EFFECT OF CYTOSTATICS ON DEVELOPMENT OF CHANGES IN SKELETAL MUSCLES FOLLOWING DENERVATION

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When skeletal muscles are paralyzed by division of a nerve or by intramuscular injection of botulinus toxin (BT) depolarization develops in the muscle cells, and the chemosensitivity of the sarcoplasmic membranes increases [1, 10-12]. By contrast with surgical denervation, however, in BT-induced muscle paralysis the nerve fibers do not degenerate and there is no phagocytic infiltration [3, 6, 7]. There is evidence that blockers of protein synthesis and mitotic inhibitors prevent postdenervational changes in muscles after division of the motor nerve. Accordingly, importance in the genesis of manifestations of denervation such as extrasynaptic acetylcholine reception and muscle fiber depolarization is attached either to the harmful action of phagocytes on muscle cell membranes [2] or to a disturbance of the controlling effect of motoneurons on protein synthesis in myocytes [5, 12]. Meanwhile, it has been shown that DNA and RNA synthesis is increased in skeletal muscles which have been surgically denervated and paralyzed with BT, and the number of cell nuclei in the muscle fibers is increased [3, 4, 7, 14, 15]. Despite the fact that true muscle nuclei are unable to divide, their source in the postnatal period is the satellite cells, which are capable of mitosis and subsequent generation into mature muscle fibers [8, 9].

For the foregoing reasons it was interesting to discover whether changes in the properties of membranes of paralyzed myocytes are connected with the incorporation of nuclei of satellite cells dividing after denervation into mature fibers [13]. For this purpose, resting membrane potentials (RMP) of muscle fibers and the chemical sensitivity to acetylcholine of the soleus muscles were investigated after their "chemical denervation" by BT, against the background of administration of cytostatic agents – the mitotic inhibitor, vincristine, and the blocker of DNA synthesis, fluorouracil.

EXPERIMENTAL METHOD

Experiments were carried out on 45 male Wistar rats weighing 120-130 g. Local paralysis was induced by intramuscular injection of a sublethal dose of type A BT into the posterior group of muscles of the right (experimental) leg [1]. The left limbs served as the control. The experimental rats were divided into three groups. Animals of group 1 were injected with BT only, the rats of group 2 received an intramuscular injection of the same dose of the toxin and also daily intraperitoneal injections of vincristine (25 μg/100 g), and the animals of group 3 received BT + daily injections of fluorouacil (5 mg/100 g). On the 5th day after injection of the toxin the coefficient of synaptic neuromuscular transmission (CSNMT) of the nerves to the soleus muscle and RMP of the fibers of the soleus muscles were investigated in all the animals [1]. At the same time isotonic contractures of the soleus muscles to different concentrations of acetylcholine were recorded *in vitro*.

EXPERIMENTAL RESULTS

As Table 1 shows, intramuscular injection of a sublethal dose of BT into the posterior group of leg muscles on the 5th day caused a disturbance of neuromuscular transmission, as shown by a sharp fall in CSNMT. This was accompanied by marked depolarization of the muscle cells. Whereas the soleus muscles of the control limbs gave only weak contractures in response to acetylcholine in concentrations of $1 \cdot 10^{-4}$ g/ml or higher, by the 5th day after injection of the toxin the paralyzed muscles responded by considerable contractions to low concentrations of acetylcholine (Fig. 1).

The same parameters of the control and experimental soleus muscles were investigated in rats receiving daily (starting from the day of injection of BT) ip injections of vincristine. Despite the similar degree of blockade of neuromuscular transmission, the fall in RMP of the fibers of the experimental muscles was considerably inhibited in the animals of this group, and their polarization was virtually indistinguishable from the control. Injections of vincristine had no effect

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TABLE 1. Synaptic Transmission from Nerves to Soleus Muscle and RMP of Soleus Muscle Fibers in Rats on 5th day of BT-Induced Paralysis after Injection of Vincristine and Fluorouracil (M ± m)

Group of animals	Muscles	CSNMT	RMP, mV
1	Experimental	0,12±0,03* ($n = 6$)	66,45±1,75* ($n = 46$)
	Control	0,91±0,02 ($n = 6$)	76,14±2,24 ($n = 21$)
2	Experimental	0,29±0,07* ($n = 5$)	74,16±1,35 ($n = 52$)
	Control	0,90±0,05 ($n = 5$)	77,71±2,15 ($n = 42$)
3	Experimental	0,28±0,07* ($n = 6$)	74,63±2,26 ($n = 30$)
	Control	0,89±0,02 ($n = 6$)	75,54±2,13 ($n = 24$)

Legend. *$P < 0.05$; n) number of experiments.

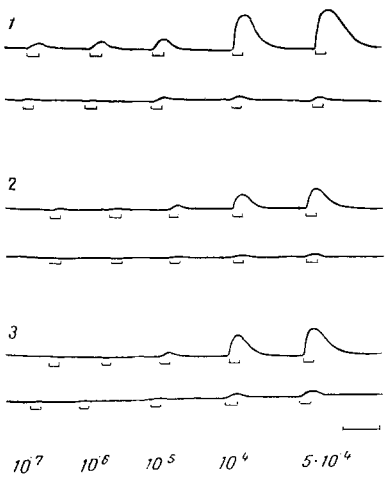

Fig. 1. Isotonic contractures of soleus muscles of rats in response to different concentrations of acetylcholine (5th day of paralysis by BT). 1) im injection of BT; 2) im injection of BT + daily ip injections of vincristine; 3) im injection of BT + daily ip injections of fluorouracil. Top traces show contractures of paralyzed muscles, bottom traces show those of muscles of control limbs. Acetycholine concentration (in g/ml) shown below traces. Time marker 20 sec.

by themselves on the polarization level of the cells of the control muscles and their chemosensitivity. The development of hypersensitivity to acetylcholine in the paralyzed muscles was delayed and their contractures could be induced by acetylcholine solutions only in high concentration (Fig. 1).

Injection of fluorouracil into the rats likewise had no appreciable effect on the level of polarization of the soleus muscle cells of the control limbs and the degree of blocking of neuromuscular transmission of the experimental limbs (Table 1). Just as in the animals of the previous group, RMP of fibers of BT-paralyzed muscles remained high and did not differ statistically significantly from RMP of muscle cells of the control limbs. The sensitivity of the experimental muscles of rats receiving daily ip injections of fluorouracil to acetylcholine was significantly lower than that of the paralyzed muscles of rats not receiving the cytostatic, although fluorouracil itself did not affect the sensitivity of the control muscles to acetylcholine (Fig. 1).

BT has no direct injurious action on nerve fibers or muscle cells. It paralyzes skeletal muscles without causing degeneration of nerves and synaptic terminals [6, 11]. Accordingly, phagocytic infiltration of the zone of "denervation" does not take place. Since in this case also injection of vincristine and fluorouracil delayed the appearance of postdenervational changes, it can be postulated that mitotic activation of undifferentiated satellites and the possible subsequent

incorporation of their cell nuclei into differentiated muscle fibers may play a definite role in their genesis. This process may participate in the mechanisms of the change in protein synthesis in the myocytes and the acquisition of more primitive functional qualities by the sarcoplasmic membranes of mature muscle cells after denervation.

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EFFECT OF PENTAGASTRIN ON PERIODIC ACTIVITY OF THE STOMACH AND DUODENUM IN DOGS WITH INTACT AND DISTURBED GASTRIC INNERVATION

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Pentagastrin (PG) and other synthetic analogs of gastrin stimulate gastric, pancreatic, and intestinal secretion and also the secretion of bile [2, 3, 5, 10]. Information in the literature on the effect of PG on the motor-evacuatory function of the gastrointestinal tract, obtained by using various preparations of PG and over a wide range of doses on different objects, is contradictory. There are no clear data on the effect of PG on periodic contractions of the gastrointestinal tract to serve as the essential physiological background for chronic experiments to study its effects and mechanisms of action.

With the introduction of a Soviet preparation of PG into clinical laboratory practice and with the possible standardization of its activity, including in chronic experiments on dogs, a wider study of the effect of PG on motor and secretory components of the periodic activity of the gastrointestinal tract has become a necessity [3, 4, 7]. The results of such investigations are given below.

EXPERIMENTAL METHOD

Chronic experiments were carried out on 11 mongrel dogs with fistulas of the fundal part of the stomach and duodenum below the point of entry of the greater pancreatic duct into it (in seven of the 11 animals). The experiments began 18-20 h after feeding, when the reaction of the gastric mucosa was alkaline. Periodic motor activity of the stomach and duodenum was recorded graphically by a balloon method with the fistulas open, so that the whole dynamics of the secretory process in the stomach and intestine could be analyzed every 15 min. PG in a dose of 6 μg/kg, which is the dose most commonly used in clinical laboratory investigations, was injected subcutaneously in some experiments during periods of contractions, and in others when the stomach was at rest 18-25 min after the end of periods of contractions. The principal

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