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EFFECT OF INHIBITORS OF PROTEIN SYNTHESIS ON DENERVATION-LIKE CHANGES IN FROG MUSCLE FIBER MEMBRANES INDUCED BY AXOPLASMIC TRANSPORT BLOCKADE BY COLCHICINE

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Blocking neurotrophic influences on muscle by denervation alters the electrophysiological properties of the muscle fiber membrane: The resting membrane potential (RMP) is reduced, input resistance (R_0) and the time constant (τ) of the muscle membrane are increased, and extrasynaptic sensitivity to acetylcholine (ACh) develops [2, 6, 8, 13]. Administration of inhibitors of protein synthesis (actinomycin D or cycloheximide) to experimental animals at the same time as the nerve is divided inhibits the development of postdenervation changes in mammals. It has accordingly been postulated that neurotrophic control is exerted through the muscle fiber gene [9].

Blockade of axoplasmic transport (AT) by colchicine, although not disturbing the conduction of excitation along nerve fibers and not interrupting neuromuscular transmission, is known to induce denervation-like changes in muscle fibers: a decrease in RMP, an increase in R_0 and τ, and the appearance of extrasynaptic sensitivity to ACh [1, 2, 7, 10]. On the basis of these investigations it has been suggested that substances carried to the muscle by AT take part in neurotrophic control of skeletal muscle fibers.

However, the question of whether the effect of AT blockade by colchicine is realized through the muscle fiber gene, as has been demonstrated for surgical denervation [4, 8, 9, 11], remains uncertain. The answer to this question is important from the point of view of possible comparison of mechanisms leading to the appearance of extrasynaptic acetylcholine sensitivity after denervation of a muscle and after AT blockade by colchicine solution. The investigation described below was devoted to this problem.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscle of *Rana temporaria* in winter, by a standard microelectrode technique. RMP was recorded and R_0 and τ of the membrane measured by the membrane voltage drop method, and the character of sensitivity of the postsynaptic membrane to ACh was studied by application of the mediator from a micropipet [11]. During the experiment the muscle was kept in a bath with circulating Ringer's solution of the following composition (in mM): NaCl 115, KCl 2.5, $CaCl_2$ 1.8 in phosphate buffer, pH 7.25, at 20 ± 0.05°C. Treatment of the nerve supplying the sartorius muscle with 10 mM colchicine solution (from Merck, West Germany) was carried out by the method described in [2]. Actinomycin D (from Reanal, Hungary) and puromycin (from Serva, West Germany) were injected intraperitoneally. The frogs were kept in a terrarium with circulating water at room temperature.

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TABLE 1. RMP, Electrical Properties, and Sensitivity to ACh of Frog Sartorius Muscle Fiber Membrane in Control, after Application of 10 mM Colchicine Solution to the Innervating Muscle with or without a Single Injection of Actinomycin D or Puromycin into Experimental Animals (M ± m)

Experimental conditions	Time, weeks	RMP, mV	R_0, kΩ	τ, msec	Maximal sensitivity to ACh, mV/nC	Zone of sensitivity to ACh, μ
Control	0	85,4±0,5 (100)	425±24 (40)	24,3±0,8 (40)	234±65 (11)	550±50 (11)
Application of colchicine	2	81,4±0,4* (100)	496±30 (30)	25,2±1,0 (30)	251±75 (8)	1760±170* (8)
	3	81,1±0,7* (100)	600±50* (35)	29,3±1,2* (35)	341±120 (12)	2340±175* (12)
Application of colchicine + puromycin	2	81,4±0,9* (40)	654±48* (40)	27,8±0,8* (40)	183±100 (11)	760±140 (11)
	3	83,2±1,3 (40)	529±25* (40)	30,0±1,5* (40)	240±75 (4)	510±100 (4)
Application of colchicine + actinomycin D	2	81,0±0,7* (50)	636±30* (40)	29,2±1,2* (40)	370±123 (10)	510±40 (10)
	3	80,5±0,9* (60)	655±65* (30)	31,0±1,6* (30)	317±85 (6)	810±150 (6)

Legend. Number of fibers studied in parentheses; *P < 0.05.

EXPERIMENTAL RESULTS

Administration of the antibiotics in doses (10 μg actinomycin D, 100 μg puromycin) preventing the appearance of extrasynaptic sensitivity to ACh after denervation of the muscle [4], together with application of colchicine to the nerve, led to a considerable mortality among the experimental frogs starting from the 9th-11th day after the operation. Accordingly the dose of actinomycin D was reduced to 5 μg and that of puromycin to 10 μg per animal. The chosen dose of actinomycin D blocked the appearance of extrasynaptic sensitivity to ACh in the denervated muscles in about half of all cases, whereas the dose of puromycin had a weak action [4].

The experiments showed that injection of the antibiotics into the experimental animals simultaneously with application of colchicine to the nerve caused the appearance of extrasynaptic sensitivity to ACh in the muscle fibers until 3 weeks after the procedure (Fig. 1; Table 1). Values of maximal sensitivity of the muscle fiber membrane to ACh likewise remained unchanged under these circumstances (Fig. 1; Table 1).

Meanwhile, with an increase in the period after colchicine application to the nerve accompanied by a single injection of actinomycin D or puromycin into the experimental animals, RMP of the muscle fibers was observed to fall and R_0 and τ of the membrane to rise (Table 1), exactly in the same way as after muscle denervation [2] or after treatment of the nerve with colchicine but without administration of inhibitors of protein synthesis (Table 1).

In these experiments the use of inhibitors of protein synthesis when AT was blocked by colchicine solution thus restrained the appearance of extrasynaptic sensitivity of the muscle fibers to ACh, as was shown previously on the denervated frog muscle [4]. However, in an earlier investigation [11] actinomycin D did not completely block the postdenervation appearance of extrasynaptic sensitivity, but simply reduced that sensitivity. This disparity may perhaps be attributable to the use of a smaller dose of actinomycin D (7.5 μg) and to differences between the experimental frogs, obtained from different geographic zones.

The results also support the view that the development of extrasynaptic sensitivity to ACh after treatment of the nerve with colchicine, just as after denervation of the muscle, was more likely to be due to the synthesis of new acetylcholine receptors than to their lateral diffusion from the synaptic zone.

It should be noted that the effective dose of actinomycin D or puromycin in the use of AT blockade was lower than in the case of anatomical denervation. This difference must evidently be accounted for by differences in the methods used to disturb neurotrophic control of the muscle fibers. For instance, when AT was disturbed neuromuscular transmission was preserved, as shown by the endplate potential (EPP) and minature EPP, which could be recorded, and contraction of the muscle in response to indirect stimulation. After division of the nerve, however, the muscle was excluded from motor activity. This difference may be

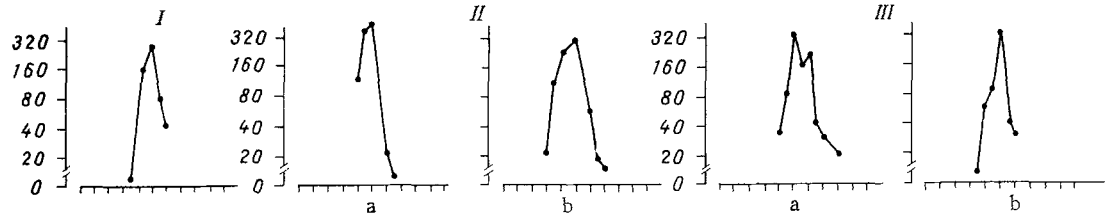

Fig. 1. Sensitivity of postsynaptic membrane of frog muscle fibers to ACh. I) Control; II, III) 2 and 3 weeks respectively after application of colchicine to nerve and injection of actinomycin D (a) or puromycin (b) into experimental animals. Abscissa, distance along muscle fiber (in μ, one division = 200 μ); ordinate, sensitivity to ACh (in mV/nC).

considerable, for there is evidence that nervous impulses and synaptic ACh may play a definite role in neurotrophic control of skeletal muscles [6, 8].

Actinomycin D is known to disturb DNA-dependent mRNA synthesis, whereas puromycin blocks ribosomal assembly of polypeptides — a later stage in protein synthesis [12]. Since the spread of sensitivity to ACh is blocked by both antibiotics, this suggests that neurotrophic regulation of synthesis of acetylcholine-receptor protein from substances carried to the muscle by AT is exerted mainly on the initial stage of protein synthesis — the stage of transcription.

It was observed previously that in frogs, unlike in mammals, no postdenervation fall in RMP is observed [11]. However, in later experiments on a much larger number of fibers, a small but significant fall in RMP was observed both after denervation and after application of colchicine to the nerve [2, 3]. This decrease was much smaller than in mammals, where it amounts to 15 mV [13]. The present investigation showed that this fall in RMP, like the rise in R_o and τ of the muscle membrane, is not blocked by administration of inhibitors of protein synthesis to the animals. Hence it follows that the mechanisms of the effect of substances carried to the muscle by AT must differ with respect to the chemosensitivity of the membrane and to electrogenesis, as has already been demonstrated for the more highly specialized tonic muscle fibers [5].

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