PHARMACOLOGY AND TOXICOLOGY

Effects of Selank on Behavioral Reactions and Activities of Plasma Enkephalin-Degrading Enzymes in Mice with Different Phenotypes of Emotional and Stress Reactions

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 2, pp. 158-161, February, 2002
Original article submitted November 27, 2001

Comparative study of plasma activities of enkephalin-degrading enzymes in mice with different phenotypes of emotional and stress reactions revealed significant differences between intact BALB/c and C57Bl/6 mice by the half-life of plasma leu-enkephalin. Selank in a dose of 100 μg/kg produced an anxiolytic effect in the open-field test and increased the half-life of plasma leu-enkephalin in BALB/c mice, but had no effect on behavioral reactions and enkephalinase activities in C57Bl/6 mice. Our results suggest that anxiolytic activity of Selank is associated with inhibition of enkephalin-degrading enzymes.

Key Words: *enkephalins; enkephalinases; enkephalinase inhibitors; Selank; open field*

Reduced functional activity of the endogenous opioid system (EOS) is closely related to disturbances in γ-GABA-benzodiazepine and cholecystokinin systems and plays an important role in behavioral manifestations of anxiety and borderline disorders [2, 7,8]. The question arises whether EOS stimulation can be used for pathogenetic correction of anxiety.

Synthetic opioid receptor agonists and inhibitors of peptidases catalyzing hydrolysis of endogenous peptides activate EOS and other peptidergic systems. Behavioral tests showed that synthetic δ-opioid receptor agonist dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg) possesses anxiolytic activity [2]. Synthetic heptapeptide Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) attenuates symptoms of anxiety [5], inhibits enkephalin-degrading enzymes, and, therefore, prolongs opioid lifetime [3].

The anxiolytic effects of dalargin and Selank depend on the phenotype of emotional and stress reactions in animals [2,5]. Selank modulates behavioral manifestations of anxiety in BALB/c mice displaying low activity in the open-field test, but has no effect on highly active C57Bl/6 mice [5].

The sensitivity of EOS to the drug depends on the state of receptors and systems of enzymatic hydrolysis. Here we studied the mechanisms of variability in anxiolytic activity of Selank. To this end we compared locomotor activity and total plasma enkephalinase activity in BALB/c and C57Bl/6 mice at rest and under conditions of unavoidable stress (open-field testing) and evaluated the effects of Selank on behavioral reactions and plasma enkephalinase activities in these mice under stress conditions.

MATERIALS AND METHODS

Experiments were performed on male BALB/c and C57Bl/6 mice (35 animals of each strain) weighing 21±2 g (Kryukovo nursery). The animals were kept under standard vivarium conditions: 20-22°C, 12-h light/dark schedule (light: 7.00-19.00), and *ad libitum* food and water supply.

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0007-4888/02/1332-0133$27.00

Locomotor activity at rest was evaluated using an automatic Rat-O-Matic system (Adea). This system allows recording behavioral reactions of animals in its habitual environment. The system represents an area similar to the standard MAK-III cage (Orion Farmaceutica) divided into 20 squares (7.5×6.0 cm). Ambulation and rearing were evaluated over 3 min.

Locomotor activity in the open-field test was estimated by the number of crossed squares [4]. The animals were intraperitoneally injected with 100 μg/kg Selank in 0.2 ml physiological saline. Control mice received an equivalent volume of physiological saline. Experiments were performed in summer at 9.30-13.30. The mice were decapitated immediately after behavioral tests. Heparinized plasma was frozen.

Plasma enkephalinase activity was estimated by accumulation of ^{3}H-leu-enkephalin degradation products [6]. Leu-enkephalin products were fractionated by thin-layer chromatography on silica gel plates (Silufol) in a 2-butanone:tret-butanol:ammonia:water system (ratio 2:2.4:1:1). Rf of leu-enkephalin, tyrosine, and other hydrolytic products were 0.8, 0.6, and 0.2-0.4, respectively. Spots corresponding to markers were cut and their radioactivity was measured on a MiniBeta liquid scintillation counter (LKB).

The results were analyzed by Student's *t* test.

RESULTS

Locomotor activity at rest was similar in BALB/c and C57Bl/6 mice (Rat-O-Matic): BALB/c mice demonstrated higher ambulation (by 20%) compared to C57Bl/6 mice (70.8±1.8 *vs.* 54.0±3.6, respectively, $p<0.01$), while rearing was similar in both strains (24.1±4.2 and 26.4±3.0, respectively). In the open-field test locomotor activity of C57Bl/6 mice was an order of magnitude higher compared to BALB/c mice (Table 1). Thus, significant differences in locomotor activity between these strains were found only under stress conditions.

We compared distances passed by animals in the open field and in Rat-O-Matic system. C57Bl/6 and BALB/c mice passed 3.8 and 4.9 m in the Rat-O-Matic system over 3 min, respectively. In the open field these animals passed 20 and 1.7 m, respectively. The number of rearing postures in the open field test decreased, especially, in BALB/c mice. Hence, open field testing induced freezing behavior in BALB/c mice which attests to their increased anxiety [5].

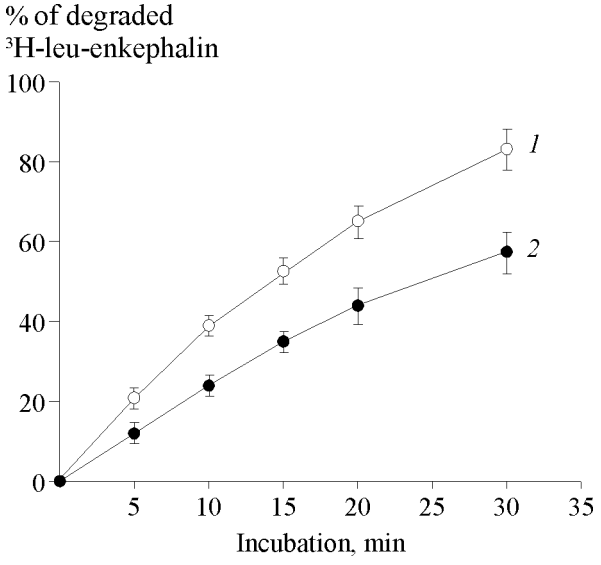

Fig. 1. Degradation of ^{3}H-leu-enkephalin in the plasma of intact BALB/c (*1*) and C57Bl/6 mice (*2*).

Changes in locomotor activity of C57Bl/6 mice in the open-field test were more pronounced (Table 1), which indicates that these animals demonstrated strong emotional reactions to stress. However, this stress reaction is not considered as a behavioral manifestation of anxiety [5]. These data show that the phenotype of emotional and stress reactions in C57Bl/6 and BALB/c mice is characterized by quantitative, but not qualitative differences.

Hydrolysis of ^{3}H-leu-enkephalin by plasma enzymes in BALB/c and C57Bl/6 mice was described by kinetic curves with a linear region, which lasted at least 15 min (Fig. 1). The rates of plasma leu-enkephalin hydrolysis in BALB/c and C57Bl/6 mice

TABLE 1. Effects of Selank on Behavioral Reactions of C57Bl/6 and BALB/c Mice in the Open Field Test (*M*±*m*)

Locomotor activity	C57Bl/6		BALB/c	
	control (*n*=10)	Selank (*n*=15)	control (*n*=10)	Selank (*n*=15)
Peripheral	167.5±15.0	206.1±13.4	14.0±5.6	40.1±10.1
Central	27.8±6.4	29.3±4.1	2.8±1.4	9.4±5.6
Vertical	17.0±3.1	12.8±2.7	0.0±0.0	0.1±0.1
Total	195.2±18.4	235.4±15.3	16.8±5.5	49.5±10.2*

Note. *$p<0.05$ compared to the control.

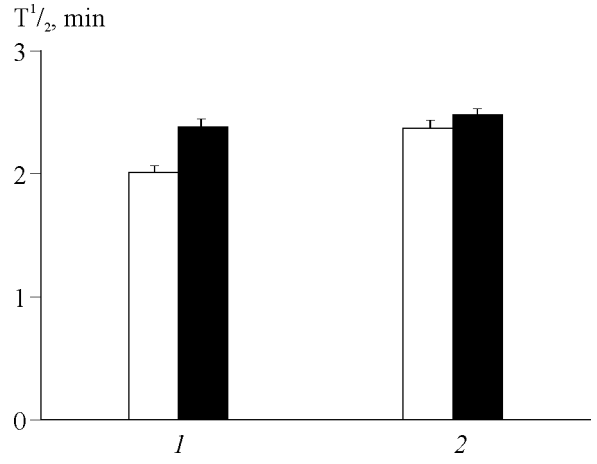

Fig. 2. Effects of Selank on plasma enkephalinase activities (leu-enkephalin half-life, $T^1/_2$) in BALB/c (*1*) and C57Bl/6 mice (*2*) after open-field testing. Dark bars: Selank. *$p<0.05$ compared to the control (light bars).

were 50±6 and 30±7 nM/min, respectively. These results show that the half-life of blood leu-enkephalin in intact BALB/c mice with pronounced behavioral manifestations of anxiety was lower than in C57Bl/6 mice (1.94±0.05 and 2.23±0.07 min, respectively, $p<0.05$).

These differences were also observed during stress. Leu-enkephalin half-life in control BALB/c mice was shorter than in C57Bl/6 mice (Fig. 2).

These differences were not manifested in behavioral reactions at rest. However, during stress accompanied by intensive release of opioids from the pituitary and adrenals rapid degradation of peptides in intact BALB/c mice weakens stress-limiting activity of EOS. Published data show that opioids enhance animal locomotor activity [9], which probably contributes to different behavioral reactions of BALB/c and C57Bl/6 mice in the open-field test (stress). High locomotor activity of C57Bl/6 mice in the open field was associated with slow degradation of endogenous opioids secreted under stress conditions. Rapid hydrolysis of opioid peptides probably determined freezing behavior of BALB/c mice (Table 1).

We hypothesized that preparations inhibiting enkephalin-degrading enzymes produce a selective anxiolytic effect on BALB/c mice. To test this hypothesis we compared the influence of Selank on behavioral reactions and enkephalinase activities in BALB/c and C57Bl/6 mice. Selank 3-fold increased locomotor activity of BALB/c mice in the open-field test, but had no effect on C57Bl/6 mice (Table 1). This is consistent with previous studies performed at the Institute of Pharmacology, Russian Academy of Medical Sciences [5].

These results show that Selank produces the anxiolytic effect on BALB/c mice with high enkephalinase activity and markedly increases the half-life of plasma enkephalin in these animals. However, the preparation has no effect on the behavior and plasma enkephalinase activity in C57Bl/6 mice (Fig. 2).

Published data show that anxiety disorders in humans are accompanied by a deficiency in endogenous enkephalinase inhibitors [1]. Selank inhibits enkephalinases [3], which suggests that the anxiolytic effect of this preparation in BALB/c mice is realized by the mechanism of substitution therapy. Selank activates EOS by prolonging enkephalin half-life against the background endogenous enkephalinase inhibitor deficiency. However, the preparation has no effect on C57Bl/6 mice with normal content of endogenous enkephalinase inhibitors. In animals with this type of emotional and stress reactions correction of the stress-induced changes can be attained via different mechanisms.

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