Naloxone-Blocked Depriming Effect of Anxiolytic Selank on Apomorphine-Induced Behavioral Manifestations of Hyperfunction of Dopamine System

V. K. Meshavkin, N. V. Kost, O. Yu. Sokolov, Yu. A. Zolotarev*, N. F. Myasoedov*, and A. A. Zozulya

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Peptide anxiolytic selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) applied intraperitoneally in doses of 0.01, 0.1, 1.0, and 10.0 mg/kg to mice reduces behavioral manifestations of dopaminergic system induced by apomorphine in the verticalization test. This effect was comparable to that of atypical antipsychotic olanzapine in near-therapeutic doses (0.1 and 1.0 mg/kg, intraperitoneally) and was blocked with nonselective opioid receptor antagonist naloxone (10 mg/kg, intraperitoneally). Radioreceptor assay showed that selank did not displace nonselective D_2-dopamine receptor antagonist ^{3}H-spiperone (EC_{50}>100 μM) and δ- and μ-opioid receptor ligand ^{3}H-DADLE (EC_{50}>40 μM) from specific binding sites on rat brain membranes. It is hypothesized that the revealed behavioral effect of selank is mediated by its modulating effect on the endogenous opioid system and specifically, by its effect on activity of enkephalin-degrading enzymes.

Key Words: *selank; apomorphine-induced verticalization*; *naloxone*; *dopaminergic system*; *opioidergic system*

Clinical trials of peptide anxiolytic selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) entered the final stage [1,4]. Behavioral experiments showed that anxiolytic action of this agent is not accompanied by the sedative and myorelaxant effects characteristic of the benzodiazepine tranquilizers. The tranquilizing action of selank is proven [4,5], but the neurochemical mechanisms of its effect remain little studied. In particular, its ability to modulate the dopaminergic system is unknown.

Our aim was to study the effect of selank on the apomorphine-induced behavioral manifestations of hyperactivity of the dopaminergic system.

Research Center of Mental Health, Russian Academy of Medical Sciences; *Institute of Molecular Genetics, Russian Academy of Sciences, Moscow. ***Address for correspondence***: meshavkin@rcmh.msk.ru. V. K. Meshavkin

MATERIALS AND METHODS

The experiments were carried out on random-bred male albino mice (n=270) weighing 22.1±0.2 g obtained from Kryukovo-Tsentral'noe Breeding Department of Russian Academy of Medical Sciences. The animals were maintained on unrestricted food and water under the vivarium conditions at 20-22°C with controlled illumination (12 h light from 7:00 to 19:00). The acclimatization period before the experiments was no less than 3 weeks.

The effect of selank on behavioral manifestations of hyperactivity of the dopaminergic system was examined using verticalization test. The test was carried out on mice placed into cylindrical chambers with a diameter of 140 mm. The bottom of the chambers was made of plexiglas, and the walls were made of stainless wires with the length of 200 mm and diameter of 2 mm (10 mm distance

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between rods). The phenomenon of verticalization was induced by subcutaneous injection of 5 mg/kg apomorphine hydrochloride (Sigma) 10 min before the test. Animal behavior was observed over an hour. The level of verticalization was scored over 10 sec every 2 min according to a 4-point scale corresponding to the number of paws in the wire wall. At the end of the experiment, the total score over the entire observation period was calculated for each mouse.

Bolus injections of selank or naloxone dissolved in 0.2 ml physiological saline were made intraperitoneally 30 min before the test. Each animal was tested once.

The affinity of selank to dopamine and opioid receptors was determined *in vitro* by its ability to compete with tritium-labeled ligands of the corresponding receptors (^{3}H-spiperone and ^{3}H-[D-Ala^2,D-Leu^5]-enkephalin, DADLE, Amersham) for binding to the receptors of membrane fraction of the rat brain.

The radioreceptor assay was performed to assess selank interaction with dopamine receptors in the striatum, where the density of D_2-receptors is high [6]. The incubation buffer (final volume 300 µl) contained (in mM): 50 Tris-HCl (pH 7.4), 120 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, striatal membrane fraction (0.15 mg protein/ml), bacitracin (50 µg/ml), ascorbic acid (0.1 mg/ml), pargyline (10 µM), ^{3}H-spiperone (40 Ci/mM), and selank (0.1-100 µM). Incubation was carried out at 25°C for 40 min.

The interaction of selank with opioid receptors was analyzed [14]. The incubation buffer (0.3 ml) contained the midbrain membrane fraction (1 mg protein/ml), peptidase inhibitor bacitracin (50 µg/ml), ^{3}H-DADLE (4 nM, 40 Ci/mM), and selank (0.1-100 µM). Incubation was carried out at 25°C for 40 min.

Bound and free label were separated on a Skatron harvester using fiberglass filters soaked in 0.1% polyethyleneimine solution. Specific binding was calculated as the difference between ^{3}H-spiperone and ^{3}H-DADLE binding in the absence and presence of 10 µM sulpiride or 2 µM dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), a synthetic analog to leu-enkephalin, respectively. The same ligands (0.1 nM-1 µM) were used for construction of calibration curves. Each point was determined in three parallels in 3 independent experiments. Protein content was estimated by the method of Lowry.

The data were analyzed statistically using Statistica software.

RESULTS

Selank in doses of 0.01, 0.1, 1.0, and 10.0 mg/kg decreased verticalization by 20-30% (p<0.001, Fig. 1). A novel antipsychotic drug olanzapine (Eli Lilly) was used as the reference preparation. In verticalization test, the comparable therapeutic doses of olanzapine (0.1-1.0 mg/kg) produced similar or even weaker effect. In a higher dose of 10 mg/kg, olanzapine completely suppressed verticalization of mice, while the effect of selank applied in this dose remained unchanged (Fig. 1). Therefore, selank can moderate (deprime) behavioral manifestations of dopaminergic system hyperactivity and it is more efficient than olanzapine when applied in doses of 0.01 and 0.1 mg/kg (p<0.05).

Combination of anxiolytic activity [4] and its activity characteristic of the neuroleptics found in this study is typical for novel antipsychotic drugs. Most these drugs (specifically, olanzapine) demonstrate pronounced anxiolytic effects in behavioral experiments [12]. However, routine anxiolytics such as diazepam mediate their effects predominantly via GABA-benzodiazepine receptors and do not affect the apomorphine-induced verticalization in experimental animals [10].

For evaluation of the mechanism underlying the effect of selank, we examined its ability to bind to dopamine receptors *in vitro*. Radioreceptor assay showed that selank does not replace ^{3}H-spiperone (non-selective antagonist of D_2-dopamine receptors) from specific binding sites on rat striatal membranes (EC_{50}>100 µM). In other words, the anti-verticalization effect of selank is not related to its direct binding to D_2-receptors.

Taking into consideration the fact that the opioid system plays a key role in modulation of the effects produced by dopaminergic system (spe-

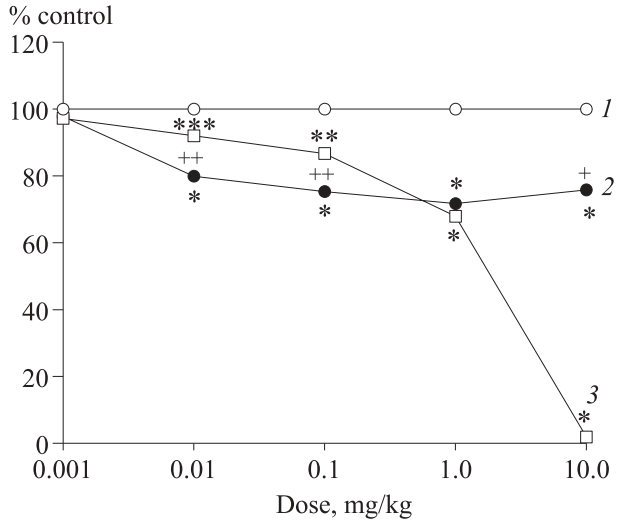

Fig. 1. Effects of selank and olanzapine on apomorphine-induced verticalization in mice. *1*) control (apomorphine 5 mg/kg); *2*) selank; *3*) olanzapine. *p<0.001, **p<0.01, ***p<0.05 compared to the control. ^+p<0.001, ^{++}p<0.05 compared to olanzapine.

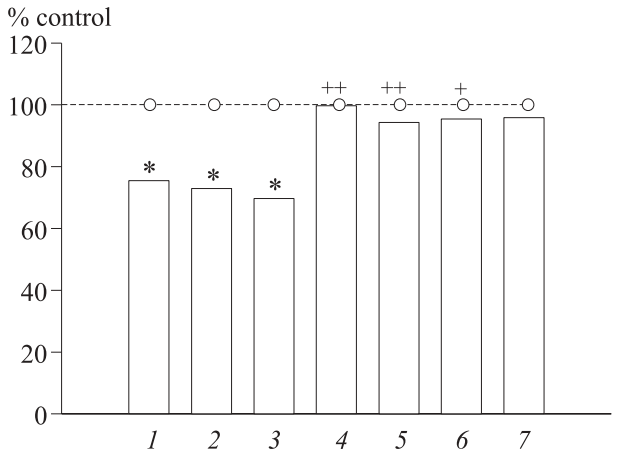

Fig. 2. Preventive effect of naloxone (10 mg/kg) on moderating action of selank on apomorphine-induced verticalization in mice. *1*) selank (0.01 mg/kg); *2*) selank (0.1 mg/kg); *3*) selank (1 mg/kg); *4*) selank (0.01 mg/kg)+naloxone; *5*) selank (0.1 mg/kg)+naloxone; *6*) selank (1 mg/kg)+naloxone; *7*) naloxone. The control level (5 mg/kg apomorphine) is shown by dashed line. *$p<0.001$ in comparison with control; $^{+}p<0.001$; $^{++}p<0.01$ relates to the blocking effect of naloxone.

cifically, apomorphine-induced verticalization, [7,8, 9]), in the next series of experiments we examined the possibility of preventing the anti-verticalization effect of selank by naloxone, an opioid receptor antagonist. Intraperitoneal naloxone (10 mg/kg) produced no effect on mouse verticalization, but prevented the action of all doses of selank used in this study (Fig. 2). This finding suggests that the effect of selank is mediated by its modulating action on the opioid system. However, the radioreceptor assay revealed no ability of selank to replace ^{3}H-DADLE (a ligand to δ- and μ-opioid receptors) from the binding sites on the rat brain membranes (EC_{50}>40 μM). Thus, the effect of selank on the opioid system cannot be produced by its direct interaction with these receptors.

We previously demonstrated that the anxiolytic action of selank can be explained by its ability to inhibit enkephalin-degrading enzymes [2,3]. In addition to the agonists and antagonists of opioid receptors, the inhibitors of enkephalinases are known to modulate the dopaminergic system [11,13]. Therefore, it can be hypothesized that naloxone-antagonized moderating effect of selank in verticalization test is mediated by inhibition of the enkephalin-degrading enzymes resulting in modulation of dopaminergic system by the opioid one.

Thus, selank moderates the pharmacologically-induced behavioral manifestations of dopaminergic system hyperfunction. Probably, this action results from ability of the drug to produce a modulator effect on the opioid system, specifically, via changes in activity of the enkephalin-degrading enzymes.

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