COMPARATIVE ANALYSIS OF ATROPHY AND REINNERVATION OF MUSCLE TISSUE IN RATS AND FROGS

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Atrophy and reinnervation of the gastrocnemius muscles in rats and frogs differ not only in their rates of development, but also qualitatively. In rats both atrophy and reinnervation of muscle takes place several times faster than in frogs. During reinnervation of the rat muscle the regenerating nerve tissue influences the muscle tissue even before reappearance of the contractile function of the muscle, atrophy of which is delayed. However, the weight of the reinnervated muscles differs considerably from the weight of the intact muscles in the late stages after restoration of function. In frogs, during reinnervation the nerve tissue has no appreciable effect on the muscle tissue, but the weight of the muscles is almost completely restored 2 months after the commencement of their function.

On the basis of considerable experimental data Studitskii developed the concept of mechanisms of tissue regulation, controlling structural processes performed by the tissues [6, 7]. One type of tissue regulation distinguished by Studitskii is neurotrophic regulation. The trophic influence of the nervous system on organs and tissues, especially muscles, has frequently been studied and continues to attract attention [1, 8, 9]. Using autografted minced muscle tissue and whole muscles as experimental models, Zhenevskaya [2, 3] demonstrated that the trophic action of a nerve can be distinguished from its functional action, i.e., conduction of the impulse, and she discovered that the trophic action of a regenerating nerve on structural processes in skeletal muscle tissue is induced in character, confirming that a neurotrophic mechanism of tissue regulation actually exists. In the writers' previous investigations this trophic effect of the regenerating nerve was confirmed by studying processes of denervation and reinnervation of the gastrocnemius muscles in rats [4, 5].

The object of the present investigation was to compare the neurotrophic tissue regulation of structural processes in muscle tissue in denervated and reinnervated muscles of lower (frogs) and higher (rats) vertebrates.

EXPERIMENTAL METHOD

Altogether 74 pond frogs (Rana ridibunda) weighing 40-70 g and 123 noninbred albino rats weighing 100-120 g were used in the two series of experiments. In series I the tibial nerve was divided in the right hind limb at a distance of about 1 cm from the gastrocnemius muscle, and to prevent reinnervation the central end was sutured to the thigh muscles. In the experiments of series II the tibial nerve was divided at the same distance from the muscle but was not sutured to the neighboring muscle, so that the injured nerve could regenerate. The animals were fixed at intervals between 3 days and 4 months after the operation. Before fixation a physiological base line for the test muscles was obtained with a type ES-3M electrical heart stimulator. The muscles were weighed and fixed in 12% formalin, then impregnated with silver by the Bielschowsky-Gros method and gilded by Lavrent'ev's method. The left intact gastrocnemius muscle

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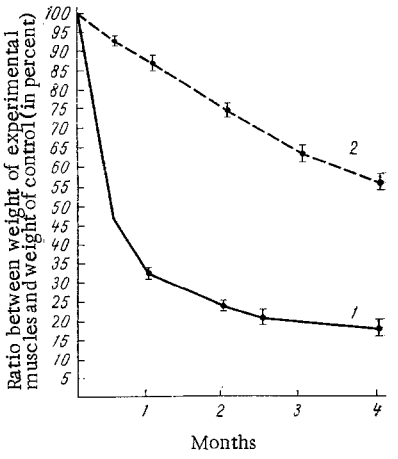

Fig. 1

Fig. 2

Fig. 1. Change in weight of atrophied muscles after division and displacement of tibial nerve in rats and frogs: 1) weight of rat muscles; 2) weight of frog muscles; vertical lines show extent of error.

Fig. 2. Regenerating axons in muscle tissue of a frog 1.5 months after division of the nerve. Impregnation, 900×.

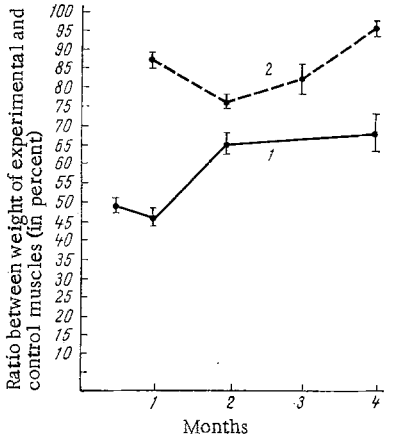

Fig. 3. Change in weight of reinnervated muscles after division of tibial nerve in rats and frogs. Legend as in Fig. 1.

was used as the control. The gravimetric data were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The results show that muscle atrophy develops very rapidly in rats after denervation. By the 3rd day after division and displacement of the nerve the weight of the muscle was reduced by 16% (Fig. 1). Histological examination showed that terminals in motor end-plates and neuromuscular spindles were degenerated. Axons were visible only in the nerve trunk entering the muscle. In frogs atrophy of the muscle fibers developed much more slowly after denervation. By the 15th day the loss of weight of the muscles was only 5% (Fig. 1). Terminals of myoneural synapsis and neuromuscular spindles also were degenerated, but occasional axons or their fragments could still be seen in the intramuscular nerve trunks.

By the 15th day the denervated rat muscles had lost half their weight, which was now only 49 ±1% of the weight of the control muscles. The denervated muscles of the frogs lost half their weight only after 4 months, when it was 55 ±2% of the weight of the control muscles.

The study of reinnervation processes showed that in rats the nerve began to grow into the muscle approximately on the 15th day after its division. On the 18th-19th day, many regenerating axons could be seen in the muscle. Primary contacts (of embryonic type) between motor nerve fibers and muscle fibers were observed: the myoneural synapse was still without its end-plate, no nuclei were visible in the synapse, the axons had no terminal branches, and they frequently ended in a "loop." A difference was observed between the weights of the reinnervated and denervated muscles, and this difference became statistically significant ($P < 0.001$) at the time of the first signs of restoration of function (22 days after division of the nerve). In frogs the nerve began to grow into the muscle approximately 1-1.5 months after division.

Reinnervation of the muscle took place less intensively than in rats. A few regenerating axons formed "loops," or pools of axoplasm, and sometimes the end of the axon was twisted into a corkscrew (Fig. 2). Primary contacts were observed between motor nerve fibers and muscle tissue, appearing as one or more loops around the muscle nucleus. However, both then and at the beginning of function of the muscles (2 months after division of the nerve), the difference between the weights of the denervated and reinnervated muscles was not significant.

A distinct contractile reaction of the muscles to nerve stimulation was detected in the rats 4 weeks after division of the nerve. On the 28th day the weight of the functioning gastrocnemius muscles was 46 ±2% of the weight of the control muscles, the difference from the weight of the denervated muscles being significant ($P < 0.001$). A rapid growth of axons took place in the muscles, many regenerating motor end-plates could be seen, and the nucleoli of many of the muscle nuclei were close to the normal size. After 4 months the weight of the reinnervated muscles reached 68 ± 5.5% (Fig. 3). In frogs appreciable recovery of contractile activity of the muscle was recorded 9 weeks after division of the nerve. The weight of the functioning muscles was then almost the same as in the denervated muscles, namely 77 ± 3% of the weight of the control muscles. The difference in weight between the denervated and reinnervated muscles became significant only 1 month after the beginning of function of the muscle. Four months after division of the nerve the weight of the reinnervated muscles was 95 ± 2% of the weight of the control muscles (Fig. 3).

Processes of atrophy thus take place more rapidly and more intensively in rats than in frogs. Reinnervation of the muscles in rats also takes place much faster than in frogs. The character of the curves respecting the change in weight of the muscles in rats and frogs after denervation and during reinnervation differed sharply (Figs. 1 and 3). The influence of nerve tissue on structural processes in the muscle tissue evidently differed considerably in these two series of animals. In rats the influence of nerve tissue on muscle tissue is manifested even in the period before function. This is shown by the fact that under the influence of the ingrowing axons there was virtually no further decrease in weight of the atrophied muscles. The action of the regenerating axons on the muscle tissue in frogs was not reflected in the weight of the muscles. Changes in the metabolism of the muscle tissue undoubtedly occurred under the influence of nerve tissue in frogs. However, these changes were so slight in degree and they took place so slowly that they did not affect the weight of the reinnervated muscles. Meanwhile the weight of the muscles in the frogs was restored more rapidly and more completely than in the rats. This suggests that in the course of evolution the neurotrophic mechanism of tissue regulation of structural processes in skeletal muscle tissue has become strengthened.

LITERATURE CITED

1. E. Gutmann and I. Hajek, in: Nervous Mechanisms of Motor Activity [in Russian], Moscow (1966), p. 9.
2. R. P. Zhenevskaya, Dokl. Akad. Nauk SSSR, 121, No. 1, 182 (1958).
3. R. P. Zhenevskaya, Experimental Histological Investigation of Neurotrophic Tissue Regulation of Structural Processes in Skeletal Muscle Tissue. Author's Abstract of Doctoral Dissertation, Moscow (1971).
4. R. P. Zhenevskaya and I. L. Novoselova, Dokl. Acad. Nauk SSSR, 185, No. 1, 214 (1969).
5. I. L. Novoselova, Byull. Éksperim. Biol. i Med., No. 10, 100 (1971).
6. A. N. Studitskii, Izvest. Akad. Nauk SSSR, Seriya Biol., No. 3, 332 (1948).
7. A. N. Studitskii, Arkh. Anat. No. 1, 29 (1964).
8. E. Gutmann, Curr. Res. Neurosci., 10, 54 (1970).
9. L. Guth, Physiol. Rev., 48, 645 (1968).