Efficacy of Peptide Anxiolytic Selank during Modeling of Withdrawal Syndrome in Rats with Stable Alcoholic Motivation

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We studied the effects of selank on the development of symptoms of acute 48-h alcohol withdrawal in outbred rats drinking 10% ethanol as the only source of fluid for 24 weeks. In alcohol-preferring animals (mean daily ethanol intake >5.0 g/kg) allowed free choice between 10% ethanol and water, single intraperitoneal injection of selank in a dose of 0.3 mg/kg eliminated anxiety induced by ethanol withdrawal in tests elevated plus maze and social interaction tests and prevented the formation of mechanical allodynia without affecting ethanol consumption. The findings suggest that selank is effective in eliminating of alcohol withdrawal symptoms in rats.

Key Words: *selank; alcohol withdrawal; anxiety; allodynia; rats*

Benzodiazepine anxiolytics temporary relieving manifestation of psychopathological symptoms associated with withdrawal syndrome play an important role in the complex medical therapy for alcoholism. However, numerous side effects, such as respiratory depression, excitement, potentiation of the narcogene effect of ethanol, and risk of drug dependence limit their use for pharmacological correction of withdrawal syndrome [8].

Apart from detoxification procedures, schemes of drug therapy for withdrawal syndrome include a wide range of neuropsychotropic drugs (neuroleptics, antidepressants, and nootropics) that exert various side effects [3]. In light of this, the search for more specific and safe antialcoholic drugs for the relief of manifestations of alcohol withdrawal syndrome is an urgent problem.

Selank, an original Russian peptide preparation, is effective in the treatment of generalized anxiety disorders and asthenic conditions. It is characterized by rapid onset of therapeutic effect and the absence of undesirable side effects [4]. Experimental studies have shown that it had a broad spectrum of psychotropic activity and restored integrative activity of the CNS impaired by neurotoxic factors of different genesis [5]. The mechanism of the central action of selank includes inhibition of enzyme degradation of enkephalins [1]. Significant therapeutic effect of selank on cognitive functions in combination with anxiolytic and stimulating effects have been demonstrated in clinical settings [6].

Here we studied the effects of selank on behavioral manifestations of withdrawal syndrome in chronically alcoholized rats.

MATERIALS AND METHODS

Experiments were carried out on 8-month-old male outbred rats with mean body weight 450-470 g in the active phase of the experiment (Stolbovaya nursery).

Alcohol abuse was modeled using the method for forced alcoholization of the animals providing a 10% ethanol solution as the sole source of fluid for 24 weeks. The animals were kept in individual ca-

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ges under standard vivarium conditions (vivarium of V. V. Zakusov Research Institute of Pharmacology) at 12-h light/dark cycle with free access to water and chow. Changes in body weight and ethanol consumption (g/kg) were recorded weekly. During forced alcoholization, ethanol was consumed in physiologically significant amount (6.5±0.2 and 5.0±0.3 g/kg at the start and end of the experiment, respectively). The experiments were performed on rats with stable alcoholic motivation that was evaluated by mean daily ethanol consumption (per 1 kg body weight per day) under free choice between 10% ethanol and water.

After 48-h alcohol deprivation, the animals were tested for alcohol deprivation effect (ADE) to assess the nature of formation of alcohol dependence. Ethanol intake was recorded over the first 90 min under conditions of free choice between water and 10% ethanol.

Anxiety-related behavior in elevated plus maze (EPM) test was evaluated as described previously [10]. The animals were placed on central platform and basic spatial and temporal parameters (time spent on the open arms, number of entries into the open arms, number of entries into the closed arms) were recorded over 300 sec. Increased number of entries into the open arms and time spent in open arms in the absence of changes in motor activity (total number of entries into the open and closed arms) were considered as a manifestation of the anxiolytic effect. The time in the open arms and number of entries in open arms were compared and calculated by the following formulas:

time in open arms=time in the open arms (sec)/300 sec×100% **(1)**,

number of entries into closed arms=number of entries into closed arms/total number of entries into open and closed arms×100% **(2)**.

Social interaction test consists in evaluation of the time of active social behavior for rats in pairs (as a measure of anxiety) to record both anxiogenic and anxiolytic properties of the drug. Testing was performed in an open field (60×60 cm area surrounded with 50-cm white walls and divided into 16 squares with a black marker) for 5 minutes under dimmed light. A pair of rats ("experimental" and "resident"; difference in body weight did not exceed 10 g) kept in individual cells prior to the experiment were placed in unfamiliar surroundings of the open field. The duration of active behavior of "experimental" rat against "resident" one (grooming, sniffing, rushing, wrestling, chasing) was registered. Passive contact (sitting and/or lying together) was not considered. Stress response to social interaction was evaluated based on individual behavioral data of "experimental" rat in accordance with previously published study protocol [9]. Anxiolytic effect was assessed by lengthening the time of active social behavior of the "experimental" rat against the "resident" rat.

Reaction threshold in response to mechanical stimulation was determined using calibrated von Frey filaments (0.04, 0.07, 0.16, 0.40, 0.60, and 1.00 g), applied through a perforated floor to the rear paws of rats placed individually in Plexiglas chambers. Fast paw withdrawal was considered as a positive response and stimulation was performed with a smaller filament or the same hair was used if the rat did not lick or shake the paw. Mechanical sensitivity threshold was determined by the minimum pressure that caused paw withdrawal response (3 of 5 attempts). Significant decrease in sensitivity thresholds upon filament application was regarded as mechanical allodynia [11].

The following preparations were used in the study: selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) in the anxiolytic dose of 0.3 mg/kg intraperitoneally (Institute of Molecular Genetics, Russian Academy of Sciences) and naloxone in a dose of 1.0 mg/kg intraperitoneally (Sigma). Water was injected once 30 min before testing in a ratio of 0.1 ml/100 g body weight of animal.

The study was performed in 5 groups of animals: intact rats (not offered ethanol; group 1), animals with stable alcoholic motivation against the background of 10% ethanol consumption (group 2), and animals with stable alcoholic motivation 48 h after ethanol withdrawal receiving water for injections (group 3), selank (group 4), or naloxone (group 5).

The data were processed using the nonparametric Mann–Whitney *U* test.

RESULTS

Evaluation of the effects of drugs on ADE severity showed that alcohol intake was not significantly affected by selank (1.08±0.12 g/kg) in contrast to nonselective opioid receptor antagonist naloxone (0.36± 0.06 g/kg *vs.* 0.88±0.05 g/kg in controls; $p<0.001$).

Ethanol withdrawal in animals with stable alcoholic motivation was associated with increased anxiety, which was seen from reduced time spent in open arms of EPM ($p<0.05$) and number of entries into open arms in comparison with the corresponding parameters in intact rats (Fig. 1). Naloxone had no effect on animal anxiety in EPM. Selank increased the time spent ($p<0.01$) and entries into open arms ($p<0.05$), which attests to the anxiolytic effect of this drug.

Ethanol withdrawal in animals with stable alcoholic motivation significantly reduced ($p<0.05$) the time of social interaction. Selank prolonged the active contact of rats in pairs ($p<0.01$) suggesting the anxiolytic effect of this drug (Fig. 2).

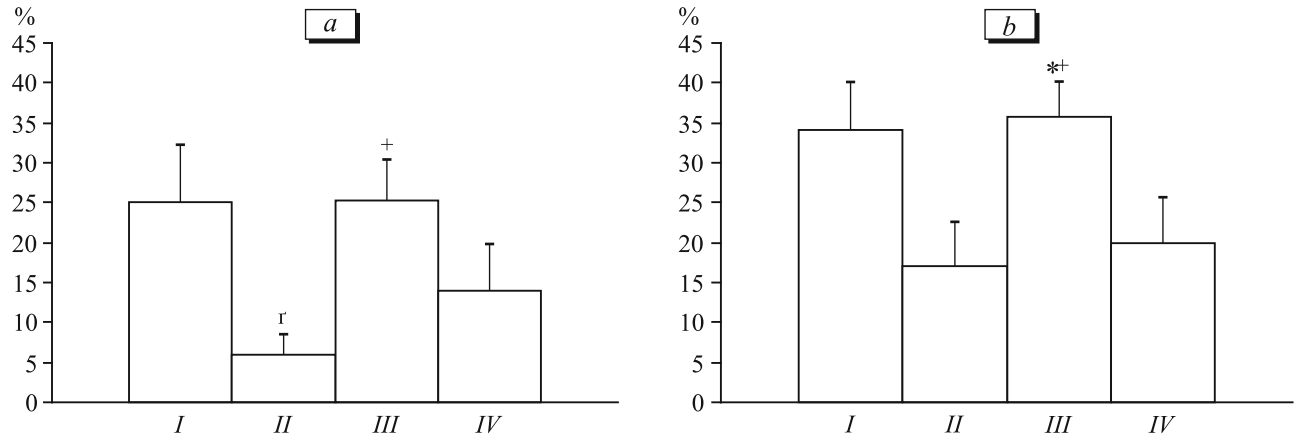

Fig. 1. Effects of selank on EPM behavior of rats with stable alcoholic motivation 48 h after ethanol withdrawal. Ordinate: *a*) time spent in open arms, *b*) entries into the open arms. *I*: group 1 (intact rats); *II*: group 3; *III*: group 4; *IV*: group 5. $p<0.05$ in comparison with *group 1 (*I*), +group 3 (*II*). Each group included 9-10 animals.

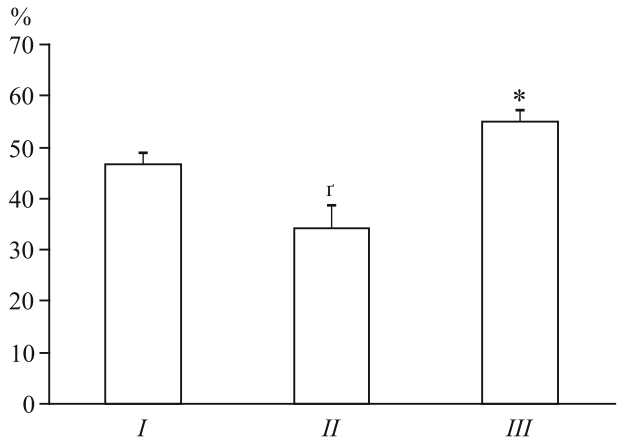

Fig. 2. Effects of selank on behavior of rats with stable alcoholic motivation 48 h after ethanol withdrawal in social interaction test. *I*: group 1; *II*: group 3; *III*: group 4; *IV*: group 5. Ordinate: time of social interaction for rats in the pair. Total observation time (300 sec) is taken as 100%. x$p<0.05$ in comparison with group 1 (*I*); *$p<0.01$ in comparison with group 3 (*II*). Each group included 8-15 animals.

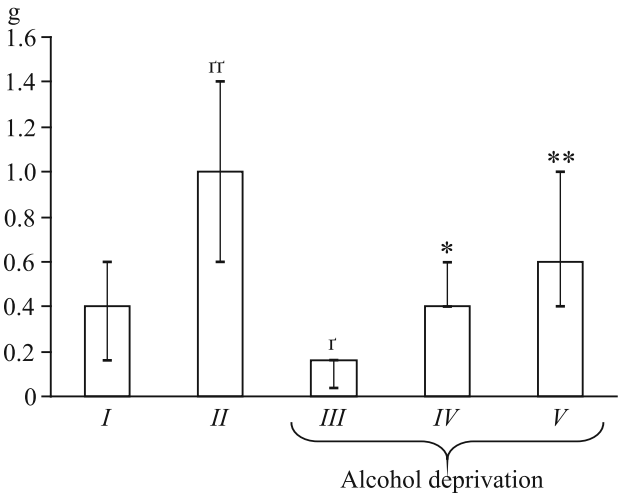

Fig. 3. Effects of selank on tactile sensitivity thresholds with non-painful mechanical stimulation 48 h after ethanol withdrawal (median, 25th and 75th quantiles). *I*: group 1 (intact rats); *II*: group 2; *III*: group 3; *IV*: group 4; *V*: group 5. Ordinate: characteristics of mechanical stimulation with von Frey filaments. x$p<0.05$, xx$p<0.01$ in comparison with group 1 (*I*); *$p<0.05$, **$p<0.01$ in comparison with group 3 (*III*). Each group included 9-10 animals.

During the first day after ethanol withdrawal, specific disorders including allodynia can be observed [11]. Our study demonstrated that mechanical sensitivity threshold to stress was increased in group 2 animals ($p<0.01$) in comparison with group 1, whereas in group 3, alcohol withdrawal dramatically decreased the response threshold, which indicated the development of allodynia (Fig. 3). Selank and naloxone (groups 4 and 5) increased the sensitivity threshold in rats with signs of withdrawal syndrome ($p<0.01$ and $p<0.001$, respectively).

According to the published reports, emotionally negative stress reaction during treatment of alcoholism can be responsible for remission failure. The search for new pharmacological targets directed at both the mechanisms of formation of stress reactions and alcohol addiction is in progress. Our earlier studies have demonstrated that naloxone and selank had opposite effects on general motor activity in inbred BALB/c and C57Bl/6 mice with "passive" and "active" phenotype of emotional stress reaction in the open field test [2]. Considering the large experience of using opiate receptor blockers in the therapy of addiction, we used naloxone as the reference drug. Naloxone reduced ethanol consumption, restored the reaction thresholds to mechanical stimulation against the background of acute alcohol deprivation, and did not affect anxious behavior in alcoholized animals.

We found that selank effectively eliminated increased anxiety and mechanical allodynia induced by

alcohol withdrawal in alcohol-preferring rats with alcohol dependence. It was proved that the effect of selank is related to its capacity to counteract functional suppression of the endogenous opioid system [5]. The content Met-enkephalin in the brain inversely correlated with ethanol consumption. Microdialysis showed statistically significant increase in Met-enkephalin in the striatum against the background of ethanol consumption (94.9±4.3 *vs*. 79.1±5.9 pg/ml in the control group) followed by its decrease to 83.9±3.8 pg/ml after alcohol withdrawal. Blood level of Met-enkephalin in "alcoholized" mice was 30% lower than in "non-alcoholized" animals [13].

The effect of selank under conditions of alcohol withdrawal can be determined by modulation of some neuropeptidergic systems via regulation of the main carboxypeptidases cleaving C-terminal lysine and arginine from precursor molecules of biologically active peptides [12] (their dysfunction under conditions of emotional stress was previously demonstrated [14]).

Alcohol deprivation reduces the content of endogenous opioids in the "positive reinforcement" system of the brain in patients with alcoholism. In this context a positive experience of the use of dietary supplements containing D-phenylalanine (enkephalinase inhibitor) for alleviating withdrawal symptoms in patients during detoxification therapy has been recently reported [7].

Thus, these data and success in using drugs developed based on endogenous regulatory peptides in pharmacotherapy for alcohol withdrawal syndrome allow considering selective anxiolytic selank as a promising tool for integrated treatment of psychoemotional disorders caused by chronic alcoholism.

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