Neuroprotective Activity of Proproten in Rats with Experimental Local Photothrombosis of the Prefrontal Cortex

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Proproten (ultralow doses of antibodies to S100 protein) exhibited neuroprotective activity in rats with experimental photochemical thrombosis of the prefrontal cortex. Proproten was more potent than standard neuroprotectors piracetam and vinpocetine in alleviating the signs of memory disorders produced by ischemic injury. Pathomorphological study of the damaged area confirmed the neuroprotective effect of Proproten.

Key Words: *Proproten; ischemic insult; neuroprotector; piracetam; vinpocetine*

Acute impairment of cerebral blood flow (stroke) ranks 3rd among other causes of death in developed countries and a leading cause of disability [2,6]. The GABAergic system is a new target for neuroprotective drugs [5]. The neuroprotective effect of GABAergic system agonists (*e.g.*, clomethiazole) is determined by prevention of membrane depolarization resulting from ischemic injury and, therefore, potentiation of the protective inhibitory effect of GABA in the central nervous system [9].

Proproten (ultralow doses of antibodies to S100 protein) was synthesized at the Research-and-Production Company "Materia Medica Holding" and since 1999 was widely used in the therapy of patients with alcohol withdrawal syndrome. This preparation alleviates signs of anxiety and improves memory and learning under conditions of alcohol dependence [4]. Blockade of GABAergic transmission considerably decreases anxiolytic activity of Proproten [1]. These data suggest that Proproten acts as a transmitter of the GABAergic system. Here we studied neuroprotective activity of Proproten.

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MATERIALS AND METHODS

Experiments were performed on adult male Wistar rats weighing 180-200 g. Bilateral focal ischemic injury of the prefrontal cortex was produced by the method of photochemical thrombosis [8] with modifications of I. V. Viktorov [3]. The animals were intravenously narcotized with 300 mg/kg chloral hydrate and the head was fixed in a stereotaxis. Bengal rose (40 mg/kg, 3% solution) was injected into the jugular vein. The skin on the head was cut, and the periosteum was separated. Light beam of a halogen lamp (24 V, 250 W) was delivered to the cranial surface above the prefrontal cortex of the left and right cerebral hemispheres using a fiber-optic light guide (diameter 3 cm); the exposure was 15-20 min for each hemisphere. The cranial surface was cooled with flowing water to prevent thermocoagulation. Body temperature was maintained at 37°C. Sham-operated rats were subjected to the same manipulations except administration of the dye and illumination. Proproten was administered

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through a gastric tube 1 h after photothrombosis. In the follow-up period this preparation was given in a daily dose of 2.5 ml/kg for 9 days. The last treatment was performed 40 min before testing. Control animals received an equivalent volume of 0.9% NaCl. The standard nootropic drug piracetam (200 mg/kg) and drugs for the correction of cerebral blood flow disturbances vinpocetine (4 mg/kg) served as reference preparations.

Impairment of integrative activity of the brain was estimated by changes in passive avoidance response (CPAR) conditioning and performance [7]. We measured the latency of transition from the light to dark compartment of the chamber. On the first day of training the rat was placed in the illuminated area (100 W lamp). The rat examined this area and moved to the dark compartment after several seconds. The door in the dark compartment was closed, and the rat remained there for 300 sec. The procedure was repeated after 1 h. During this session the rat was immediately removed from the dark compartment. On the next day, the training sessions were performed 2 times at a 1-h interval. When the rat repeatedly entered the dark compartment, the door was closed. Electric current (1 mA, 50 Hz) was delivered via a metal-grid floor for 5 sec. CPAR learning was considered to be completed when the latency of transition was not less than 300 sec. CPAR performance was tested 9 days after photothrombosis. Locomotor activity of rats in the open field (automatic RODEO-1 device) was studied before CPAR learning and on day 9 after surgery. We recorded the number of squares crossed over 300 sec.

Macroscopic and microscopic (histological) examination of the brain was performed on control animals (photothrombosis+0.9% NaCl) and rats with ischemic injury receiving Proproten on day 9 after surgery (photothrombosis+Proproten). Paraffin sections of the brain were stained with hematoxylin and eosin and Nissl's cresyl violet to visualize pathomorphological changes in neurons. The area of damage in a macroscopic preparation was estimated using AUTOCAD 2000 software.

TABLE 1. Effects of Proproten, Piracetam, and Vinpocetine on CPAR Performance in Rats with Cerebral Ischemia Produced by Local Photothrombosis of the Prefrontal Cortex (*M*±*m*)

Group	CPAR latency, sec	
	before ischemia	after ischemia
Intact animals, 0.9% NaCl (*n*=20)	300	225.0±24.5
Sham-operated animals, Proproten (*n*=20)	300	300
Photothrombosis, 0.9% NaCl (*n*=12)	300	127.0±38.2*
Photothrombosis, Proproten (*n*=12)	300	284.0±17.3**
Photothrombosis, piracetam (*n*=12)	300	155.0±45.5*
Photothrombosis, vinpocetine (*n*=7)	300	159.0±37.3*

Note. $p<0.05$: *compared to intact animals; **compared to photothrombosis.

The results were analyzed by Student's *t* test for dependent and independent variables.

RESULTS

In intact rats receiving physiological saline CPAR extinction was observed on day 9 after training. The latency was shortened by 1.3 times (Table 1). The CPAR extinction was not revealed in sham-operated animals receiving Proproten. Ischemic injury to rat prefrontal cortex shortened the latency of CPAR by 2.4 times compared to the baseline level. The latency of CPAR returned to normal after administration of Proproten. Piracetam and vinpocetine produced a less significant effect. In rats receiving these preparations the latency of CPAR did not differ from that in control animals with ischemia.

TABLE 2. Effects of Proproten, Piracetam, and Vinpocetine on Locomotor Activity of Animals with Cerebral Ischemia Produced by Local Photothrombosis of the Prefrontal Cortex (Automated Open Field, *M*±*m*)

Group	Locomotor activity	
	before ischemia	after ischemia
Intact animals, 0.9% NaCl (*n*=20)	275.0±12.2	101.0±78.4
Sham-operated animals, Proproten (*n*=20)	229.0±3.5	112.0±25.4
Photothrombosis, 0.9% NaCl (*n*=12)	194.0±15.3	169.0±65.3
Photothrombosis, Proproten (*n*=12)	220.0±10.5	176.0±24.7
Photothrombosis, piracetam (*n*=12)	265.0±16.8	240.0±15.7
Photothrombosis, vinpocetine (*n*=7)	254.0±13.2	185.0±17.5

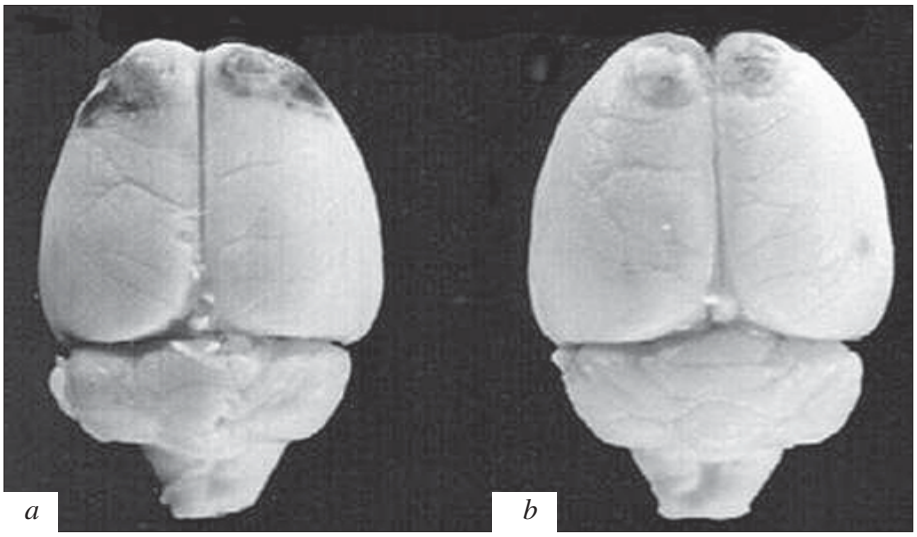

Fig. 1. Damaged area in rat prefrontal cortex on day 9 after bilateral photothrombosis: control, photothrombosis+2.5 ml/kg 0.9% NaCl intragastrically for 9 days (*a*); Proproten, photothrombosis+2.5 ml/kg Proproten intragastrically for 9 days (*b*); macrophoto.

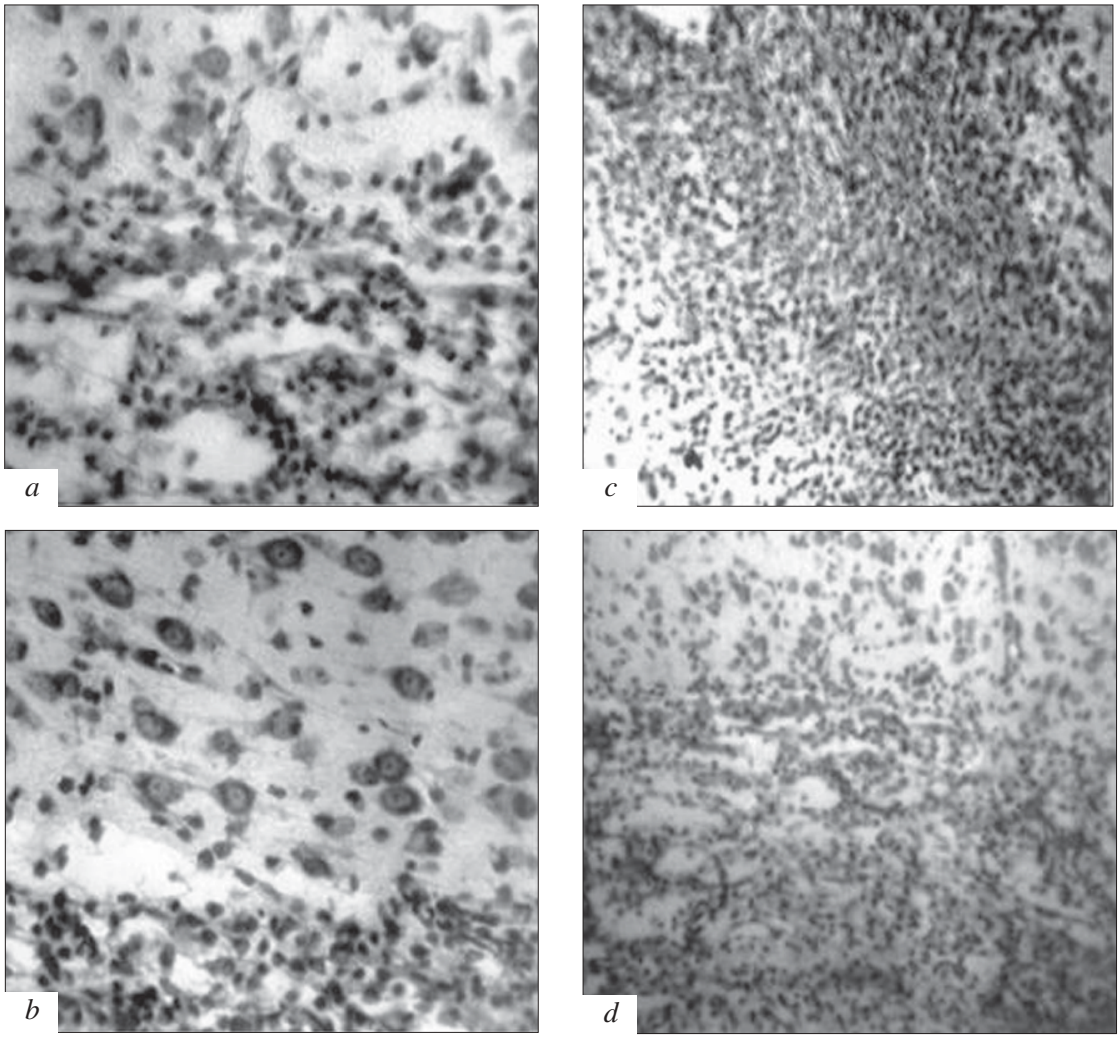

Fig. 2. Histological section of rat brain on day 9 after bilateral photothrombosis of the prefrontal cortex. Staining with hematoxylin and eosin or cresyl violet. Section at the interface of the perifocal area and zone of necrosis: control (*a*) and Proproten (*b*); 1 cm=40 μ. Section of the perifocal area: control (*c*) and Proproten (*d*); 1 cm=20 μ.

The test preparations had little effect on locomotor activity of rats with ischemia (Table 2).

Pathomorphological study showed that Proproten produces a positive effect on brain tissue integrity. The zone of focal ischemic injury in the frontal lobes of control rats was more extensive than in animals receiving Proproten for 9 days (Fig. 1). The zone of injury in control rats (area of surface projection) was 39.9% greater than in animals of the Proproten group. In control rats ischemic injury involved deeper layers of the nervous tissue.

Signs of ischemic damage to neurons were revealed in the perifocal area (penumbra) of control animals. We found a well-defined focus of necrosis with gliomesodermal scar and glial cysts (Fig. 2, *a*). The perifocal area included a wide leukocytic border (magnification 20 μ, Fig. 2, *c*). Proproten decreased the severity of cysts and gliomesodermal scar in the zone of necrosis. The penumbra contained a lower number of hyperchromic neurons and higher number of normal nuclear neurons (Fig. 2, *b*). Proproten improved the state of the leukocytic border in the perifocal area (compared to the control, Fig. 2, *d*).

Our results indicate that Proproten is more potent than standard neuroprotectors piracetam and vinpocetine in alleviating the signs of memory disorders produced by ischemic injury to the brain cortex. A pathomorphological study of the damaged area confirmed a strong neuroprotective effect of Proproten. Proproten exhibited neuroprotective activity during experimental focal ischemic thrombosis, which simulates clinical signs of ischemic stroke. The data show that Proproten holds much promise for the complex therapy of patients with this disorder.

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