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EFFECT OF DISTURBANCE OF MOTOR FUNCTION ON PROPERTIES OF THE SKELETAL MUSCLE FIBER MEMBRANE

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Denervation of frog muscle depresses the resting membrane potential (RMP), modifies the passive electrical properties of the membrane, and leads to the appearance of extrasynaptic sensitivity to acetylcholine (ACh) [2, 3]. It is suggested that the neurotrophic control of the above-mentioned parameters of the muscle membrane is effected with the participation of substances carried to the muscle by axoplasmic transport [2, 3]. Denervation-like changes arising in the membrane after blocking of axoplasmic transport by colchicine differ quantitatively to some extent from those arising after denervation of the muscle. It has been postulated that these differences are due to the fact that after division of a nerve or blockade of axoplasmic transport the muscle finds itself in different situations: in the first case it is immobile, whereas in the second its motor activity is preserved [1].

Immobilization of frog muscle by botulinus toxin is also known to cause a denervation-like increase in postsynaptic sensitivity to ACh [5], although to a lesser degree than denervation. In this connection the view is held that nervous impulses and motor activity of the muscle connected with them, are essential for maintaining the differentiated state of the muscle membrane. However, experiments with immobilization of a muscle by acting on its nervous apparatus do not solve the problem of what is more important: motor activity of the muscle as such or the presence of a certain type of nervous impulsation [2]. To solve this problem, it is therefore interesting to conduct experiments with immobilization of a muscle without any direct action on neuromuscular transmission. Experiments of this kind are of practical importance for various forms of immobilization of the limbs are used in clinical practice as a method of treatment after trauma to them.

The aim of the present investigation was to study the effect of tenotomy and immobilization of the limb on the properties of the frog muscle fiber membrane.

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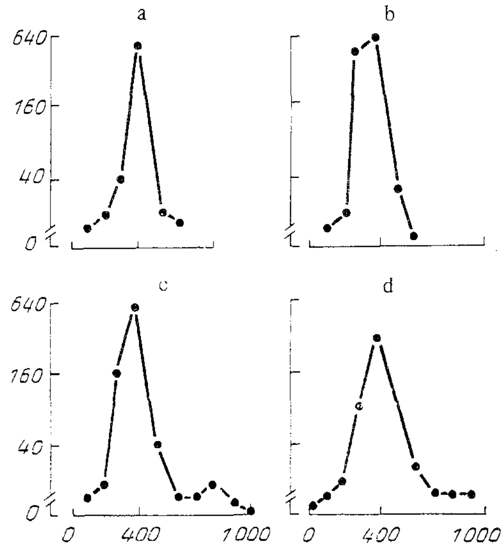

Fig. 1. Sensitivity of muscle fibers to ACh in control (a), 2 weeks after tenotomy (b), and 2 (c) and 3 weeks (d) after immobilization of limb by metal splint in position of extension. Abscissa, distance along fiber (in μ); ordinate, sensitivity to ACh (in mV/pC).

EXPERIMENTAL METHOD

Experiments were carried out in the fall on the sartorius muscle of *Rana temporaria*. RMP, input resistance (R_0), and the time constant (τ) of the membrane were measured by the voltage drop on the membrane method; the sensitivity of the muscle fibers to ACh was measured by application of the mediator from a micropipet. When values of maximal sensitivity to ACh were calculated, a correction was introduced for the magnitude of the RMP [9].

In the animals of one group, the tendon of the sartorius muscle attaching it to the knee joint was divided in the right hind limb through a skin incision. In the other groups the right hind limb of the animals was immobilized by means of a metal splint so that all three joints — ankle, knee, and hip — were immobilized. The limb was kept in the extended position (the muscle was stretched) and parallel to the long axis of the trunk. All operations on the animals were conducted under ether anesthesia. After the operation the animals were kept in a terrarium with a supply of running water at room temperature. The corresponding muscles of intact frogs served as the control. During the experiments the muscle was kept in Ringer's solution of the following composition (in mM): NaCl 115, KCl 2.5, $CaCl_2$ 1.8, in phosphate buffer, pH 7.3, at room temperature.

EXPERIMENTAL RESULTS

The writers showed previously that division of a motor nerve reduces RMP 13-15 days after the operation and increases R_0 and τ of the membrane. The zone of postsynaptic sensitivity of the muscle fibers to ACh is widened under these circumstances without any change in maximal sensitivity to the mediator [3].

After tenotomy, the value of RMP, R_0, and τ of the membrane did not differ from the control at this same time. There were likewise no changes in the character of postsynaptic sensitivity to ACh (Fig. 1, Table 1).

Disturbance of the motor function of the muscle by tenotomy thus does not lead to the appearance of denervation-like changes in the muscle membrane. The results agree fully with those of previous experiments on rats to study the effect of tenotomy on properties of sensitivity of the muscle membrane to ACh and its selective ionic conductance [8].

TABLE 1. RMP, Passive Electrical Properties, and Sensitivity to ACh of Muscle Fiber Membrane of Intact Frog Sartorius Muscle and Also of Muscle 2 Weeks after Tenotomy and 2 and 3 Weeks after Immobilization of Limb in Extension by Metal Splint (M ± m)

Experimental conditions	RMP, mV	Input resistance, kΩ	Time constant, msec	Maximal sensitivity to ACh, mV/pC	Zone of sensitivity to ACh, μ
Control	85,4±0,5 (100)	425±24 (40)	24,3±0,8 (40)	234±65 (11)	550±50 (11)
Tenotomy	85,8±0,7 (60)	409±53 (37)	26,5±1,5 (37)	211±90 (7)	460±46 (7)
Immobilization					
2 weeks	86,2±0,5 (75)	427±49 (40)	25,3±1,7 (40)	247±67 (13)	975±51 (13) $P<0{,}001$
3 weeks	87,5±1,0 (55)	601±100 (30)	27,0±2,9 (30)	148±40 (8)	913±91 (8) $P<0{,}001$

Legend. Number of fibers studied shown in parentheses. Level of significance taken as 0.05.

However, according to one view, tenotomy is not an absolutely adequate model for immobilization of a muscle because it does not prevent its shortening. In the next series of experiments a different model of immobilization was accordingly used, namely immobilization of the whole of the animal's limb in the position of extension, when the sartorius muscle is stretched.

Under these conditions, on the 13th-15th day after the operation there was likewise no decrease in RMP and no increase in R_0 and τ of the membrane. However, the width of the zone sensitive to ACh was significantly greater than in the control, namely about 1 mm, but it was less than at the corresponding time after denervation of the muscle, when the width was more than 1.5 mm. Maximal sensitivity of the membrane to ACh was unchanged under these circumstances (Fig. 1, Table 1). Widening of the sensitive zone of the muscle fibers indicates that inactivation of the muscle by immobilization of the limb in the position of extension may lead to denervation-like changes, although to an appreciably lesser degree than denervation itself.

To test the hypothesis that changes may develop more slowly in this version of muscle inactivation than after division of the nerve, the effect of immobilization of the limb on properties of the muscle fiber membrane was studied a longer period after application of the splint. On the 19th-21st day the values of RMP, R_0, and τ of the membrane as before were indistinguishable from the control (Table 1). The width of the zone of postsynaptic sensitivity of the muscle fibers to ACh was greater than in the control but was unchanged compared with that after an interval of 2 weeks (Fig. 1, Table 1). Values of maximal sensitivity to ACh remained the same as before (Table 1). Consequently, the results did not confirm the writers' previous hypothesis that differences between the effect of immobilization of the muscle in the stretched state and division of the nerve are purely temporary or quantitative in character.

Experiments with tenotomy and mechanical immobilization of the muscle thus lead to the conclusion that motor activity of the muscle as such is not a decisive factor maintaining the differentiation of the frog muscle fiber membrane at a level assigned by the nervous system. This conclusion is in good agreement with data in the literature on the effect of inactivation of the mammalian limb (without any action directed toward the nervous apparatus of the muscle) on the properties of the muscle fiber membrane [6, 7].

During immobilization of the limb, we did, however, observe some widening of the chemosensitive zone of the fibers. Stretching a muscle is known to excite its proprioceptors, which influence the firing pattern of the motoneurons through the gamma-afferent pathway [4, 10]. The character of the spike discharge also is known to influence neurotrophic control of the properties of the muscle membrane [2]. It can therefore be tentatively suggested that the appearance of a denervation-like widening of the zone of chemosensitivity to ACh on the muscle fiber membrane during immobilization of the limb may be connected with changes in the motoneuronal firing pattern. This hypothesis also is confirmed by the fact that immobilization affects the properties of the muscle membrane differently depending on whether the muscle is in a free or stretched state.

Consequently, some quantitative differences in the denervation-like changes arising after blockade of axoplasmic transport and after denervation of the muscle, and also changes of denervation-like character appearing after disturbance of neuromuscular transmission are attributable not to the presence or absence of motor activity as such, but to the necessity of neuronal impulsation and of electrical activity of the muscle for effective neurotrophic regulation of the properties of the muscle fiber membrane.

The results confirm the view that motor activity itself is not the factor that determines the level of differentiation of the muscle membrane. That level is assigned by motoneuronal activity.

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