RESTORATION OF CONTRACTILE PROPERTIES OF MUSCLE AFTER PARTIAL AND TOTAL DENERVATION

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Data on the mechanisms and phenomenology of reinnervation of skeletal muscles provide a basis for the elaboration of clinical and instrumental diagnostic criteria. The time course of restoration of the contractile properties of muscles after total denervation has been studied [2, 9, 15]. However, in neuromuscular diseases denervation is usually partial and not total. Morphological and functional characteristics of the course of reinnervation after partial and total denervation differ significantly, suggesting that there are differences also in recovery of the contractile properties of the muscles.

This paper describes the results of investigations of the contractile properties of the tibialis anterior muscle (TAM) of rats and also of the number of motor units (nMU), the average voltage, in millivolts (mMU), and the strength of its functioning motor units (MU) at the initial stages of reinnervation after partial and total denervation.

EXPERIMENTAL METHOD

Experiments were carried out on 53 young, sexually mature albino rats weighing 102-132 g. Complete denervation of TAM was produced by crushing the sciatic nerve under sterile conditions at the level of the upper third of the thigh (group 2); partial denervation was produced by crushing nerve root L4, according to [8], on the opposite side (group 1). The control investigations were carried out on 18 TAM of nine intact animals and on eight TAM of the opposite, "sound" limb of eight animals of group 1. TAM in the rat receives its motor innervation mainly from root L4 and partly from L5 [10, 12]. On the basis of existing data on the course of reinnervation in the leg muscles in rats and mice after analogous total and partial denervation [4, 6, 7, 14] the animals were used in the experiments on the 7th, 14th, 28th, 42nd, and 63rd days after crushing. The methods of investigation of the contractile characteristics and electromyographic counting of nMU and mMU in experiments in vivo were described previously [2, 3, 5]. The following parameters were determined: M — the weight of the muscle (in mg); P_t/M — the strength of single, and P_0/M the strength of tetanic contraction in response to sciatic nerve stimulation, calculated per unit weight of muscle (in g/mg), P'_t/M — in response to direct stimulation correspondingly; CT — contraction time to the maximum (in msec); 1/2 RT — the half-relaxation time (in msec); P_t/P_0 — the ratio of the strength of single contraction to the strength of tetanic contraction; P_t/P'_t — the coefficient of functional innervation as described in [11]; pMU — the average strength of a single contraction developed by one MU (P_t/nMU, g). After the physiological investigations had ended the muscles were frozen in liquid nitrogen and cryostat sections were cut and stained histochemically [13] for succinate dehydrogenase (SDH) activity. The following types of fibers were identified: A) white, B) intermediate, and C) red. Taking account of differences in the histochemical composition of the control TAM, groups of fibers of one type in the middle part of the reinnervated TAM (more than 10) were interpreted as a pathological feature [14]. The total number of muscle fibers (which, in the animals used, was 3-4 times less than the average figure given in [10]) was counted in each preparation and the type composition determined (in %).

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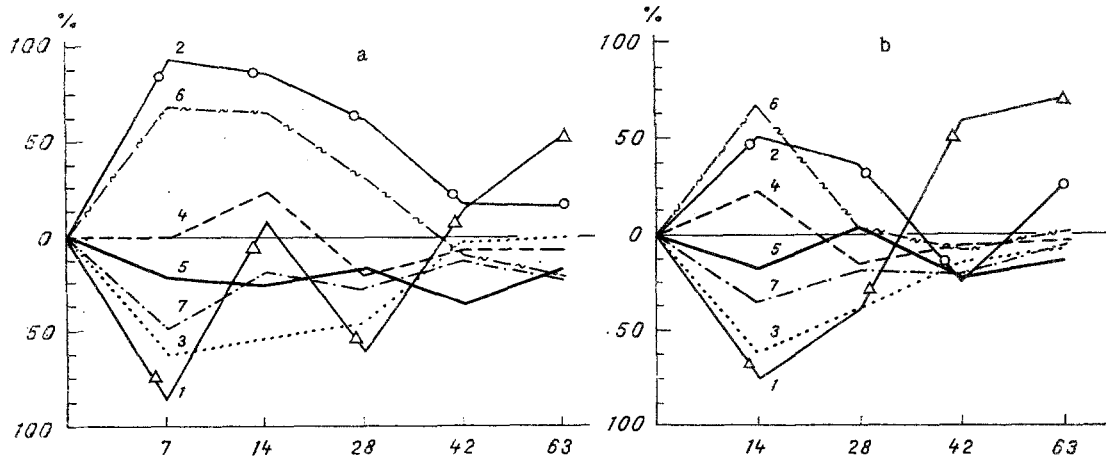

Fig. 1. Time course of changes in some contractile and electromyographic characteristics of rat TAM during regeneration after partial (a) and total (b) denervation. Abscissa, time (in days); ordinate, changes relative to control (in %). 1) mMU; 2) 1/2 RT; 3) P_0/M; 4) P_t'/M; 5) nMU; 6) P_t/P_0; 7) P_t/M.

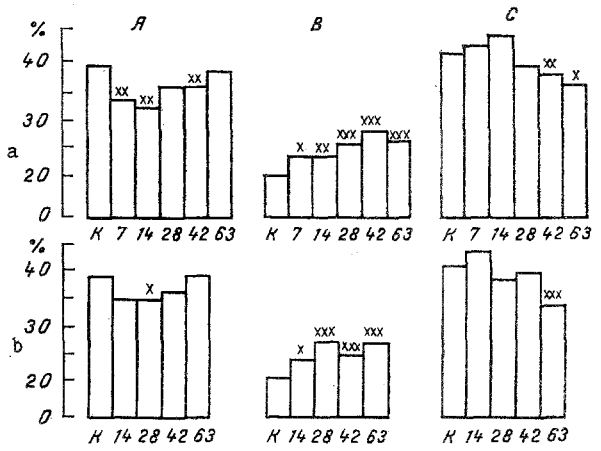

Fig. 2. Time course of changes in relative percentage of muscle fibers of types A, B, and C according to SDH activity in rat TAM at different times after partial (a) and total (b) denervation. K) Control; 7-63) days after denervation; x) $p < 0.05$, xx) $p < 0.01$, xxx) $p < 0.001$ compared with control.

EXPERIMENTAL RESULTS

In the animals of group 1, i.e., after partial denervation of TAM, neuromuscular junctions were restored initially by compensatory regeneration from intact axons of root L5 (sprouting). After 7 days, which according to existing data [4] can be considered to be the time when the formation of functioning synapses is complete, in the absence of any significant reduction in P_t/P_t', a decrease was found in P_t/M, P_0/M, nMU, mMU, and pMU, and an increase in P_t/P_0, and 1/2 RT (Fig. 1). The observed changes in the contractile properties are characteristic of denervation [9, 15]. A decrease in mMU and pMU in MU whose axons are contained in the preserved root L5 was noted when these MU produced a compensatory renervation of the muscle by sprouting. This decrease reflects the process of active restructuring of the MU [1]. Groups of fibers of one type at this and subsequent times were not observed in the animals of group 1. Only a small increase in the relative percentage of type B fibers was found (Fig. 2).

After 14 days, in the animals of group 1 compensatory reinnervation can be assumed to be complete and "true" reinnervation by regenerating axons to have begun [4]. Changes in the contractile properties preserved the same tendencies as before (Fig. 1). Meanwhile some increase in P_t/M, recovery of mMU and pMU, and a decrease in nMU were observed. This indicates continuing reorganization of the architectonics and "enlargement" of functioning MU.

The next stage (28 days) is characterized by completion of "true" reinnervation [4]. Under these circumstances some muscle fibers lose their compensatory reinnervation and make connections with regenerating axons. Meanwhile many muscle fibers have a polyneuronal innervation at this stage [4]. Changes in contractile properties preserved their previous tendencies at this time, but for the first time reduction of P_t'/M, characteristic of this period of reinnervation after total denervation [9], began to appear (Fig. 1); nMU remained depressed, but a decrease in mMU reappeared; this probably reflects the time course of replacement of neuromuscular connections.

On the 42nd day most functional characteristics were back to normal; nMU continued to be reduced, but pMU was increased. On the 63rd day a decrease in P_t/M was found and a decrease in P_t/P_0 for the first time. According to existing data, at this time not only is a certain number of reduced MU found, formed on account of "true" reinnervation of MU, and also polyneuronally innervated muscle fibers, but other MU enlarged due to sprouting may also be present [4].

In the animals of group 2, i.e., after total denervation of TAM, reinnervation began on the 14th day on account of regenerating "indigenous" axons [14]. Synapse formation continued until the 25th day. On the 15th day 25% of muscle fibers had polyneuronal innervation, but after the 25th day the process of liquidation of "surplus" synapses began [6]. At this time the number of muscle fibers of intermediate type increased, and by the 42nd day, groups of fibers of one type were discovered [14]. After 60 days the muscle fibers had mononeuronal innervation [6]. The time course of changes in the contractile characteristics continued even after 60 days [2]. Investigation of the contractile properties (Fig. 1) and histochemical type composition (Fig. 2) of the animals of group 2, continued until the 63rd day, and their comparison with the corresponding findings in the animals of group 1 revealed a marked similarity of the tendencies of the time course of their recovery. Differences were observed in the times of the changes (after partial denervation the recovery process took longer because it began sooner) and they were quantitative in character (Fig. 1). Meanwhile, comparison of the time course of changes in mMU and pMU in the two groups revealed significant differences, especially in the initial stages of reinnervation. Groups of fibers of one type — a sign formed during reinnervation of the stable MU also, were found only in the animals of group 2, starting with the 42nd day. In the animals of group 1 they were not found at these times, whereas data showing the presence of "enlarged" MU were obtained both by ourselves (Fig. 1) and by other investigators [4] by physiological methods. Histochemical transformation of the muscle fibers of those MU which were formed after partial denervation takes place late in the rat TAM [10] — much later than in MU formed after total denervation [12]. Consequently, the dissociation in time between the electromyographic and muscle histochemical features of restructuring of MU during compensatory reinnervation, observed by ourselves and other workers [14], was greater than during "true" reinnervation.

Thus in the initial stages of reinnervation after partial and total muscle denervation differences are found in the electromyographic and muscle-histochemical characteristics of MU, quantitative differences are found in the time course of recovery of the contractile properties of the whole muscle, while the general tendencies of their changes characteristic of denervation are preserved. These differences reflect particular features of the nervous regulation of the contractile functions of a muscle during the course of reinnervation, which differs after partial and total denervation. The results can be used in the development of new diagnostic criteria for the course of human neuromuscular diseases.

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EXPERIMENTAL BASIS FOR USE OF ULTRASONIC SURGICAL INSTRUMENTS IN NEUROPHYSIOLOGY

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Improvement in surgical techniques and application of the latest advances in science have led to the development of instruments for working with biological tissue, whose cutting edge makes microscopic longitudinal oscillations of ultrasonic frequency, of between 23,000 and 70,000 Hz, with an amplitude of up to 0.1 mm or more [1]. More recently ultrasonic instruments (USI) have begun to be used in surgery. The reason is the many important advantages of USI over traditional instruments: reduction of the force required when working on solid tissues, the fact that they have a hemostatic effect, so that operations can be performed in regions difficult of access, and reduction of operative trauma [2, 3].

The study of the experimental physiological basis of the action of USI on the brain must lead to improvement in existing types of instruments and the development of new ones, and must widen the range of their application in neurosurgery [4, 5]. Meanwhile it is not yet clear what late aftereffects may arise following the use of USI on brain tissue.

The aim of this investigation was to study the results of the action of USI on the functional state of the brain 8-9 days after injury to brain structures.

EXPERIMENTAL METHOD

Chronic experiments were carried out on 11 adult cats weighing 3-3.5 kg. The somatosensory projection area (SI) and the visual area (VI) of the cortex were destroyed by ultrasound or extirpated with the aid of a curette. For ultrasonic destruction of the above projection areas, an experimental ultrasonic neurosurgical system, developed at the Department of Neurosurgery, Central Postgraduate Medical Institute, in conjunction with the Acoustic Institute, Academy of Sciences of the USSR, was used.

Electrodes were first inserted into the animals (under pentobarbital anesthesia, 40 mg/kg) under aseptic conditions into the following cortical and thalamic structures: symmetrical area SI and VI of the intact hemisphere; the parietal region (P) of both hemispheres, bilaterally into the thalamic nuclei — the specific nucleus ventralis posterolateralis (VPL), the specific optic nucleus — the lateral geniculate body (LGB), and the association nucleus lateralis posterior (LP). Evoked potentials (EP) were derived by a monopolar technique and subsequently averaged on an APT-1000 analog computer. Peripheral stimulation was carried out unilaterally. The skin of the forelimb was stimulated by electrical discharges (19-20 V; 0.5 msec) and flashes from an FS-02 photostimulator (0.3 J, 0.05 sec) also were used. After the end of the experiment the animals were killed, the brain was fixed in 10% formalin solution, and after postfixation in alcohols, paraffin blocks were made and sections cut to a thickness of 20 μ, and stained by Nissl's method.

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