Nootropic and Antiamnestic Effects of Tenoten (Pediatric Formulation) in Immature Rat Pups

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The antiamnestic effects of tenoten (pediatric formulation) was demonstrated on the model of scopolamine-induced amnesia of passive avoidance reflex and the nootropic effect of this preparation was demonstrated on the model of incomplete conditioning and in rat pups with experimental attention deficit syndrome. The efficiency of the preparation was comparable to that of piracetam and phenibut and even surpassed it by some parameters.

Key Words: *tenoten (pediatric formulation); immature rats; conditioned passive avoidance; scopolamine-induced amnesia; attention deficit syndrome; hyperactivity*

Difficulties in the use of psychotropic preparations in pediatrics dictate the need in new effective and safe drugs. Here we studied nootropic activity of tenoten (pediatric formulation, TP), a preparation containing ultralow doses of antibodies to S100 protein. Previous studies showed that preparations containing S-100 protein (proproten-100, tenoten, and TP) exhibit anxiolytic, antidepressant, and stress-protective properties and produce no side effects [3,5,6].

The search for preparations for rehabilitation of children after brain damages and infectious and other diseases impairing memory and learning capacity and for the treatment of children with attention deficit/hyperactivity syndrome (ADHS) is an urgent problem. In foreign countries, ritalin (methylphenidate), a preparation of the amphetamine group, is used for the treatment of ADHS. Despite its high efficiency, ritalin has side effects and contraindications (including child age <6 years) and induces addiction [1]. Ritalin is unallowed for the use in Russia, where ADHS is usually treated with phenibut.

There is no generally accepted experimental model of ADHS; for this purpose some investigators use SHR rats [7], rats with bisphenole-A-induced endocrine disorders [9], *etc*. In our study we used highly active immature rat pups with inadequate behavior as the model of ADHS. Moreover, the nootropic effect of the preparations was studied on the model of disturbed passive avoidance conditioning and antiamnestic activity was studied on the model of disruption of conditioned passive avoidance response (PAR). Piracetam and phenibut were used as the reference preparations.

MATERIALS AND METHODS

The studies were performed on outbred albino male and female rats aging 30-35 days, which corresponded to 4.8-10.5 years of human life [8,10]. In experimental series I, amnesia of conditioned PAR was modeled by subcutaneous injection of scopolamine (1.4 mg/kg) 15 min before PAR testing [4]. In series II, rat pups with incompletely conditioned PAR due to the use of suboptimal conditioned negative reinforcement were used. For series III, rat pups with high motor activity and inadequate behavior were chosen. Highly active animals were chosen using the open field test. Then, rat pups with impulsive inadequate behavior were selected from highly active animals using Brady and Nauta test-stimuli and scores.

In series I and II, TP (experimental group) and distilled water (control) were administered in a volume

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of 2.5 ml/kg for 10 days. In series III, the preparations were administered in the same volume for 7 days. Piracetam (400 mg/kg in a volume of 2.5 ml/kg) was used as the reference preparation in series I and II and phenibut (125 mg/kg in a volume of 2.5 ml/kg) served as the reference preparation in series III. The last dose of the test preparation was given 40 min before testing.

The nootropic and antiamnestic effects of TP were evaluated by PAR conditioning in a Lafayette Instrument Co setup. The pup was placed into the experimental chamber and the latency of the first entry into the dark compartment (L1) was recorded over 3 min. After the animal entered the dark compartment, the door was closed and the animal was subjected to inescapable electrocutaneous painful stimulation via the electrode floor. Twenty-four hours after learning, the latency of entry into the dark compartment (L2) was recorded. If mnestic processes were not disturbed, the animal remembered the painful electrocutaneous stimulation inflicted in the dark compartment and entered this compartment later than during learning.

For each experimental series, the regimen and the strength of painful electrical stimulation were chosen individually bearing in mind the fact that pain sensitivity of rat pups differs from that of adult rats. Different experimental series showed that 8 electrical stimuli (0.6 mA) in 2 sessions with a 2-day interval are required for PAR conditioning in 30-35-day-old rat pups with medium motor activity and usual behavior. For incomplete PAR conditioning (series II), 5 stimuli (0.45 mA) were enough. In rats with high motor activity and inadequate behavior, complete PAR conditioning required 5 stimuli (0.45 mA).

RESULTS

In intact rats, L2 increased by 12.4 times compared to L1 ($p<0.05$; Table 1), while during PAR conditioning with the use of suboptimal conditioned negative reinforcement (0.45 mA, 5 stimuli) L2 remained practically unchanged (Table 2), which attests to the absence of PAR conditioning in this group. In rats with scopolamine-induced amnesia of PAR, L2 increased by only 1.6 times ($p<0.05$). After administration of TP to rats with PAR amnesia and to rats with incomplete conditioning, L2 increased by 3.4 and 1.7 times, respectively ($p<0.05$; Tables 1 and 2). This fact probably suggests that TP affects not only the recovery of suppressed conditioned response, but also facilitation of conditioning. Administration of piracetam in series I and II revealed different effects. Piracetam increased L2 by 3.7 times ($p<0.05$; Table 1) in rats with scopolamine-induced amnesia, but had no significant effect on facilitation of PAR conditioning in the model of incomplete conditioning (Table 2).

TABLE 1. Antiamnestic Effects of TP and Piracetam in Scopolamine Amnesia of PAR in Rat Pups (*M±m, n*=12)

Preparation	Before learning	Testing after 24 h	
	L1, sec	L2, sec	ΔL, sec
Intact (distilled water)	8.83±3.15	109.25±25.03	98.25±23.87
Control (distilled water+scopolamine)	9.08±2.28	14.83±3.12^{+}	5.08±1.95^{+}
TP+scopolamine	7.17±2.25	50.83±17.43*	43.50±16.88*
Piracetam+scopolamine	10.17±1.82	55.58±14.42*	45.42±14.02*

Note. Here and in Tables 2 and 3: $p<0.05$ compared to: *control, $^{+}$intact rats.

TABLE 2. Effect of TP and Piracetam on PAR Conditioning with the Use of Suboptimal Negative Reinforcement (*M±m, n*=15)

Preparation	Before learning	24 h after learning	
	L1, sec	L2, sec	ΔL, sec
Control	10.3±1.1	15.1±2.3	4.8±1.9
TP	7.2±1.1	26.4±4.6*	19.2±3.9*
Piracetam	9.2±2.1	19.2±4.8	10.3±5.7

TABLE 3. Effect of TP and Phenibut on PAR Conditioning in Highly Active Rat Pups with Inadequate Behavior (*M*±*m*)

Preparation	L2, sec	Number of transitions	Number of animals not entering the dark compartment, %
Control (*n*=27)	26.61±11.26	2.4±0.67	0
TP (*n*=18)	91.33±34.14*	0.78±0.34*	39*
Phenibut (*n*=10)	73.4±36.56*	0.8±0.26*	20

In series III, rat pups with high motor activity and inadequate behavior after PAR conditioning entered the dark compartment with electrical painful stimulation more rapidly than series I rat pups with medium motor activity and normal behavior (by 4 times, $p<0.05$). This difference is probably related to the increased total motor activity of pups in this group. TP not only increased L2 in highly active rats by 3.4 times ($p<0.05$ compared to the control group), but also 3-fold reduced the number of transitions between the light and dark compartments ($p<0.05$ compared to the control group, Table 3). The direction and degree of changes in these parameters were comparable for phenibut and TP. However, the number of animals in whom the nootropic effect of phenibut was observed was similar to that in the control group, while the nootropic effect of TP was recorded in a significantly greater number of animals compared to the control (Table 3). It can be hypothesized that TP improves learning of rat pups by reducing their hyperactivity, *i.e.* increasing their "assiduity".

Thus, were demonstrated a significant nootropic and antiamnestic effects of course administration of TP to immature rat pups in PAR conditioning. TP improves conditional relationship between the suboptimal stimulus and reaction, *i.e.* facilitates conditioning in animals, including highly active rat pups with inadequate behavior, by reducing their inadequate activity and restores disrupted conditional response. The efficiency of TP was comparable to nootropic activity of piracetam and phenibut.

REFERENCES

1. E. D. Belousova and M. Yu. Nikanorova, *Ross. Vestn. Perinatol. Pediatr*., No. 3, 39-42 (2000).
2. R. I. Kruglikov, *Neurochemical Mechanisms of Learning and Memory* [in Russian], Moscow (1981).
3. Proproten-100. *Ultralow Doses of Affinity-Purified Antibodies to S-100 Protein* [in Russian], Moscow (2002).
4. *Manual on Experimental (Preclinical) Study of New Pharmacological Substances*. Ed. R. U. Khabriev [in Russian], Moscow (2005).
5. V. K. Shamrei, V. I. Kuriatov, I. Yu. Khabarov, and P. D. Shabanov, *Psikhofarm. Biol. Narkol*., **6**, Nos. 1-2, 1212-1219 (2006).
6. V. Castagne, M. Lemaire, I. Kheyfets, *et al., J. Pharm. Pharmacol*. **60**, No. 3, 309-316 (2008).
7. A. C. Chess and J. T. Green, *Behav. Neurosci.,* **122**, No. 1, 63-74 (2008).
8. Y. Masuo, M. Morita, S. Oka, and M. Ishido, *Regul. Pept.*, **123**, No. 1-3, 225-234 (2004).
9. R. Quinn, *Nutrition*, **21**, No. 6, 775-777 (2005).
10. M. A. Suchow, S. H. Weisbroth, and C. L. Franklin, *The Laboratory Rat.* Amsterdam *etc.* (2006), pp. 149, 153.