and the mechanisms involving physical factors are fundamentally different.

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The Effect of Etimyzol on the Development of the Deafferentation Pain Syndrome

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Administration of etimyzol in a dose of 4 mg/kg to rats with deafferentation pain syndrome reduces the incidence of the syndrome and its severity. This effect is associated with activation of the hypothalamic-hypophyseal-adrenal system. Systematic administration of the preparation models repeated stress, thus developing adaptation.

Key Words: *pathological pain; etimyzol; stress, adaptation*

Previously, it was demonstrated that repeated or long-term weak stressors (injection of normal saline or handling) alleviate chronic deafferentation pain syndromes or slow its development as a result of stress analgesia [10-13].

The Russian-manufactured drug etimyzol has been shown to activate the hypothalamic-hypophyseal-adrenal system [8,9], which is involved in the development of the stress reaction.

Our goal was accordingly to test the possibility of preventing the development of the deafferentation pain syndrome (DPS) induced in rats by cutting the sciatic nerve.

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MATERIALS AND METHODS

Experiments were performed on 92 male Wistar rats weighing 150-180 g. DPS was induced by cutting the sciatic nerve at the level of the popliteal fossa, after which the central segment of the nerve was firmly ligated and placed in a polyethylene capsule [4]. Etimyzol was injected intramuscularly as follows: 15 days before and 15 days after surgery (schedule I) and during a 30-day period starting from the first day after surgery (schedule II). The control animals were given normal saline (0.1 ml intramuscularly in the intact paw) according to the two schedules.

The animals were divided into 6 groups: group 1 rats (n=10) were injected with etimyzol in a dose of 4 mg/kg according to schedule I, group 2 rats (n=21) were injected with normal saline according to sched-

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ule I, in group 3 (n=10) etimyzol was injected in a dose of 4 mg/kg according to schedule II, in group 4 (n=10) the preparation was injected in a dose of 15 mg/kg according to schedule II, group 5 rats (n=22) were given normal saline according to schedule II, and group 6 rats (n=19) with cut sciatic nerve received no treatment (control).

The animals were observed during 200 days. The severity of DPS was evaluated by the intensity of autotomy [6]. Terminal blood flow was studied by microcopy of the mesentery under Nembutal anesthesia (5 mg/100 g body weight). The morphofunctional state of mast cells in the mesentery was assessed after vital fixation of the area under the light guide with 96° ethanol and staining with Toluidine Blue. Venular permeability was assessed by the method of "labeled vessels" [2,4].

The surgery and biomicroscopy were performed in the morning. The rats were maintained in the vivarium on a standard diet.

The results were analyzed using Student's t test and the χ^2 test.

RESULTS

In group 6 (no treatment), the incidence of autotomies was 21% on the 2nd postoperative day and 100% by the 133rd day. In group 2 (administration of normal saline), autotomy was delayed until the 23rd day, and by the 146th day its incidence had risen to 67%, remaining at this level until the end of the study.

Administration of 4 mg/kg etimyzol according to schedule I (group 1) delayed the development of DPS compared with group 6 rats (no treatment) by 35 days, and compared with the control for this series (group 2) by 11 days, the incidence of autotomies at the end of the observation period being 30% (Fig. 1, *a*). It should be noted that the incidence of autotomy in groups 1 and 2 was significantly (p<0.01) lower than in group 6 throughout the experiment. In addition, starting from day 90 after surgery, the incidence of autotomies in rats treated with etimyzol (group 1) was significantly lower (p<0.05) than in rats given normal saline (group 2) (Fig. 1, *a*).

Administration of 4 mg/kg etimyzol according to schedule II (group 3) delayed the development of DPS by 6 and 7 days compared with groups 5 and 6, respectively. The incidence of autotomies in group 3 was significantly (p<0.01) lower than in group 6 and, starting from day 50 after the last administration, significantly (p<0.05) lower than in group 5 (Fig. 1, *b*).

During the first week after surgery, the number of animals with autotomies in group 4 (administration of 15 mg/kg etimyzol according to schedule II) was higher than that in group 6. After the end of treatment, the incidence of autotomies increased 20% and then remained unchanged until day 140 after surgery, i.e., it was lower than in untreated rats (group 6) but higher than in group 5.

Administration of normal saline according to schedule II (group 5) did not delay the development of DPS compared with group 6 rats, but, starting from the third week after surgery, the number of animals with autotomies was significantly lower than in group 6 (p<0.05, Fig. 1, *b*).

Comparison of the effects of etimyzol administered according to the two schedules showed that preventive use produced a more favorable effect on the incidence of autotomies.

Administration of etimyzol according to schedule I markedly reduced the severity of DPS in comparison with group 6 throughout the observation period and in comparison with group 2 starting from postoperative day 85 (Fig. 2, *a*). The decrease in the severity of DPS in rats given normal saline is associated with the influence of the repeated weak stress stimuli induced by the injections themselves. This effect was preserved until the 85th day after surgery. Etimyzol had a similar effect, but one which was observed during the entire observation period (200 days). This difference is probably to be attributed to the preparation, which activates the hypothalamic-hypophyseal-adrenal system to a greater extent than repeated injections of normal saline.

Administration of etimyzol (4 mg/kg) according to schedule II significantly reduced the severity of DPS compared with group 6 rats from day 40 until the end of the observation period and compared with group 5 from day 70 until the end of the study (Fig. 2, *b*).

When administered in a dose of 15 mg/kg according to schedule II (group 4), etimyzol increased the severity of DPS during the period of administration. After the end of treatment, the severity of autotomies in these animals markedly decreased and remained virtually unchanged throughout the observation period, without differing significantly from that recorded in groups 5 and 6 (Fig. 2, *b*).

From a comparison of the effective doses and schedules of etimyzol administration, it is concluded that the lower dose and schedule I are more conducive to lessening DPS severity (Fig. 2).

Presumably, the stress of surgery had less of an effect on the course of DPS during the development of adaptation by preventive administration of etimyzol.

At high doses the preparation produced a different effect on DPS: during the period of administration it accelerated the development of DPS and exacerbated it, whereas after the end of administration it inhibited it in comparison with group 5 (Figs. 1, *b* and 2, *b*). In addition, the 15 mg/kg dose affected behavior:

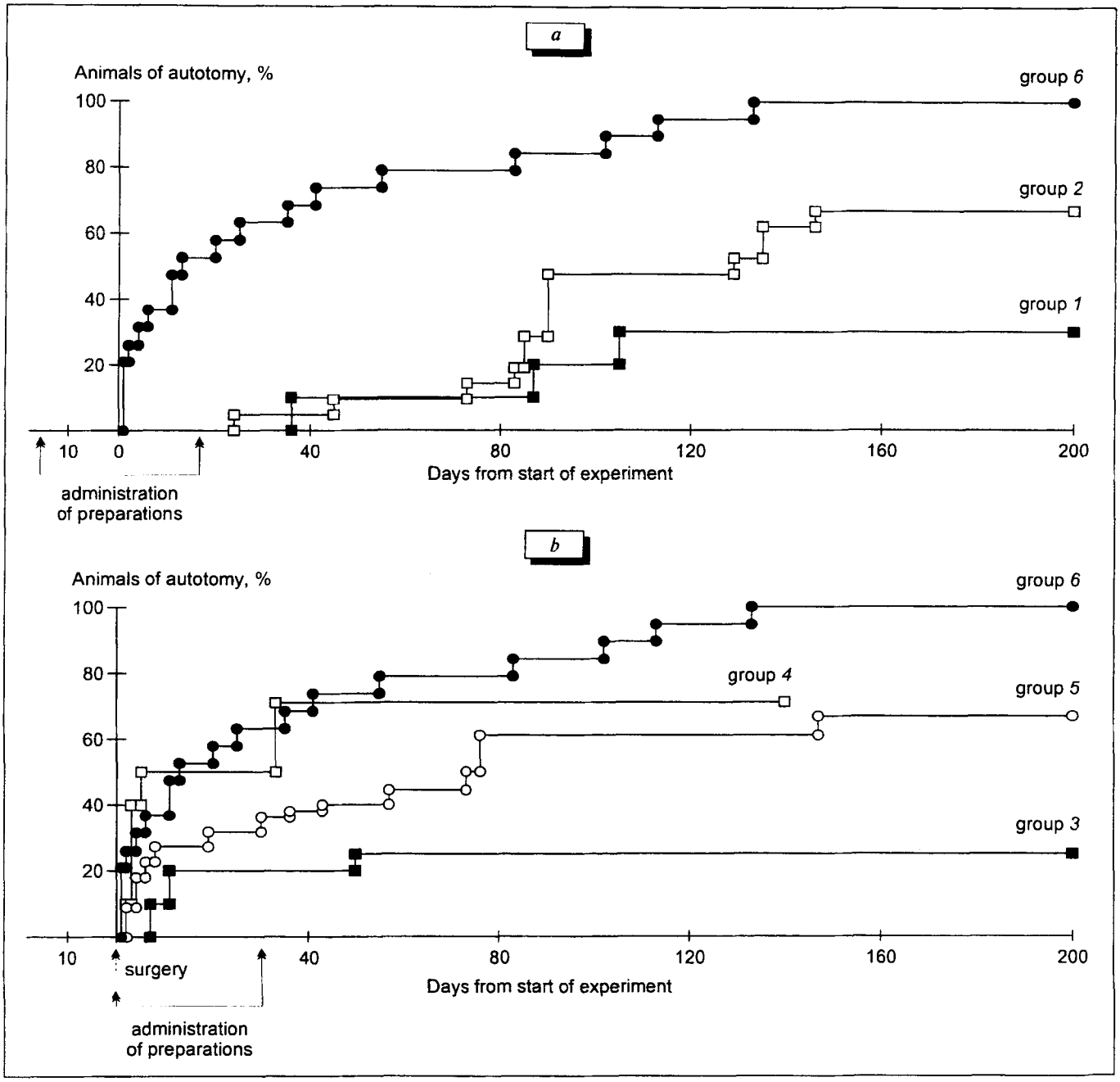

Fig. 1. Effects of etimyzol and normal saline on the development of DPS. *a*) schedule I; *b*) schedule II.

starting from the first few days of administration anxiety, vocalization, motor activity, tactile sensitivity, and aggressiveness increased. Presumably, this was associated with hyperactivation of the hypothalamic-hypophyseal-adrenal system, which facilitated the development of DPS. These data are consistent with evidence [7] indicating that strong stress factors promote the development of DPS. In addition, it is known that strong stressors cause microcirculatory disturbances [1] which accelerate and intensify DPS [6].

Biomicroscopy of the mesentery and evaluation of venular permeability and the morphofunctional state of mast cells confirmed our previous finding that the degree of microcirculatory disturbances is determined by the severity of autotomy [3]. In animals with pronounced DPS the blood flow rate decreased, aggregated erythrocytes were seen in capillaries and venules, leukocytes adhered to the walls of venules, and venular permeability for India ink particles increased, as did degranulation of mast cells.

The results obtained in groups where normal saline was injected according to both schedules are consistent with reports indicating that DPS can be retarded by repeated weak stressors [10-12].

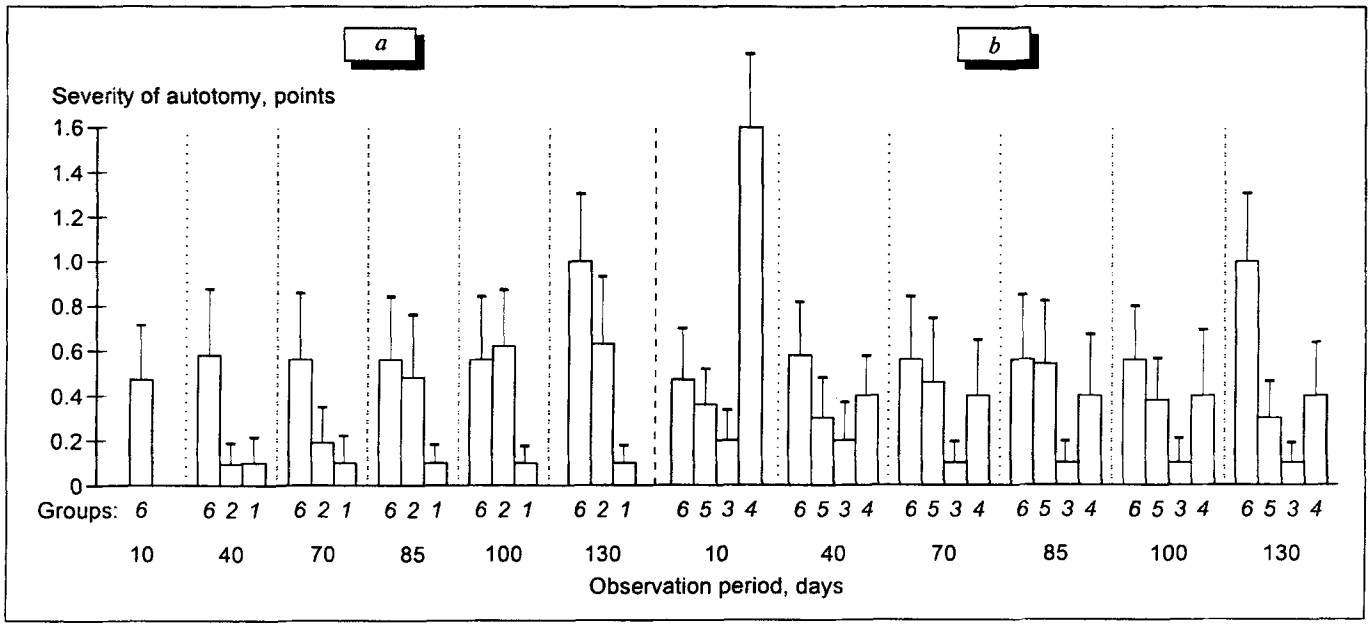

Fig. 2. Effects of etimyzol and normal saline administered according to schedules I (*a*) and II (*b*) on the severity of autotomy.

The prolonged (6 months) decrease in the incidence of autotomies in rats after the last injection of normal saline and etimyzol is probably due to the development of a long-term adaptation to stress [5].

It should be emphasized that administration of etimyzol in a dose of 4 mg/kg according to both schedules was more effective in preventing DPS or reducing its severity compared with administration of normal saline. These data can serve as a basis for clinical investigations of patients with DPS.

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