Study of Neurotrophic Activity of Thrombin on the Model of Regenerating Mouse Nerve

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Experiments demonstrated a dose-dependent facilitating effect of thrombin and peptide thrombin receptor agonist PAR1 (TRAP6) on regeneration of mouse peripheral nerve after its crushing. The maximum neurotrophic effect was observed at low concentrations of thrombin (10 nM) and TRAP6 (10 μM).

Key Words: *nerve regeneration; thrombin; peptide thrombin receptor agonist*

In modern neurology thrombin and other serine proteinases attracted much attention as possible neurotrophic agents promoting regeneration of neurons and neuronal processes [5]. Expression of thrombin in various regions of intact nervous system and its ability to cross the blood-brain barrier during vascular pathology were reported [9,10]. Neurons and glial cells have membrane receptors for thrombin, termed as proteinase-activated receptors, PARs [6,8]. PAR were recently detected on the surface of damaged peripheral neurons and axons [11]. Effect of thrombin on regenerating nerve fibers is unknown.

Our aim was to study the effect of exogenous thrombin and hexapeptide TRAP6 (a synthetic thrombin receptor agonist Ser-Phe-Phe-Leu-Arg-Asn-NH_2), selective PAR1 activator, on regeneration of sensory axon of mouse peripheral nerve after its crushing *in vivo*.

MATERIALS AND METHODS

Experiments were carried out on male albino mice (n=77) weighing 20-25 g. The animals were narcotized with ether and *n. peroneus communis* in the left knee-joint region was crushed with ophthalmic forceps with flat branches (1 mm) coated with plastic tips. The length of the crushed fragment was 1 mm.

Bovine α-thrombin and TRAP6, a peptide thrombin receptor agonist PAR1, were synthesized in Russian Cardiology Research-and-Production Complex, Ministry of Health, Russian Federation. Both preparations were encapsulated in 33% pluronic gel (70 μl, Sigma). Single applications of both agents were performed onto the damaged site of the nerve immediately after crushing. The control mice were treated with pluronic gel filled with equal volume of physiological saline. Thrombin was used in concentrations 3 nM-1.3 μM, and TRAP6 was applied in concentrations of 5 and 10 μM.

Axon growth and regeneration were tested electrophysiologically on day 11 after crush. To this end, 15 mm-long segment of the sensory branch (*n. fibularis superficialis*) of the peroneal nerve was isolated, the crushed fragment being in the middle of the isolated segment. The monopolar compound action potential (CAP) was recorded with suction electrode from the distal end of the nerve in response to anterograde supramaximal electrical stimulation of its proximal part mounted on silver chloride wire electrodes.

The latency, rise time, duration, and amplitude of CAP were analyzed, and the ability of the nerve to reproduce rhythmic CAP bursts at a rate of 50 Hz was tested [2].

The data were processed with Mini-Analysis software using Mann—Whitney U test.

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RESULTS

In our previous experiments nerve growth to the periphery and reinnervation the original targets in muscles, skin, and other tissues were observed on day 11 after trauma [1]. At this term, functional parameters of axons in the regenerating nerve still significantly differ from those of intact nerve (Fig. 1, *a*, *b*). The amplitude of CAP in intact nerve did not change during rhythmic stimulation (50 Hz, 30 sec), while in regenerating nerve it rapidly dropped to 25% of the initial level (Fig. 1, *c*).

On day 11 after the surgery, the parameters of CAP were recorded in mice treated with thrombin in concentrations of 3 nM, 10 nM, 30 nM, and 1.3 μM (experimental group) and in control mice not receiving the drug. In addition, the same parameters were recorded in mice with intact nerve.

No significant differences of the CAP parameters were observed in control and experimental mice treated with thrombin in a concentration of 1.3 μM. However, lower concentrations of the drug shortened the latency and increased the amplitude of CAP (Fig. 1). In the narrow range of low concentrations (3-30 nM), thrombin produced positive shifts in the examined parameters (Fig. 1). The most pronounced effect was observed at 10 nM: the latency of CAP decreased by 38% ($p<0.01$), and its amplitude increased by 137% in comparison with the control group ($p<0.01$, Fig. 1).

Similar dose-dependent effects of thrombin were revealed, when we analyzed changes in CAP amplitude under conditions of rhythmic stimulation of the nerve at a rate of 50 Hz. After application of thrombin in a concentration of 10 nM, the nerve became more capable to maintain the amplitude of CAP. The effect was significant ($p<0.01$) from stimulation second 2.

The application of TRAP6 in low concentrations to the nerve slightly shortened the latency of CAP. In a concentration of 10 μM it significantly shortened CAP latency by 49% ($p<0.001$). In a lower concentration (5 μM) TRAP6 was less effective: the latency decreased by only 32% ($p<0.05$, Fig. 2). By contrast to thrombin, application of TRAP6 produced no changes in the amplitude of single CAP and in the decrement of CAP amplitude during rhythmic stimulation.

Thus, both preparations accelerated recovery of functional activity of the regenerating axons (conduction velocity, latency, *etc*.) only in low concentrations (thrombin, 10 nM and TRAP6, 10 μM). Thrombin produced more pronounced effects and improved all examined parameters of the nerve. These findings agree with published data, that thrombin promotes survival of hypoxia-damaged neurons *in vivo* [7,8] and *in vitro* [4,12] and activates antiinflammatory reactions in the nervous tissue [3,5] only in low concen-

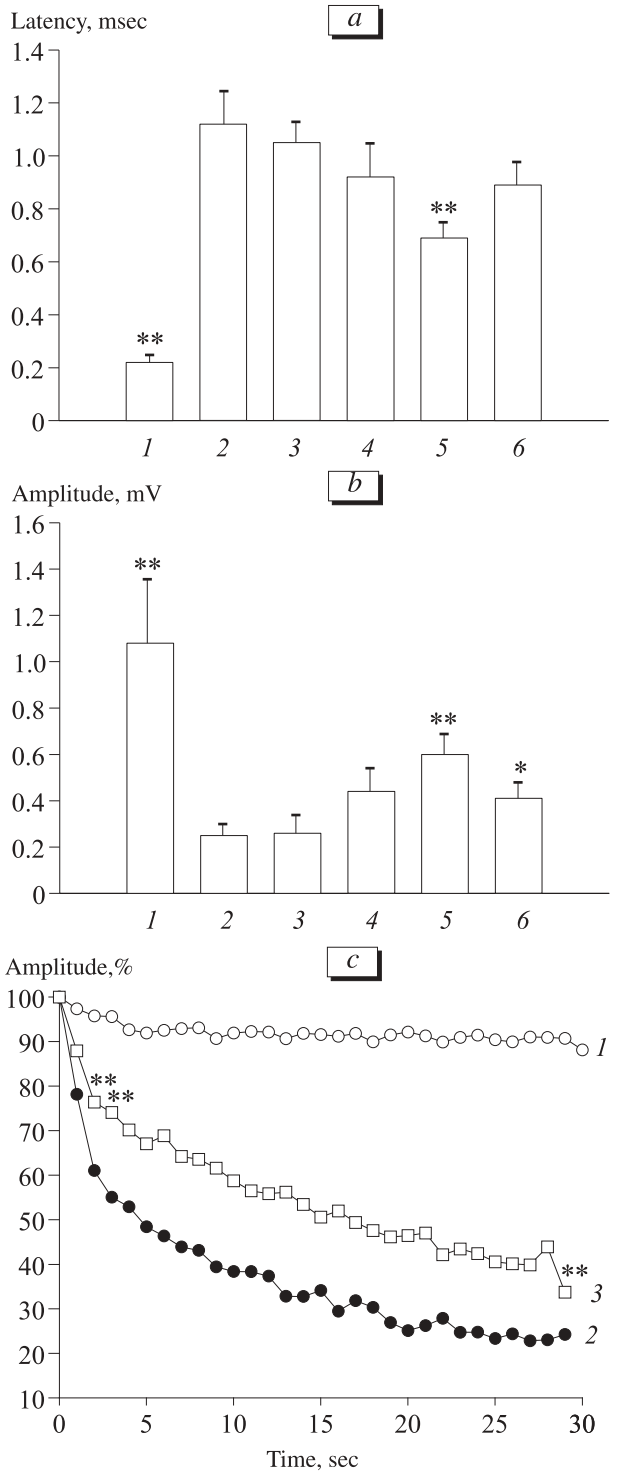

Fig. 1. Latency (*a*) and amplitude of compound action potential (CAP, *b*) in intact nerve (*1*, *n*=7), control crushed nerve on day 11 after crushing (*2*, *n*=7), and crushed nerved treated with thrombin in concentrations of 1.3 μM (*3*, *n*=7), 30 nM (*4*, *n*=6), 10 nM (*5*, *n*=11), and 3 nM (*6*, *n*=7). *c*) Decrement of CAP amplitude during high-frequency (50 Hz) stimulation in intact nerve (1, *n*=7), in control crushed nerved on day 11 after crushing (*2*, *n*=7), and in crushed nerved treated with thrombin in concentration of 10 nM (*3*, *n*=11). Here and in Fig. 2: **p*<0.05, ***p*<0.01 compared to *2* (Mann—Whitney test).

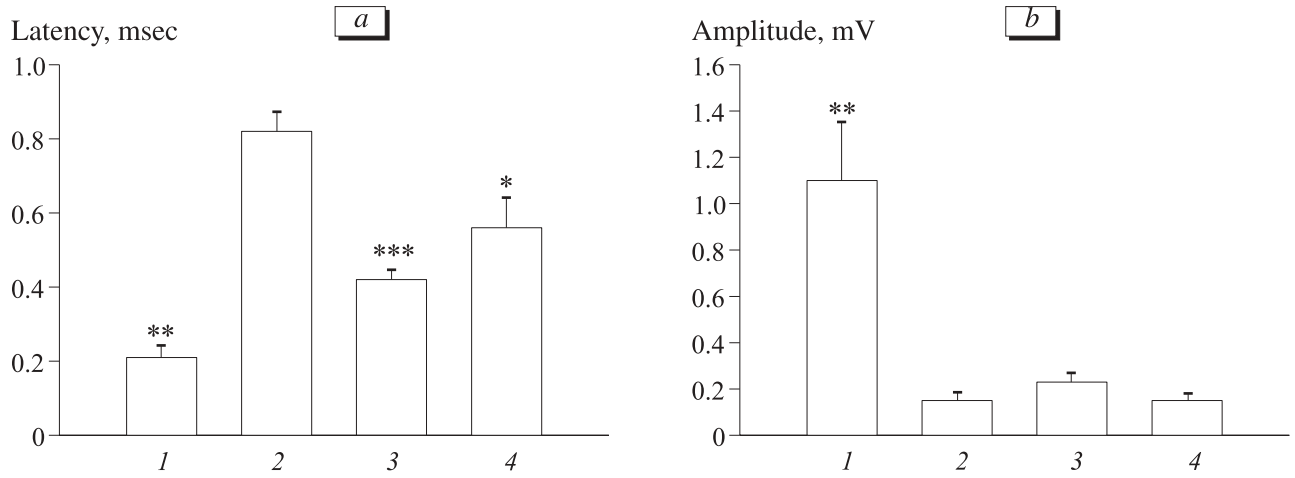

Fig. 2. Latency (*a*) and amplitude of compound action potential (*b*) in intact nerve (*1*, *n*=8), control crushed nerved on day 11 after the crushing (*2*, *n*=7), and crushed nerved treated with TRAP6 in concentrations of 10 μM (*3*, *n*=8) and 5 μM (*4*, *n*=6).

trations. The efficiency of low concentrations of thrombin and similarity of its effect to that of TRAP6 peptide attest to the presence of specific receptors for thrombin in regenerating *n. peroneus* in mice, which mediate the neurotrophic effects of both agents.

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