from diversion of the course of the tibial nerve was compared with the process of reinnervation of the muscle after division of the same nerve. In the period before resumption of functional activity of the muscle, the process of atrophy was retarded by the regenerating axons. Experiments in which the nerve was redivided showed that when the first contacts were established between the regenerating axons and the muscle fibers, and before the onset of functional activity in the muscle, the nerve tissue had an inductive effect on the muscle tissue similar to that of embryonic induction, maintaining the weight of the muscle for several days after the second denervation.

Research is going on in A. N. Studitskii's laboratory to study the neurotrophic regulation of plastic processes in tissues [1, 3, 4, 6, 7]. These experiments have shown that the trophic action of a regenerating nerve on repair in muscle tissue is inductive in character, like embryonic induction [2]. It has been suggested that the main role in this trophic effect is played by the motor innervations [3, 7, 8]. The writer has established the trophic influence of nerve on muscle, using the rat gastrocnemius muscle reinnervated by the peroneal nerve as the model [5].

The object of the present investigation was to study this phenomenon during reinnervation of the gastrocnemius muscle by the tibial nerve, which innervates its normally, and also to determine the limits of the period when the trophic action of the regenerating nerve is inductive in character.

EXPERIMENTAL METHOD

Experiments were carried out on 330 noninbred rats weighing 100-120 g, in three series. In series I, atrophy of the muscle was studied after denervation. The tibial nerve in the right hind limb was divided 1 cm from the muscle and the proximal end of the divided nerve was brought out and sutured to a superficial muscle of the thigh. The distal end of the divided nerve (about 0.5 cm) was removed. Material was investigated from 1 to 35 days later. In the experiments of series II, reinnervation of the muscle was studied. The tibial nerve of the right hind limb was divided at the same distance (about 1 cm) from the muscle, without diverting its proximal end. In the experiments of series III, changes in the weight of the muscles were studied after redivision of the regenerating nerve. For this purpose the tibial nerve was divided just as in series II, and the nerve was then redivided on the 15th, 19th, and 22nd days after this operation. In this series the muscles were fixed on the 3rd day after the second operation. The physiological state of the reinnervated muscles was investigated by means of a type ES-ZM cardiac stimulator. In all this series of experiments, both gastrocnemius muscles were weighed. The weight of the right (atrophied and reinnervated) muscles was expressed as a percentage of the weight of the left (control) muscles. Student's criterion was used for the statistical analysis of the results. The muscles were fixed in 12% formalin and then impregnated with silver by the Bielschowsky-Gros method and gilded by Lavrent'ev's method.

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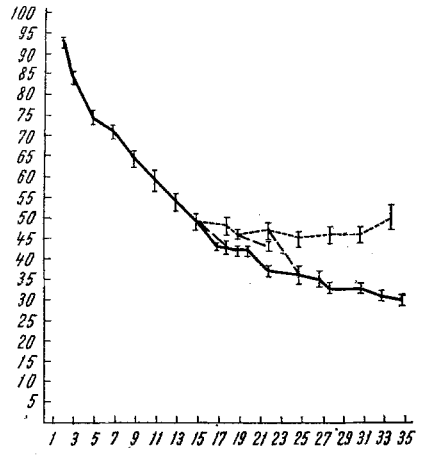

Fig. 1. Changes in weight of atrophied and reinnervated muscles after diversion of the tibial nerve, and after primary and secondary division of the nerve. Continuous line shows weight of muscles after diversion of nerves; line of dots shows weight of muscle after division of nerve; line of dashes shows weight of muscle after redivision of nerves. Vertical lines show limits of error. Abscissa, time of investigation (in days); ordinate, ratio of weight of experimental muscles to weight of controls (in %).

Fig. 2. Primary contact between nerve and muscle on 19th day after division of nerve. Impregnation, 400 ×.

EXPERIMENTAL RESULTS

The experiments on series I showed that atrophy of the gastrocnemius muscle after denervation takes place very rapidly in young rats. The weight of the denervated muscle 3 days after the operation was 84% of the weight of the left, control muscle; this difference in weight is statistically significant. By the 15th day the denervated muscles had lost half their initial weight (49% of the weight of the control muscles). The process of atrophy gradually slowed down: one month after denervation the weight of the atrophied muscles was 33%.

In the experiments of series II, atrophy of the muscles continued until the 15th day just as in series I. Histological study of the material showed that on the 15th day regenerating axons were visible as a rule only in the nerve trunk approaching the muscle. No regenerating axons could be seen at that stage in the intramuscular nerve trunks. Slowing of the process of atrophy of the reinnervated muscles was observed on the 18th–19th day after division of the nerve (Fig. 1). Meanwhile, investigation of the contractile response of the muscles to stimulation of the regenerating tibial nerve showed that in most animals the muscles had not yet begun to contract at this time of the investigation. The weight of the regenerated muscles was 46% of the weight of the control muscles, whereas the weight of the denervated muscles was 42%. Histological examination showed that on the 19th day after division of the nerve some nerve trunks contained a few axons, although some trunks still contained none. Growing axons emerged from the nerve trunks and continued as single fibers, terminating in "bulbs of growth." Sometimes primary contacts between axons and muscle fibers were observed. These primary contacts consisted of simple division of an axon into two branches, sometimes with loops (Fig. 2). Serial sections through the muscle at this time of the investigation showed no motor end-plates. It was noted that in the part of the muscle where no growing axons were seen, the nucleoli in the muscle nuclei were enlarged, as is usual in a denervated muscle. In the part of the muscle which contained numerous regenerating axons, the nucleoli were enlarged to a still greater degree. Approximately two-thirds of the muscles tested 22 days after the operation had begun to respond by contraction to electrical stimulation of the nerve. Only those animals in which the muscle contracted in response to stimulation of the nerve were investigated. At this time the weight of the reinnervated muscles was 50% of the weight of the control muscles, while the weight of the denervated muscles was only 37%. This difference in the weight of the muscles is statistically significant. Histological examination of the material showed that at this time of the investigation most of the intramuscular nerve trunks contained axons, many growing axons with bulbs of growth were present along the muscle fibers, and numerous reinnervated motor end-plates could be seen. Terminals of the axons had not yet begun to divide in some of them, but they formed loops (Fig. 3). Fully formed motor end-plates also were found.

Fig. 3. Regenerating motor end-plate 22 days after division of nerve. Impregnation, 900 ×.

In the experiments of series III, when the nerve was redivided on the 15th day after the first division, at a time when regenerating axons had not yet formed contacts with the muscle fibers, the weight of the muscles fell during the first three days after the operation by almost the same amount in the experiments of series I with diversion of the nerve: in series I by 9%, and in series III by 7%. When the nerve was redivided 19 days after primary division, at a time when the regenerating nerve axons had reached the muscle fibers and were forming primary contacts, but the muscle had not yet begun to contract in response to stimulation of the nerve, the weight of the muscle three days after redivision was reduced by only 3%, this decrease not being statistically significant. Meanwhile, in the experiments of series I with diversion of the nerve the weight of the muscles by this period was reduced significantly by 5%. Despite redivision of the nerve, development of atrophy was inhibited. If the nerve was redivided 22 days after primary division, when motor end-plates had regenerated in the muscle and functional activity was clearly defined, three days after the second operation the weight of the muscles was reduced by 11%, so that it coincided with the weight of the atrophied muscles in the experiments of series I at the same time of the investigation.

These results show that both after diversion of the nerve and after its simple division, the process of atrophy of the gastrocnemius muscles in rats follows a similar course until the regenerating axons start to establish primary contacts with the muscle fibers. Before functional activity of the muscles is resumed, atrophy of the muscle fibers is slowed by the action of the regenerating axons, and as the time of functional activity draws close, the weight of the reinnervated muscles differs significantly from the weight of the denervated muscles. The considerable increase in size of the nucleoli in the part of the muscle into which nerves had grown suggests that the metabolism of the muscle tissue is altered by the regenerating axons even before function is restored. The effect of the nerve at this time is inductive in character. This is shown by the fact that the processes developing in the muscle under the influence of a regenerating nerve continue, as if by inertia, for some time after contact ceases between nerve and muscle. These processes inhibit the development of muscle atrophy. That is why redivision of the nerve on the 19th day of reinnervation leads to a much smaller decrease in weight than in the denervated muscle. After recovery of functional activity of the muscle, the influence of the nerve on the muscle loses its inductive character. Redivision of the nerve at this time leads to an appreciable loss of weight of the muscles, just as after primary division.

The results of this investigation thus confirm the existence of a trophic influence of the regenerating nerve on plastic processes in the muscle and they indicate that the trophic effect is inductive in character before functional contact is established between the nerve and the skeletal muscle.

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