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The Effects of Repeated Administration of the Micellar Complex of Methylprednisolone on the Locomotor Activity of a Terrestrial Snails

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We studied the effects of repeated injections of methylprednisolone and its micellar complex with block-copolymer on locomotor activity of a terrestrial snail. It was shown that methylprednisolone solution injected into the hemolymph of the animal produced a direct effect on the muscle system of the animal as soon as 1 h after administration: it slowed down snail locomotion and reduced contractile activity of the foot muscles. The micellar complex of methylprednisolone with block-copolymer prevented this effect during the first 2 days of injection and negatively affected locomotion only in 2 days after injection, the decrease in locomotion in this case was not accompanied by a decrease in contractile activity of the foot muscle.

Key Words: *methylprednisolone; amphiphilic trifunctional block-copolymer; snail; locomotion; contractile activity of the muscles of the mollusk sole*

Methylprednisolone is a controversial drugs that provide high neuroprotective assistance in the treatment of various neurodegenerative conditions. The use of synthetic glucocorticosteroid methylprednisolone sodium succinate (MP) is a standard therapy after acute spinal cord injury in humans [3]. The possible mechanisms of its neuroprotective effect are inhibition of LPO and stabilization of processes triggered by disturbances in homeostasis of sodium, potassium, and calcium ions, impaired mitochondrial metabolism, pathological glutamate release leading to increased excitability of spinal cord neurons [5]. In addition to the neuroprotective effects, prednisolone and its analogues affect muscle cells. For instance, analogues of prednisolone, in particular α-MP, increase intracellular calcium concentration in myoblasts [11,13] and the number of myofibril precursor cells [8] in patients Duchenne muscular dystrophy.

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However, MP have a number of side effects that can lead to pneumonia, sepsis, some other complications during its intravenous administration [6]. To solve this problem, various systems for local delivery of MP have been proposed during the last decade [2]. A promising system for local delivery of MP to nerve cells is its micellar complex with an amphiphilic trifunctional block-copolymer (TBS). TBS is a carboxylated product of the polymerization of ethylene oxide and propylene oxide with glycerin [7].

The effects of new pharmacological preparations were tested on terrestrial snail *Helix lucorum*. Locomotion is one of the important forms of behavior of gastropods. Moving in space, mollusks coordinate waves of muscle contractions that propagate along the sole of the foot via axons of central neurons and peripheral ganglia neurons [10]. Locomotor activity of gastropod mollusks allows assessing the state of smooth muscles of the sole of the foot and coordinated work of the nervous and muscular system of mollusks [10,14]. That is why locomotor activity of invertebrates is a unique model for studying the pharmacological effects of both transmitters and neurospecific

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substances on the coordinated work of the nervous and muscular systems [1,4,9].

Here we studied chronic effects of MP and its micellar complex with amphiphilic trifunctional block-copolymer on locomotor activity of snails *Helix lucorum*.

MATERIALS AND METHODS

The experiments were carried out on the land gastropod pulmonary mollusk *Helix lucorum*. These animals are characterized by diverse behavior and pronounced locomotor activity [1,10,14]. Prior to the experiments, the snails were kept in a glass terrarium in a humid atmosphere at room temperature for at least two weeks. All experimental procedures were carried out in accordance with Directive 2010/63/EC of the European Parliament and the Council of Europe On the Protection of Animals used for Scientific Purposes (September 22, 2010).

The studied drugs were injected with a thin needle through insensitive part of snail skin in the region of the sinus node once a day over 4 days. MP and micellar complex TBS+MP were injected in doses of 0.015 and 0.1 mg/g body weight, respectively. Animals of active control group were injected with saline. The injection volume of all solutions did not exceed 0.1 ml.

The animals were divided into 3 groups: active control group (n=8), injection of MP (n=9), and injection of TBS+MP (n=4). The data obtained during testing before injection of the test preparations served as intact control.

Motor activity was tested out before administration of studied substances, 1 h after the first injection, and 24 h after each injection over 4 days. The locomotion rate was evaluated by measuring the distance traveled by the snail in the active state over 1 min. Measurements were performed on a vertical wall of the glass terrarium where the beginning and end of the straight path of each animal were marked with a marker, and then these segments were measured with a ruler. The rate of propagation of a single contractile wave was estimated by the time of propagation of the investigated wave along the entire length of the mollusk sole. The mean length of the sole during movement and the mean number of waves during movement were also evaluated [1].

The mean speed of locomotion, speed of propagation of the contractile wave, and change in the length of the sole of the mollusk were compared. The dependence of the movement speed on the length of the sole of the mollusk was evaluated. The values obtained at different terms after injections of the test substances and initial values (intact control) were compared using one-way repeated measurements ANOVA. The differences were considered significant at $p<0.05$.

RESULTS

Analysis of the dynamics of the influence of a single injection of the studied substances on locomotor activity of snails showed that the rate of locomotion of animals receiving MP significantly decreased in 40 min after injection to 0.73±0.07 mm/sec (*vs* 1.01±0.07 in intact animals; $p<0.05$) (Fig. 1, *a*). In 1 h after injection of MP, the rate of locomotion decreased to 63.6±5.5% of the initial value (before injection), while after injection of saline, it was 90.6±4.9% of the original. After injection of the TBS+MP complex, the rate of locomotion was reduced to 80.2±12.6% (Fig. 1, *b*). In 1 h after MP injection, the rate of propagation of a single contractile wave decreased to 5.6±0.2 mm/sec (*vs* 6.2±0.2 mm/sec in intact animals; $p<0.05$); after

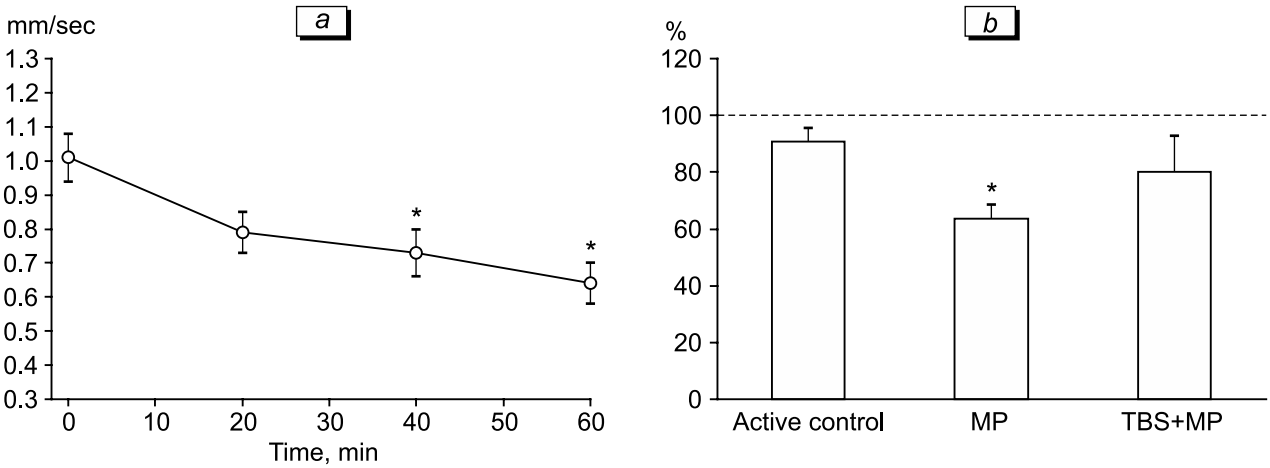

Fig. 1. Dynamics of changes in the locomotion speed within 1 h after injection of MP (*a*) and locomotion speed in snails of different groups in 1 h after injection of substances (*b*; % of the speed of intact animals). *$p<0.05$ in comparison with intact animals.

injection of saline and TBS+MP complex, no changes in this parameter were observed.

The study of the effects of chronic administration of the studied substances showed that on the first day after the first injection of MP, the speed was reduced to 0.69±0.05 mm/sec and remaining reduced for all 4 experimental days (0.50±0.05 mm/sec on day 4; $p<0.001$ in comparison with intact control). The rate of locomotion of snails injected with the TBS+MP complex significantly decreased on day 3 to 0.5±0.05 mm/sec (*vs* 1.1±1.19 mm/sec in intact animals; $p<0.001$). The locomotion speed in snails injected with saline did not significantly change on days 1-3, but was reduced on day 4 (Fig. 2, *a*). It was also shown that, together with a decrease in the rate of locomotion, MP injection caused a significant decrease in the length of the snails sole. Significant changes in sole length were observed as soon as on the first day after MP injection and the maximum decrease were observed on day 4: 7.4±0.1 cm vs 8.1±0.1 cm in intact control ($p<0.05$). In other groups, no significant changes in sole length were observed during chronic administration of MP.

The speed of propagation of a single contractile wave in the sole also decreased after MP injections: 5.2±0.3 mm/sec on the next day after the first injection of MP and 4.8±0.2 mm/sec on day 4, which significantly differed from this parameter in intact animals (6.2±0.3 mm/sec). In the group of animals treated with TBS+MP complex, no significant changes in the speed of propagation of the contractile wave were detected. However, in active control group, this parameter was reduced on day 4 (Fig. 2, *b*).

Analysis of the dependence the locomotion speed on the sole length in intact snails revealed a direct dependence between these parameters: $y=0.24x+6.40$, which indicated that the snail crawled faster when its sole is longer (Fig. 3). A similar dependence was observed in active control group on days 3 and 4: $y=0.22x+6.30$. However, in snails injected with MP, the dependence was negative on days 3 and 4: $y=-0.24x+8.13$, *i.e.* with increase in foot length, the

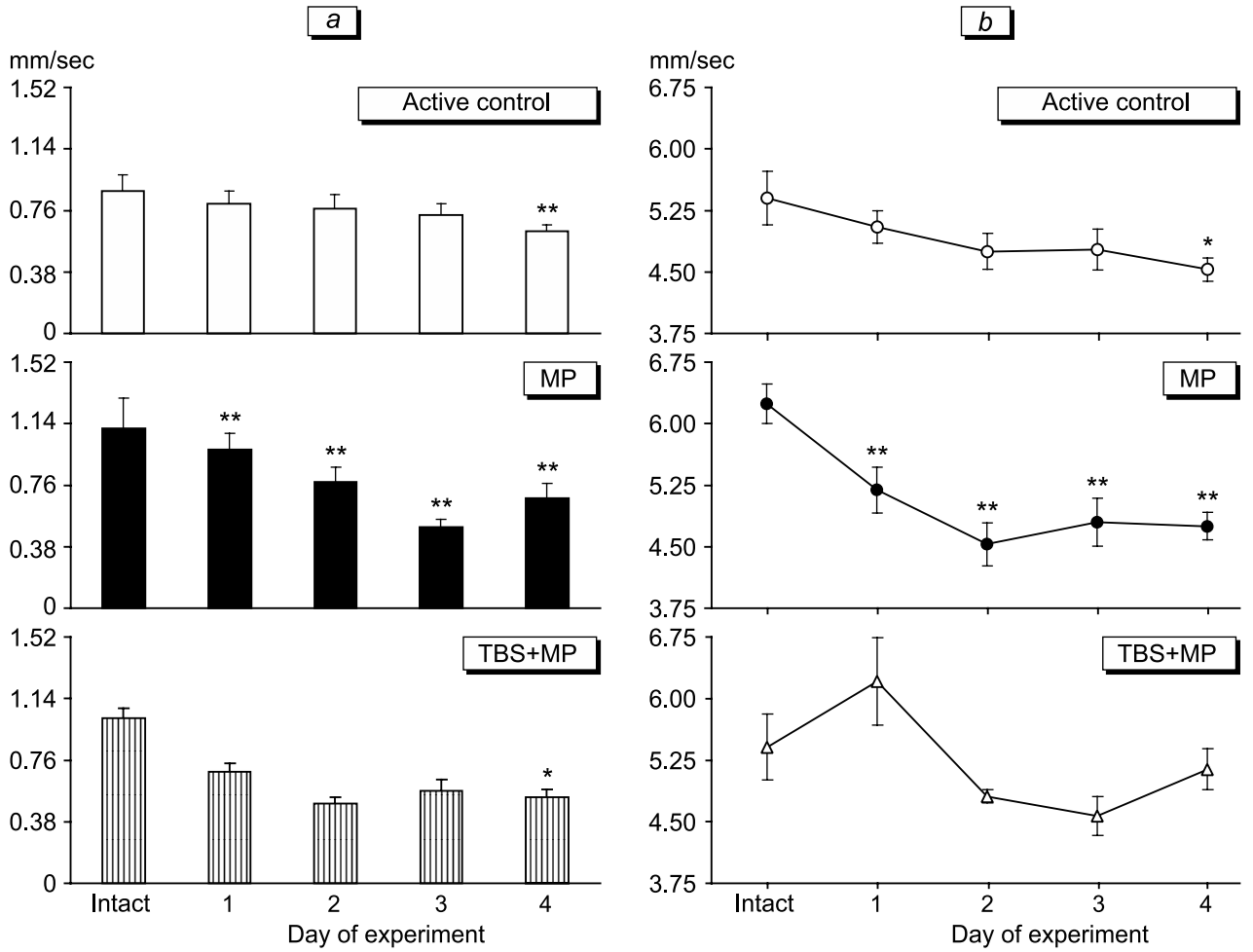

Fig. 2. Dynamics of changes in the locomotion speed (*a*) and the speed of propagation of a single contractile wave (*b*) in snails of active control group and animals treated with MP or TBS+MP. *$p<0.05$, **$p<0.01$ in comparison with intact animals.

locomotion speed slightly decreased or remained unchanged. In the group of animals injected with TBS+MP complex, no dependence of locomotion speed on the sole length were revealed: y=-0.12x+7.88 (Fig. 3).

The regulation of the speed of locomotion in terrestrial mollusks is mediated by the speed of propagation of the contractile wave of muscle contraction. On the other hand, muscle contractions are controlled by a pool of neurons of the central and peripheral nervous system and this control is mediated by the dopamine and serotonergic systems [9,12]. Serotonin stimulates locomotion and significantly increases its speed, without changing the frequency of contraction of the sole. At the same time, dopamine regulates the speed by shortening the length of the sole and decreasing the amplitude of contraction of the muscle fibers of the sole involved in the formation of the contractile wave [10]. At the same time, administration of neurotoxin (to serotonin) 5,6-DOT daily in small doses for a week or the same total dose of neurotoxin 30 mg/kg body weight per injection is accompanied by a gradual decrease in the rate of locomotion that persisted for 7 days [1]. In our previous studies, a similar effect was observed after administration of neurotoxin (to dopamine) 6-OHDA that reduced the speed of locomotion on day 8 by about 2 times. At the same time, serotonin, like dopamine, regulates the speed by shortening the length of the sole and decreasing the amplitude of contraction of the muscle fibers in the sole involved in the formation of the contractile wave [10]. Our experiments showed that MP injected into hemolymph of the animal had a direct effect on the locomotion speed as soon as in 40 min after administration and reduced the speed of propagation of the contractile wave in 1 h after injection, *i.e*. the speed of locomotion decreased due to a decrease in the frequency of steps. It can be hypothesized that MP directly affects contractile activity of muscle cells of the sole.

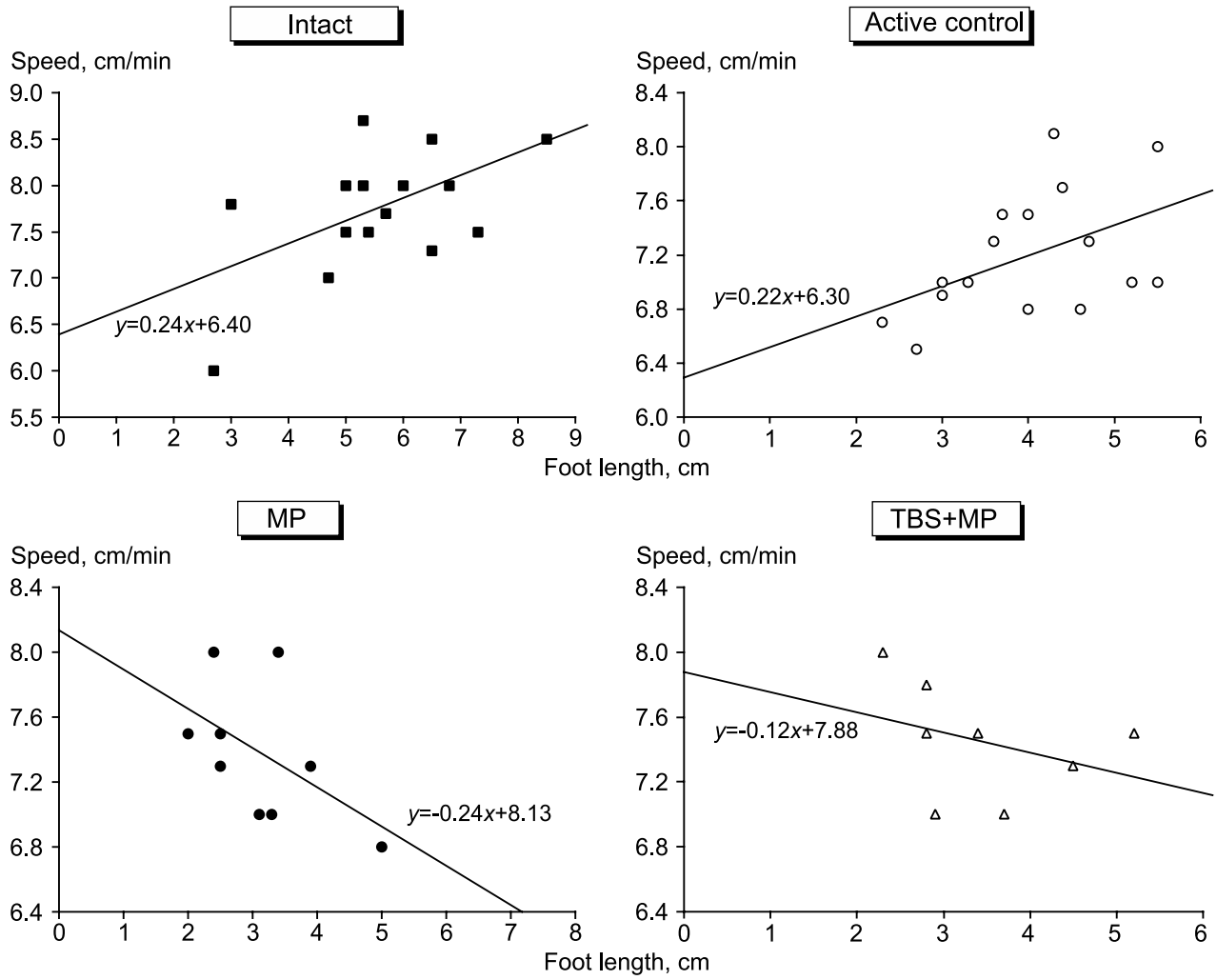

Fig. 3. Dependence of locomotion speed on foot length in intact snails, animals of the active control group, and animals treated with MP and TBS+MP after a series of injections.

The simultaneous decrease in the speed of locomotion and speed of propagation of the contractile wave observed throughout the course of MP administration and attaining its maximum on day 4 probably indicates a cumulative effect of MP.

In snails treated with TBS+MP complex, the decrease in the locomotion speed was observed later than after injection of MP and was not accompanied by a decrease in the speed of propagation of single contractile wave. The micellar complex abolished the short-term effects of MP, probably due to facilitation of the direct delivery of MP to intracellular targets, where it modulated protein synthesis.

The dependence of the speed of locomotion of the mollusk on the length of the sole of the foot, depending, in turn, on the contraction force of the muscle fibers of the sole was demonstrated in a number of studies [1,9]. In our experiments, this correlation persisted in intact animals or snails injected with saline, however, the correlation dependence was opposite on days 3 and 4 of MP injection, despite the fact that the mean length of the sole in animals of this group at this term was significantly shorter. In animals treated with TBS+MP complex, no linear dependence of the locomotion speed on the foot length and no changes in the foot length were observed.

Thus, MP solution injected into the hemolymph of snails had a direct effect on the muscle system as soon as in 1 h, reducing the locomotion speed and the contractile activity of the muscles of the sole. While micellar complex TBS+MP prevented this effect during the first two days and has a negative effect on locomotion only starting from day 3; the decrease in locomotion in this case was not accompanied by a decrease in contractile activity of the muscle.

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