fibroblast growth factor [4]. In fact, under the influence of dalargin, the intensity of chemotaxis and the phagocytic and secretory activity of the macrophages were appreciably increased, and junctions between macrophages and fibroblasts also were more numerous. The role of dalargin in stimulation of the immune mechanisms of wound healing, expressed morphologically as infiltration of the granulation tissue with lymphocytes and plasma cells at certain periods, likewise cannot be ruled out. Early proliferation of fibroblasts under the influence of dalargin leads to their more rapid differentiation, proteoglycan and collagen biosynthesis, fibril formation, maturation of granulation tissue, and its conversion into fibrous scar tissue. Dalargin also has an undoubted stimulating effect on epithelial regeneration.

It is evidently unnecessary to use dalargin for a long period of time, for even when a 6-day course of local application was tested, some slowing of maturation of granulation tissue was observed on the 4th-7th day as a result of prolonged proliferation of fibroblasts and endothelium. Dalargin, with its trigger mechanism of action, evidently induces a cascade of inflammatory-reparative reactions, and shortens all phases of wound healing.

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EFFECT OF ACETYLCHOLINESTERASE INHIBITORS ON REINNERVATION OF MOUSE SKELETAL MUSCLE

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KEY WORDS: reinnervation; neuromuscular transmission; acetylcholinesterase inhibitors; extensor digitorum longus.

Acetylcholinesterase inhibitors (AChEI) are widely used in clinical medicine to potentiate synaptic transmission. It is well known that many neuromuscular disorders are accompanied by processes of denervation and reinnervation [2]. Consequently it is important to understand the action of AChEI on the time course of reinnervation of muscle fibers. No special electrophysiological investigations have hitherto been undertaken to study the effect of AChEI on the course of reinnervation in functionally mature muscles of animals.

This paper gives data on the action of AChEI on the state of neuromuscular transmission under conditions of evoked denervation and reinnervation.

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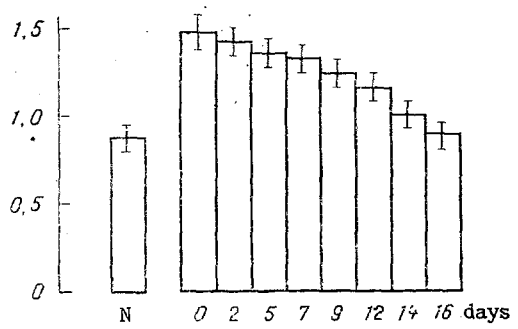

Fig. 1. Weakening of potentiation of amplitude of MEPP under the influence of AChEI phosphacol when administered daily to animals. Abscissa: normal (N) average amplitude of MEPP in intact (not receiving AChEI) mouse; numbers denote days of administration of AChEI; ordinate (here and in Figs. 2 and 3) amplitude of MEPP (in mV).

EXPERIMENTAL METHOD

Experiments were carried out on a fast skeletal muscle — the extensor digitorum longus (EDL) of laboratory albino mice. A denervation-reinnervation syndrome in EDL was evoked by mechanical crushing of the lateral popliteal nerve at the level of the knee. As the control, a mock operation was performed on the opposite limb. AChEI of Soviet origin were used: the organophosphorus derivatives phosphacol and GD-65, blocking AChE irreversibly, and the synthetic carbamate preparation neostigmine, with reversible action. The following doses and times of intramuscular injections were chosen bearing in mind the toxicity and duration of action of the drugs: phosphacol in a concentration of 2.5 μg/kg was injected daily, GD-65 in a concentration of 2 μg/kg once every 5 days, the neostigmine in a concentration of 25 μg/kg was given daily in the morning and evening. AChEI were injected together with atropine in a concentration of 20 μg/kg. The action of AChEI on the character of neuromuscular conduction was analyzed on an isolated nerve-muscle preparation of EDL at different times after the beginning of chronic administration of the compounds. The preparation was placed in a constant-temperature chamber at 28°C with a continuous flow of oxygenated Liley's solution at pH 7.2-7.4. Synaptic potentials were recorded by a standard microelectrode technique.

EXPERIMENTAL RESULTS

In the experiments of series I the action of AChEI was studied on parameters of miniature end-plate potentials (MEPP) and muscle fibers with their innervation intact.

A single injection of the AChEI led to an increase in the mean values of amplitude and rise time of MEPP by 1.5-1.8 times, which lasted throughout the time of action of the test compound. During continuous administration of the AChEI to animals for 4-5 days the parameters of MEPP were maintained at the same elevated level. No differences in principle (except times during repeated injections) were found for the different types of AChEI. Starting with the 6th day, with continuous administration of AChEI gradual weakening of their potentiating effects was found (Fig. 1). Weakening of the action of the AChEI can be explained by the damaging effect of an excess of acetylcholine on nerve endings and postsynaptic structures, leading to a decrease in transmitter release and reduction of the density of postsynaptic acetylcholine receptors [3, 7].

In the experiments of series II the character of action of the AChEI on reinnervation of EDL were studied by determining restoration of the normal parameters of synaptic conduction.

Crushing the motor nerve for 2 days led to complete disappearance of the MEPP and of end-plate (EPP) and action (AP) potentials evoked by stimulation of the nerve. This procedure was accompanied by other changes characteristic of denervation: a fall of membrane potential, the appearance of extrasynaptic sensitivity to acetylcholine, etc [1]. With this mechanical injury of the nerve, it regenerated quite quickly. As early as on the 7th-8th day the first signs of functional recovery of synaptic transmission were observed. In this period MEPP could be recorded in 10-15% of the muscle fibers. Their amplitude was considerably depressed (0.12 ±

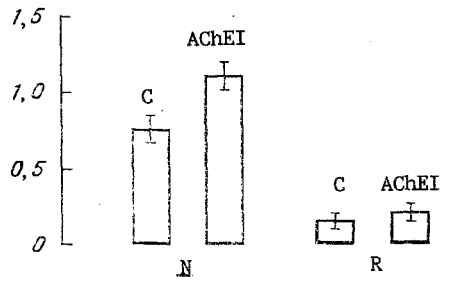

Fig. 2

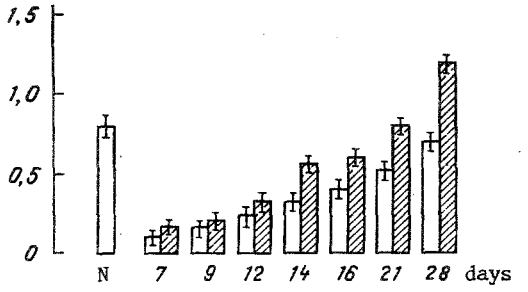

Fig. 3

Fig. 2. Average values of amplitude of MEPP recorded in synapses of normally innervated (N) EDL and in EDL on 7th day after crushing of nerve (reinnervation — R). C — control, AChEI — 6 h after administration of a single dose of phosphacol.

Fig. 3. Increase in mean values of amplitude of MEPP during reinnervation of EDL and restoration of neuromuscular transmission without injection of phosphacol into mice (unshaded columns) and during chronic administration of phosphacol (shaded columns). Abscissa: N) mean amplitude of MEPP in intact normally innervated muscle; numbers denote days after crushing nerve.

0.09 mV) compared with the control (0.7 ± 0.1 mV). The frequency of MEPP in this early period of reinnervation was 0.3 ± 0.1 Hz, compared with 1.0 ± 0.1 Hz normally. These MEPP were characterized by a slow rise and half-decay time. Stimulation of the motor nerve led to generation of slow low-amplitude EPP, unable to induce AP generation. Later an increase in amplitude and shortening of the temporal parameters of MEPP and EPP were observed. On the 15th-16th days after crushing of the nerve 10-15% of the muscle fibers could generate AP. Effective synaptic conduction was restored after 40-44 days.

During chronic continuous administration of AChEI the first signs of recovery of neuromuscular transmission were found at the same times after crushing of the nerve or after the 4th day as in the group of animals not receiving AChEI, i.e., on the 6th-7th day after crushing of the nerve. The MEPP which could be recorded at this time had rather higher amplitude and longer duration, but the potentiating effect of AChEI in this case was much weaker than when acting on intact synapses of normal muscle (Fig. 2). The reason why the AChEI administered over a period of 5-7 days of reinnervation was less effective had nothing to do with the long administration of the compound. For instance, single injection of AChEI into the animal on the 7th day of reinnervation led to equally weak potentiation. Most probably this reflects the presence of an insufficient amount of AChE itself in the synaptic region at this period. This stagement is in agreement with the increased duration of synaptic potentials at these early times of recovery of neuromuscular transmission, as was stated above.

Subsequent administration of AChEI in the course of reinnervation caused the amplitude of MEPP on the 15th-16th day to be 1.3-1.4 times greater than at these times for EDL in the absence of AChEI. On the 21st day after crushing of the nerve more than 80% of the muscle fibers had MEPP with an amplitude indistinguishable from that of intact synapses (Fig. 3). In response to single stimulation of the nerve, AP generation took place in virtually 100% of fibers. Prolonged administration of AChEI against the background of reinnervation, incidentally, was not accompanied by any signs of inhibition of neuromuscular transmission. For instance, the amplitude of MEPP showed no tendency to fall even after a month.

Chronic administration of AChEI against the background of denervation and reinnervation thus has no effect on the initial times of restoration of neuromuscular transmission and has a very weak potentiating action in the early period of formation of synaptic conduction. In the latter stages of reinnervation potentiating effects of AChEI become strong and greatly shorten the times taken for actual restoration of normal working parameters of neuromuscular conduction. In the 3rd week of reinnervation, for instance, they are comparable with parameters characteristic of much later (5 weeks) stages of reinnervation of EDL in the absence of AchEI.

AChEI can be used to accelerate restoration of effective neuromuscular transmission after nerve injury of different kinds.

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EFFECT OF THYMOSIN (FRACTION 5) ON TESTICULAR ENDOCRINE FUNCTION IN MICE

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KEY WORDS: thymosin; gonads; testosterone; prostaglandins.

Much attention has been paid in recent years to the study of the connection between the immune and endocrine systems of the body. The idea has developed that there exists a hypothalamus–pituitary–gonad–thymus axis, responsible for the central modulation of many immunological functions [4]. Direct participation of sex steroids in reactions of cellular and humoral immunity is now known to take place; this problem has been reviewed by Grossman [5]. However, the effect of the immune system on the gonads and, in particular, on the testes, has not yet been adequately studied. Among the principal regulatory factors capable of realizing this effect may be included the polypeptide hormones of the thymus. The active substances of the thymus (thymosin, T-activin) are widely used in clinical practice to stimulate immunological functions, but data on their action on other vitally important systems, including the endocrine system, are extremely scanty. The aim of this investigation was to study the effect of thymosin (fraction 5), a thymus hormone, on the hormonal function of the testes.

EXPERIMENTAL METHOD

Male BALB/c mice aged 2 months, kept under standard animal house conditions, were used. Thymosin (fraction 5) was obtained at the Research Institute of Technology of Blood Substitutes and Hormonal Preparations, Moscow, by Goldstein's method [3] and was given in doses of 0.1 and 1 μg; control animals received bovine serum albumin (BSA) in equimolar doses. Indomethacin ("Serva"), an inhibitor of prostaglandin synthesis, was used in a dose of 100 μg per mouse. All preparations were injected intraperitoneally in 0.1 ml of sterile physiological saline. The animals were killed by decapitation. The blood was centrifuged to obtain plasma. The gonads were removed and placed in bicarbonate buffer (Krebs–Ringer solution saturated with carbogen) and incubated for 3 h at 37°C. Testicular hormonal function was characterized by the plasma testosterone level and by the specific production of this hormone by the testes in vitro, determined by radioimmunoassay using standard kits for testosterone determination (Minsk). The results were subjected to statistical analysis by monofactorial dispersion analysis, using Fisher's test.

EXPERIMENTAL RESULTS

A single injection of thymosin into male mice led to a fall in the peripheral blood plasma testosterone level of the experimental animals compared with the controls. A reduced

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