MORPHOLOGICAL AND HISTOLOGICAL CHARACTERISTICS OF FROG SKELETAL MUSCLE ON APPLICATION OF COLCHICINE TO THE MOTOR NERVE

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After experimental application of colchicine to the motor nerve of a muscle to disturb fast axoplasmic transport the area of cross section of the muscle fibers was reduced, the number of fibers with low succinate dehydrogenase (SD) activity was increased, and the muscle fibers were more homogeneous as regards their optical density after staining for SD activity. Similar changes also were found after division of the motor nerve. However, denervation was accompanied by blocking of conduction and degenerative disturbances in the nerve endings. In preparations treated with colchicine the conduction of excitation in the nerve and through the synapse continued and miniature end-plate potentials (MEPP), end-plate potentials (EPP), and action potentials (AP) of the muscle fibers were recorded. It is concluded that fast axoplasmic transport supplies the muscle with substances maintaining the differentiated state of its fibers.

KEY WORDS: *motor nerve; colchicine; axoplasmic transport; conduction of excitation.*

The application of Vinca alkaloids and colchicine to a motor nerve blocks fast axoplasmic transport taking place through the microtubules of the axon [7, 9, 15]. Under these conditions a conduction of nervous impulses and transmission of excitation in the myoneural synapses are essentially undisturbed [3, 4, 12]. However, blocking of the fast axoplasmic current stops the intra-axonal transport of substances which, in the opinion of several workers [5, 8, 14], are synthesized in the perikaryon of the motoneuron and, when supplied to the muscle along the axon, exert a "trophic" influence on it. The removal of this influence leads to the appearance of denervation phenomena in the skeletal muscle [4, 8, 11].

Consequently, by using colchicine, which destroys microtubules, it is possible to study neuromuscular transmission and the "trophic" influence of the nerve on skeletal fibers separately.

TABLE 1. Areas of Cross Section and Relative Percentages of Muscle Fibers with Different Levels of SD Activity in Frog Sartorius Muscle (15 days after procedure)

Type of muscle fibers with respect to SD activity	Application of 30 mM colchicine solution to motor nerve				Divison of motor nerve of muscle				Application of Ringer's solution to motor nerve			
	areas of cross section of muscle fibers in conventional planimetric units		relative number of muscle fibers, %		areas of cross section of muscle fibers in conventional planimetric units		relative number of muscle fibers, %		areas of cross section of muscle fibers in conventional planimetric units		relative number of muscle fibers, %	
	control	experiment	control	experiment	control	experiment	control	experiment	control	experiment	control	experiment
α	3,42±0,07	2,09±0,06	46,06	57,41	3,34±0,07	1,84±0,05	38,81	50,39	3,40±0,07	3,44±0,08	39,88	41,08
αβ	1,32±0,04	0,96±0,02	35,48	28,24	1,32±0,04	1,05±0,02	36,10	26,96	1,30±0,04	1,34±0,05	32,99	31,78
β	0,67±0,02	0,52±0,01	18,46	14,35	0,84±0,02	0,63±0,02	25,09	22,65	0,73±0,02	0,67±0,02	27,13	27,14

Legend: α) low SD activity; αβ) average, β) high activity.

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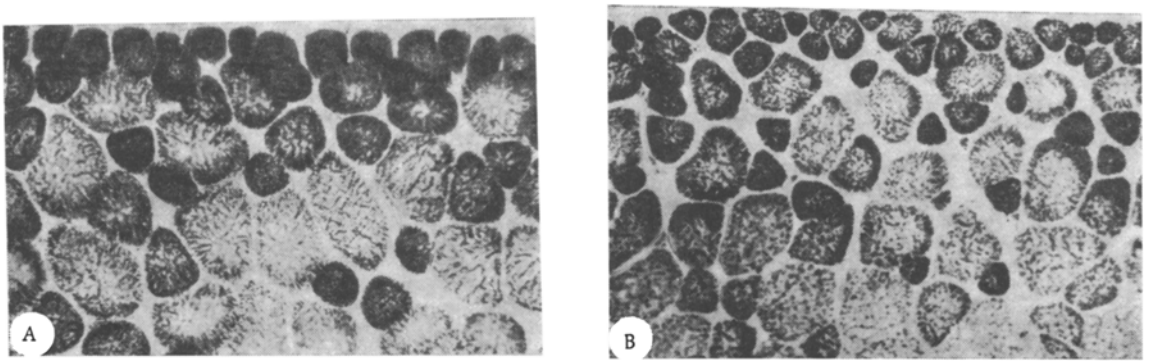

Fig. 1. SD activity in fibers of frog sartorius muscle: A) control; B) 15 days after application of colchicine to motor nerve. Stained with nitro-BT, photomicrograph, 63×.

In this investigation this method was used to study skeletal muscle by the application of morphometric, histochemical, and electrophysiological methods, permitting an objective assessment of whether "denervation" phenomena do in fact arise when the fast axoplasmic current is blocked but myoneural transmission of excitation is preserved.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscle of *Rana ridibunda*. A swab soaked in a 30 mM solution of colchicine (Merck), made up in Ringer's solution for cold-blooded animals, pH 7.0, was applied for 20 min to the surface of the nerve leading to the muscle at a distance of 2-3 cm from it. After removal of the swab the skin wound was sutured. In a series of experiments anatonical denervation of the muscle was performed by dividing its motor nerve, and a swab soaked in Ringer's solution only was applied to the dissected nerve.

The animals were killed 2 weeks after the procedures, experimental and control muscles were isolated, transverse sections were cut through the muscles and succinate dehydrogenase (SD) activity was demonstrated in them by the method with nitro-BT. The sections were photographed and the negatives were scanned in the IFO-451 recording microphotometer. The optical density values were conventionally divided into nine groups in diminishing order.

The positives were recorded and the area of cross section of the muscle fibers determined by means of a planimeter. Measurements were made separately for three types of fibers differing in the intensity of their staining for SD. Pale fibers, i.e., those with low SD activity, were conventionally described as α fibers, those with average activity as $\alpha\beta$ fibers, and the dark fibers, i.e., those with high SD activity, as β fibers [10].

Nerve endings in the muscle were studied by Jabonero's impregnation method. End-plate potentials (EPP), miniature end-plate potentials (MEPP), and action potentials (AP) of the muscle fibers were recorded with the aid of microelectrodes. A type UBP-2-03 biopotential amplifier with cathode follower at the input was used. The potentials were recorded from the screen of an S1-18 oscilloscope by means of the FOR camera. The nerve was stimulated by an ESL-2 stimulator with high-frequency output stage. All the (40) experimental animals were kept at room temperature.

The numerical data were subjected to statistical analysis using parametric [2] and nonparametric [1] methods.

EXPERIMENTAL RESULTS

The results showed (Table 1) that after the application of colchicine to the motor nerve the area of cross section of the muscle fibers was less than in the control. This was true of all three types of fibers. The changes observed were highly significant ($P < 0.001$). After anatomical denervation a significant decrease in the area of cross section of these types of muscle fibers also was found ($P < 0.001$). In experiments in which Ringer's solution was applied to the motor nerve no significant differences were found in the area of cross section of the muscle fibers of the experimental and control groups.

The relative percentages of the various fibers in the muscle whose motor nerve had been treated with colchicine showed an increase in the number of α fibers and a decrease in the number of $\alpha\beta$ and β fibers (Table 1; Fig. 1A, B). The differences between the numbers of α

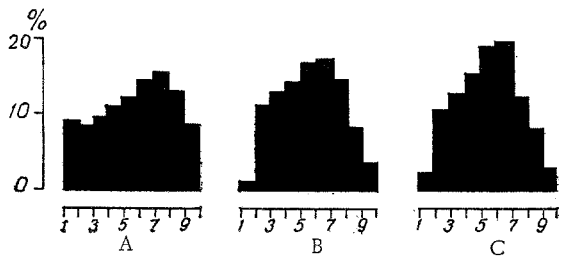

Fig. 2. Distribution of muscle fibers in frog sartorius muscle by optical density: A) after application of Ringer's solution to motor nerve; B) after application of colchicine to motor nerve; C) after division of nerve. Abscissa, optical density (conventional units); ordinate, distribution of muscle fibers, in per cent, by level of optical density.

fibers in the experimental and control muscles were significant ($P < 0.05$). No significant differences could be found separately for the αβ and β fibers. Similar changes were observed in the anatomically denervated muscle. In experiments in which Ringer's solution was applied to the motor nerve no changes could be found in the relative numbers of the different types of fibers in the muscles of the experimental and control limbs.

In experiments with anatomical denervation and application of colchicine to the motor nerve of the muscle (Fig. 2B, C) a sharp decrease was observed compared with the control (Fig. 2A) in the number of fibers belonging to the 1st and 9th optical density groups and an increase in the number of fibers belonging to the 5th and 6th groups. This is evidence of increasing homogeneity of the fibers with respect to optical density. It can accordingly be postulated that SD activity in the β fibers was reduced and in the α fibers increased, so that both types of fibers came to resemble the αβ type more closely with respect to this index.

After application of colchicine to the motor nerve no degenerative changes were found in the nerve endings, whereas the distal portion of the divided nerve underwent degeneration. Changes discovered in the skeletal muscle after anatomical denervation and after the application of colchicine to its motor nerve were thus similar. In both cases well-marked atrophic changes were observed. However, after anatomical denervation the conduction of nervous impulses is disturbed and the spontaneous secretion of the mediator in the synapses ceases [6, 13]. The present experiments showed that after application of colchicine to the axon the spread of excitation along the membrane of the nerve fiber, its transmission in the neuromuscular synapse, and also the spontaneous secretion of acetylcholine by the nerve endings are preserved, as is shown by the possibility of recording the MEPP, EPP, and AP of the muscle fiber.

The results of these experiments indicate that the changes observed in a muscle after the application of colchicine to its motor nerve are not connected with removal of the effect of acetylcholine, but are due to a disturbance of the fast axoplasmic current, which is probably responsible for transporting to the muscle the active substances which are needed to maintain the fine functional differentiation of muscle fibers.

The essential point to note is that the changes of a denervation character recorded in the muscle were the same whether due to the blocking of the fast axoplasmic current or to anatomical division of the nerve.

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