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EFFECT OF CHRONIC PRESYNAPTIC NEUROMUSCULAR TRANSMISSION BLOCK ON PROPERTIES OF FROG MUSCLE FIBER MEMBRANES

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Denervation of a muscle, disturbing neurotrophic control, changes the properties of the frog muscle fiber membrane [4]. Extrasynaptic sensitivity to acetylcholine (ACh) appears, the resting membrane potential (RMP) falls, and the cable properties of the membrane are modified [2, 4, 18].

Chronic postsynaptic blocking of neuromuscular transmission by curare or α-bungarotoxin has been shown not to change the properties of frog muscle fiber membranes [6]. This suggests that nervous impulses and synaptic ACh do not play a decisive role in maintenance of the functionally necessary state of muscle fibers determined by the nervous system. Meanwhile it has been shown that presynaptic blocking of ACh secretion by botulinum toxin causes a series of denervation-like changes in the muscle membrane, although to a less marked degree than denervation of the mucle [8], and this is regarded as proof of the participation of ACh in trophic influences on muscle. Meanwhile in experiments with botulinum toxin not only does ACh secretion cease, but the outflow of substances brought into nerve terminals by axonal transport, and exerting a trophic influence on muscle, from the terminals also is disturbed [10]. At the same time the muscle is excluded from motor activity. All these considerations make it difficult to evaluate the role of both synaptic ACh and of nervous impulses and the associated motor activity of the muscle in neurotrophic control of muscle fibers.

It was accordingly decided to make a further study of the contribution of ACh and nervous impulses in neurotrophic control of the muscle fiber membrane in frogs, using different experimental approaches for the purpose, and details of this study are given below.

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EXPERIMENTAL METHOD

Experiments were conducted in winter on the sartorius muscle of frogs (*Rana ridibunda*). RMP and input resistance were measured by means of a standard microelectrode technique, the time constant (τ) of the membrane was measured by the voltage drop on the membrane, and sensitivity of the muscle fibers to ACh was determined by application of the mediator from a micropipet [18]. When values of maximal sensitivity to ACh were calculated a correction was introduced for the value of RMP [18].

Under ether anesthesia, in animals of group 1 the motor nerve was divided through a skin incision at a distance of 1.5-2 cm from its point of entry into the muscle. In the animals of group 2, a glass capsule 10 mm long and 2 mm in diameter, with two end holes 20-30 μ in diameter, and containing 20-30 μl of tetrodotoxin (from San Kyo, Japan) in a concentration of 10^{-2} M, was placed alongside the nerve supplying the sartorius muscle. In this way conduction of action potentials along the nerve could be blocked for more than 1 week [16]. The frogs also received daily subcutaneous injections of 0.2 ml of 3×10^{-4} M tetrodotoxin solution, which immobilized them completely for several hours. To disturb ACh synthesis in nerve endings the animals of group 3 received daily subcutaneous injections of an aqueous solution of hemicholinium-3 (from Sigma, USA) in a dose of 30 μl per animal [11]. All the frogs were kept in a terrarium with running water at room temperature. Corresponding muscles of intact frogs served as the control. The animals were used in the experiments on the 13th-15th day after the operation or beginning of the injections. During the experiment the muscle was kept in a bath with continuously flowing 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

A reduction of RMP, an increase in R_o and τ of the membrane, and the appearance of extrasynaptic sensitivity to ACh were observed 2 weeks after division of the nerve (Table 1, Fig. 1). Maximal values of sensitivity of the muscle membrane to mediator remained unchanged (Table 1). This is in agreement with earlier observations [2], but the size of the zone sensitive to ACh was smaller at this time after denervation of the muscle in absolute magnitude than usually [2, 4, 18]. The size of the zone of postsynaptic sensitivity to ACh also was smaller than usual in intact frogs. As regards this parameter the latter were similar to "summer" frogs, in which the size of the zone of post-synaptic sensitivity to ACh is half that of "winter" frogs [3]. This was evidently a particular feature of this batch of animals and may also have been attributable to the unusually mild weather in the fall and winter.

Injection of hemicholinium-3 into the frogs for 2 weeks, disturbing neuromuscular transmission and immobilizing the animal, did not change RMP or τ, but increased R_o of the muscle membrane. The size of the zone of the postsynaptic membrane sensitive to ACH in most fibers studied was within the limits of the control values (Table 1, Fig. 1), but in some cases it was larger, namely 700-800 μ, suggesting the appearance of extrasynaptic sensitivity to ACh. Maximal sensitivity to ACh under these circumstances was reduced in magnitude, in agreement with data in the literature [12, 20].

Disturbance of neuromuscular transmission by injection of hemicholinium-3 thus gave rise to a denervation-like increase in R_o of the muscle membrane and, in some cases, to the appearance of extrasynaptic sensitivity to ACh.

Hemicholinium-3 is known to disturb ACH synthesis in nerve endings by preventing reuptake of choline from the synaptic space, as a result of which the outflow of ACh from the presynaptic membrane is reduced. As a result conduction of excitation from nerve to muscle is blocked [12, 20]. This raises the question of what is responsible for the denervation-like changes in the muscle membrane which we observed: a deficiency of synaptic ACh or disturbance of the motor function of the muscle.

No changes in RMP, τ of the membrane, or the size of the zone of post-synaptic sensitivity to ACh and values of maximal sensitivity of muscle fibers to the mediator were found 2 weeks after the beginning of immobilization of the muscle by tetrodotoxin (Table 1, Fig. 1). Tetrodotoxin blocks potential-sensitive sodium channels of axonal [7] and muscle membranes [18] strictly selectively, disturbing action potential generation in nerve and muscle fibers but without affecting axonal transport of materials [19] or the sensitivity of the postsynaptic membrane to ACh, as our own data show (Table 1).

TABLE 1. RMP, R_o, τ, and Sensitivity to ACh of Frog Sartorius Muscle Fiber Membrane in Control and on 13th-15th Day after Denervation of Muscle or Its Immobilization by Daily Injections of Hemicholinium-3 or Implantation of Capsule with Tetrodotoxin beneath the Nerve (M ± m)

Experimental conditions	RMP, mV	R_o, kΩ	τ, msec	Zone of sensitivity to ACh, μ	Maximal sensitivity to ACh, mV/pC
Control	85,8±0,4 (100)	452±37 (40)	25,2±1,0 (40)	300±23 (10)	239±45 (10)
Denervation	81,3±0,6 † (100)	607±63* (40)	28,9±1,5* (40)	700±45 † (11)	314±110 (11)
Hemicholinium-3	86,3±0,6 (80)	625±53* (40)	25,2±1,2 (40)	510±110 (8)	78±31* (8) $P<0,05$
Tetrodotoxin	85,2±0,5 (70)	620±63* (30)	25,1±0,8 (30)	390±39 (8)	176±53 (8)

Legend. Number of fibers tested given in parentheses. *$P < 0.05$, †$P < 0.001$ compared with control.

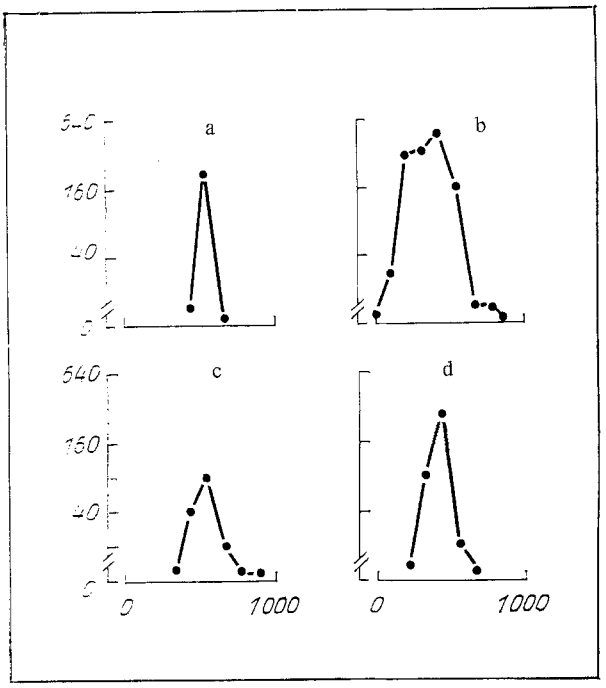

Fig. 1. Sensitivity of muscle fibers to ACh in control (a), on 13th-15th day after denervation of muscle (b), its immobilization by daily injections of hemicholinium-3 (c), or implantation of capsule with tetrodotoxin beneath nerve (d). Abscissa, distance along fiber (in μ); ordinate, sensitivity to ACh (in mV/pC).

It can thus be tentatively suggested that in experiments both with hemicholinium-3 and with tetrodotoxin the appearance of a denervation-like increase in R_o of the muscle membrane was due, not to absence of ACh, but to disturbance of the flow of nervous impulses and the associated disturbance of motor activity of the muscle. Evidence against participation of ACh in neurotrophic control of the frog muscle membrane is given by experiments with blocking of axonal transport, when denervation-like changes in muscle fibers were observed although quantum [1] and nonquantum ACh secretion [5] were preserved, and ACh has no effect on the development of changes in denervated muscle fibers in experiments *in vitro* [14].

As has already been pointed out, chronic postsynaptic blocking of neuro-muscular transmission does not lead to denervation-like changes in the muscle membrane [6]. Hence it can be concluded that what is important for the neurotrophic control of muscle fibers is not so much cholinergic transmission of excitation from nerve to muscle itself and motor activity of the muscle as the presence of nervous impulses and a preserved mechanism of neurosecretion in nerve endings. It has been shown that peptides capable of being carried to the muscle with axonal transport are secreted from motor nerve endings in the fraction with ACh [17]. Nervous impulses stimulate and their absence, conversely, inhibits the release of peptides from nerve endings in the fraction with ACh, which have a neurotrophic action [21]. The appearance of denervation-like changes in the muscle membrane in the presence of a chronic disturbance of neuromuscular transmission by hemicholinium-3 or blocking of conduction along the nerve by tetrodotoxin may be connected both with a disturbance of evacuation of materials from nerve endings, which are carried by axonal transport and exert a trophic influence [2, 4], and with absence of nervous impulses. The latter may have an effect of its own on the mechanism of neurotrophic control of muscle fibers through Ca^{++} ions [9, 13], secreted from the sarcoplasmic reticulum at the time of passage of the action potential along the muscle membrane.

In experiments on warm-blooded animals with chronic presynaptic blocking of neuromuscular transmission by hemicholinium-3 [11] or with disturbance of the conduction of excitation along the nerve by tetrodotoxin [16, 19] under similar experimental conditions several denervation-like changes develop in the muscle fibers, and in particular, extrasynaptic acetylcholine receptors appear on the muscle membrane. However, these changes are less marked than in the denervated muscle. In mammals, synaptic ACh and nervous impulses probably play a more important role in the neurotrophic regulation of muscle fibers than in frogs, and this is evidently a distinguishing feature of the neurotrophic regulation of muscle fibers in amphibians. Meanwhile all the changes may be connected with the fact that absence of nervous impulses and disturbance of ACh secretion in the synapse may affect the process of release of substances from nerve terminals which are carried there by axonal transport and which have a trophic influence on the muscles [4].

It can be concluded from the results of these experiments that immobilization of a frog muscle by disturbing the conduction of excitation along the motor nerve or presynaptic blocking of the transmission of excitation from nerve to muscle by disturbance of ACh synthesis is not equivalent to its denervation.

Neurotrophic control of the frog muscle fiber membrane is effected mainly by substances carried to the muscle by axonal transport [4] and it does not depend on the character of the spike discharge or synaptic ACh. It must be pointed out in this connection that the presence of conduction of excitation along nerve fibers and a preserved neurosecretion apparatus in motor endings are an important condition for the neurotrophic influence of the motoneuron on muscle fibers.

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EFFECT OF CHEMICAL SYMPATHECTOMY ON THE DEVELOPMENT OF DOCA-SALT HYPERTENSION IN RATS

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The level of the arterial pressure (BP) depends mainly on the cardiac output (CO) and total peripheral vascular resistance (TPVR). During the development of DOCA-salt hypertension, which is sodium- and volume-dependent, CO rises initially, but this is followed by an increase in TPVR, which leads to stabilization of BP at a high level [11]. The mechanism of the rise in TPVR has not been finally established. In particular, there are conflicting data on the role of the sympathetic nervous system in this process [6, 9, 14]. According to Provoost [14] neonatal sympathectomy accelerates the development of DOCA-salt hypertension.

The object of this investigation was to study the role of the sympathetic nervous system in mechanisms of development of DOCA-salt hypertension and also to study the functional and structural changes taking place in the blood vessels in this condition.

EXPERIMENTAL METHOD

Experiments were carried out on four groups of Wistar rats: 1) normotensive rats (NR) with an intact sympathetic nervous system, 2) NR subjected to neonatal sympathectomy (NR_s), 3) rats with induced DOCA-salt hypertension with an intact sympathetic nervous system (DSR), 4) rats with DOCA-salt hypertension subjected to neonatal sympathectomy (DSR_s). Neonatal sympathectomy was simulated by subcutaneous injection of guanethidine (from "Pliva," Yugoslavia), starting with the first day after birth, and daily for 3 weeks in a dose of 25 mg/kg. Previous experiments [1] showed that as a result of de-sympathization under these conditions fewer than 1% of nerve cells remained in the stellate ganglion. At the age of 10-12 weeks unilateral nephrectomy was performed on all the animals. Arterial hypertension was induced by subcutaneous implantation of DOCA tablets in a dose of 40 mg per rat (the same operation was repeated 3 weeks later); instead of water, these animals were given 1.5% NaCl solution to drink. BP was measured in the caudal artery of the waking rats after 1, 3, 5, and 7 weeks by means of an automatic electroplethysmograph (Natsume KN 209, Japan). In the second week (prehypertensive stage) and seventh week, simultaneous perfusion of the blood vessels of the posterior part of the body with modified Tyrode solution, through a catheter introduced into the abdominal aorta [2, 10], was performed on the animals of all groups in pairs. The rate of perfusion was maintained at a constant level by means of a peristaltic pump (Harvard Model 1201, USA). The perfusion pressure, which under conditions of a constant blood flow,

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