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Proline-Containing Dipeptide GVS-111 Retains Nootropic Activity after Oral Administration

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Experiments on rats trained passive avoidance task showed that N-phenyl-acetyl-L-prolyl-glycyl ethyl ester, peptide analog of piracetam (GVS-111, Noopept) after oral administration retained antiamnesic activity previously observed after its parenteral administration. Effective doses were 0.5-10 mg/kg. Experiments on a specially-developed model of active avoidance (massive one-session learning schedule) showed that GVS-111 stimulated one-session learning after single administration, while after repeated administration it increased the number of successful learners among those animals who failed after initial training. In this respect, GVS-111 principally differs from its main metabolite cycloprolylglycine and standard nootropic piracetam.

Key Words: *dipeptides; GVS-111; passive and active avoidance*

Positive effects of some neuropeptides on cognitive processes (memory, attention, and concentration) prompted the search for correctors of cognitive dysfunctions among these substances. Practical use of mnemotropic neuropeptides (vasopressin, ACTH, and others) was unsuccessful because of their low biological stability and low permeability of the blood-brain barrier for these substances. Some modifications of oligopeptide structure increasing its stability were proposed [1]. The approach developed at the Institute of Pharmacology (Russian Academy of Medical Sciences) for the past 15 years is construction of neurotropic oligopeptides structurally similar to the corresponding non-peptide prototype [5]. For instance, structural and conformational analysis of piracetam and dipeptides containing pyroglutamate or proline led to creation of highly efficient nootropic compounds. N-Phenyl-acetyl-L-prolyl-glycyl ethyl ester (GVS-111, Noopept) exhibited the highest nootropic activity: it improved learning and attenuated the effects of damaging factors on conditioning and memory in doses 3 orders of magnitude lower than the corresponding doses of piracetam [10-12]. These effects of GVS-111 were observed after its parenteral administration. The aim of the present study was to elucidate whether nootropic activity of GVS-111 is retained after oral administration. There are many pathological states characterized by chronic cognitive dysfunction, such as a (postconcussion syndrome, recovery after cerebral ischemia, and initial and moderate dementia of neurodegenerative and vascular genesis). In light of this, the search for new oral nootropic drugs (most convenient for long-term treatment) possessing no toxicity because of their endogenous origin is of crucial importance.

MATERIALS AND METHODS

Nootropic activity of GVS-111 was studied on the models of passive and active avoidance conditioning.

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Experiments were carried out on outbred male albino rats weighing 180-200 g.

Passive avoidance conditioning was performed on a Laffaete Instruments setup. The rat was placed on an illuminated platform (250×70 mm) facing away from a square door (60×60 mm) leading to a dark compartment (400×400×400 mm) with an electrified floor. After finding the door the rat entered the dark compartment due to innate preference of darkness (a hole reflex). The latency of the first entrance and the time spent in the dark compartment were recorded for 3 min. After 3 min, the door was closed and the animal received an electrical shock (8 pulses, 0.45 mA, 1 sec each delivered with 2-sec intervals). Retention was tested 24 h after training; the latency of the first entrance and the time spent in the dark compartment were recorded. The antiamnesic activity of GVS-111 was tested on the model of electroshock amnesia. Transcorneal electrical shock (70 V, 300 msec) was applied immediately after training.

GVS-111 was administered intragastrically in doses of 0.3-30 mg/kg 20 min before training. For evaluation of antiamnestic activity of GVS-111, the animals were randomly divided into 3 groups: passive control (0.9% NaCl before training and false electrical shock after it), active control (0.9% NaCl before training and transcorneal electrical shock after it), and experimental group (GVS-111 before training and the electrical shock after it). The groups for each dose included no less than 20 rats.

The data were analyzed statistically using Mann-Whitney *U*-test. To compare the antiamnestic effects of different doses, the index of relative antiamnestic activity was calculated by the formula:

$$(L_O\text{-}L_{AC}/L_C\text{-}L_{AC})\times 100\%,$$

where L_O, L_{AC}, L_C are the mean latencies of entrance into the dark compartment in the retention test in the experimental, active control, and passive control groups, respectively.

The effects of GVS-111 on learning and memory were studied on a model of two-way active avoidance in a shuttle-box. The box (45×23×25 cm) with acrylic opaque walls and electrified floor was divided into two equal compartments with a square door between them (6×6 cm). Light flashes served as a conditioned stimulus and electrical stimulation (20-25 V) as an unconditioned stimulus. The animals were kept in a vivarium with free access to food and water. Twenty-four hours before active avoidance training they were deprived of food. During training the animal was placed in one of the compartments for 5 min to explore the shuttle box, then a flicker was switched on and after 6 sec electrical current was applied to the floor. If the rat ran into the dark compartment, CS and US were switched off, if not – the current was switched off after 6 sec. The intervals between conditioned and unconditioned stimuli were 30 sec.

In the present study we used massive training schedule: the animals were presented up to 50 combinations per session. Runs into the dark compartment after CS and before US presentation were considered as avoidance. The criterion of successful learning was 10 avoidance responses to 10 consecutive CS presentations. If the learning criterion was reached after 40 trials, the number of trials was increased to 60.

The effects of GVS-111 on active avoidance conditioning were evaluated using different experimental protocols. In series I, the rats received GVS-111 in a dose of 0.5 mg/kg intraperitoneally 15 min before training. In series II, GVS-111 was administered *per os* 20 min before training. The dose-response dependence for this route of administration was determined by using GVS-111 in doses of 0.3, 0.5, 1.0, 5.0, 10.0, and 30 mg/kg. In series III and IV, the animals received single intraperitoneal (0.5 mg/kg) or oral (10 mg/kg) doses of GVS-111, respectively, (these doses were previously shown to be optimal); controls received saline. During 9 consecutive days after training good learners received 0.9% NaCl, while poor learners continued to receive GVS-111. In the control group, both good and poor learners received 0.9% NaCl. After 9 days the animals of all groups were tested for active avoidance retention.

To clarify whether the changes in active avoidance were associated with changes in locomotor activity, in an additional experimental series the rats were treated with GVS-111 (10 mg/kg, group 1) or 0.9% NaCl, (0.2 ml/kg, group 2) for 9 consecutive days. Each group consisted of no less than 10 rats.

Fisher exact probability and χ^2 tests were applied for statistical analysis of active avoidance indices in rats receiving saline and GVS-111.

RESULTS

Oral administration of GVS-111 produced an antiamnestic effect (Fig. 1, *a*). The dose-effect relationship in a 0.3-1.2 mg/kg dose range was described by a bell-shaped curve with a peak at 0.5 –0.7 mg/kg. The antiamnestic effect disappeared at a dose of 1.2 mg/kg and appeared again at higher doses. The second peak corresponded to 10 mg/kg, while at 30 mg/kg the antiamnestic activity decreased. Therefore, the dose-dependence of GVS-111 effect was described by a two-peak curve.

The reports on the effects of nootropics on active avoidance behavior are contradictory. The initial reports on learning-facilitating effects of piracetam [9]

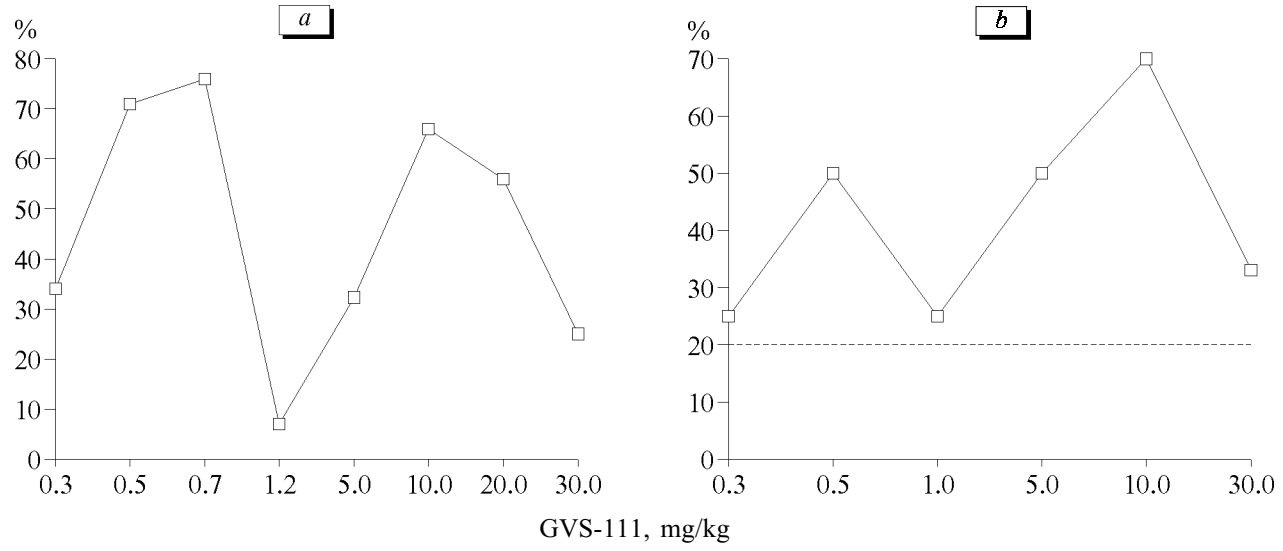

Fig. 1. The antiamnestic effect of oral GVS-111 on the model of electroshock amnesia in a passive avoidance test (*a*) and its learning-stimulating effect in active avoidance test (*b*). Ordinates: antiamnestic activity (*a*) and the number of animals achieving learning criterion (*b*); *$p<0.05$ compared to the control (dotted line).

were not confirmed by further studies [14]. According to M. Sansone *et al.* [13], the nootropic activity in this test can be manifested only under conditions of preliminary subchronic administration. Our previous experiments showed that the nootropic-induced facilitation of active avoidance learning was an unstable phenomenon requiring a special method of disturbance (collide) to be revealed [6]. We hypothesized that low reproducibility of this effect was due to ineffective training schedule. When studying a conditioned freezing response we found that massive training schedule was less efficient for learning than the usual schedule with 3-4 sessions a day, 15 trials each [3]. Proceeding from this and generally accepted idea that the initial deficit of learning is a necessary condition for the manifestation of nootropic effects, we applied this massive schedule for active avoidance conditioning.

In the control group, the majority of animals (15-30% in different experiments) did not achieve the learning criterion. A single intraperitoneal injection of GVS-111 (0.5 mg/kg) before training increased the number of animals achieving the learning criterion from 25 to 60% (these experiments were carried out because the effects of parenteral GVS-111 in massive training schedule were not studied). Oral GVS-111 dose-dependently increased the number of good learners (Fig. 1, *b*). The dose-dependence curve had two peaks (similar to those in passive avoidance test), but the effect of GVS-111 in a dose of 0.5 mg/kg was insignificant.

Our previous findings on the passive avoidance model showed that GVS-111 stimulated all stages of memory processing, in contrast to piracetam affecting primarily memory acquisition [11]. The positive effect on the early stages of memory formation is also typical of cycloprolylglycine (CPG), the main GVS-111 metabolite. [4]. In this context, we investigated the effects of repeated administrations of GVS-111 after active avoidance training and compared them with the effects of CPG and piracetam. All these drugs after single administration improved learning, but only GVS-111 administered in a subchronic regimen increased the number of animals attaining the learning criterion among poor learners. Under these conditions

TABLE 1. Effects of GVS-111, Its Main Metabolite CPG, and Piracetam on Learning Active Avoidance after Single and Subchronic (9 days) Administration (% Animals Attaining Learning Criterion)

Experimental conditions	Control (NaCl)	GVS-111, mg/kg		Piracetam, 200 mg/kg, intraperitoneally	CPG, 0.5 mg/kg, intraperitoneally
		0.5, intraperitoneally	10, *per os*		
Single administration	20	50	70	53	50
Subchronic administration	28	100	90	57	33

piracetam was ineffective, while CPG even decreased this parameter (Table 1), which agrees with the data on negative effect of CPG on memory retrieval [4]. These findings confirm the important role of consolidation processes in completion of learning [7]. They also imply that GVS-111 exhibits a wider spectrum of positive mnemotropic effects compared to piracetam.

The positive effect of GVS-111 on learning can result from its specific mnemotropic activity, rather than from general activation. This is confirmed by the absence of significant changes in locomotor activity: the mean number of horizontal movements in rats receiving 10 mg/kg GVS-111 for 10 days was 1708.8± 56.06 0.9% *vs.* 1506.6±45.0 in animals treated with NaCl ($p<0.1$).

There are data that dipeptides are more resistant to intestinal brush border enzymes, compared to peptides with a more complex structure [8]. The presence of cyclic amino acid proline and stabilizing substitutions at N- and C-terminals may increase GVS-111 resistance to these enzymes. The results of the present study confirm this assumption. Our data are in line with the data of pharmacokinetic studies showing that GVS-111 after oral administration is rapidly absorbed and enters systemic and cerebral circulation, and that its concentration in the brain far exceeds its plasma concentration [2]. This attests to neurotropism and high bioavailability of GVS-111. Oral GVS-111 in single and subchronic administration was effective not only in passive avoidance test (common for the screening procedure), but also in our version of active avoidance training. In this situation, GVS-111 stimulated memory consolidation and promoted attaining of learning criterion in poor learner animals.

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