From the data obtained the conclusion may be drawn that NaOCl induces lipid peroxidation in LDL, which leads to disturbances in the structure of the surface proteolipid layer of the particles. This manifests itself, on the one hand, as restricted mobility (an increase of parameters S and τ), and, on the other, as an increased polarity (drop of parameter h) of the microenvironment of fatty-acid chains of phospholipids as deep as the 12th CH_2 group (approximately 1.7 nm from the lipid-water interface). Marked changes in deeper regions (16th CH_2 group) become detectable only at concentrations of NaOCl exceeding 1 mM. It should be noted that earlier we observed similar qualitative changes in the mobility and polarity of the phospholipid acyl chains depending on the degree of LDL autooxidation [5,13,14].

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Behavioral Effects of β-Casomorphin-7 and Its Des-Tyr-Analogs

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Early investigations of the effects of opioid peptides on behavior and the exploratory reaction demonstrated that intracerebral administration of morphine, endorphins, and Met-enkephalin causes muscular rigidity and immmobility in experimental animals [4,16]. Further studies revealed a more complex character of opioid influence. Depending on the type of peptide, the mode of its administration into the organism, the species of experimental animal, and the peptide dose, it is possible to register an entire spectrum of effects, ranging from significant motor excitation to a complete inhibition of locomotor activity and catotonia [14].

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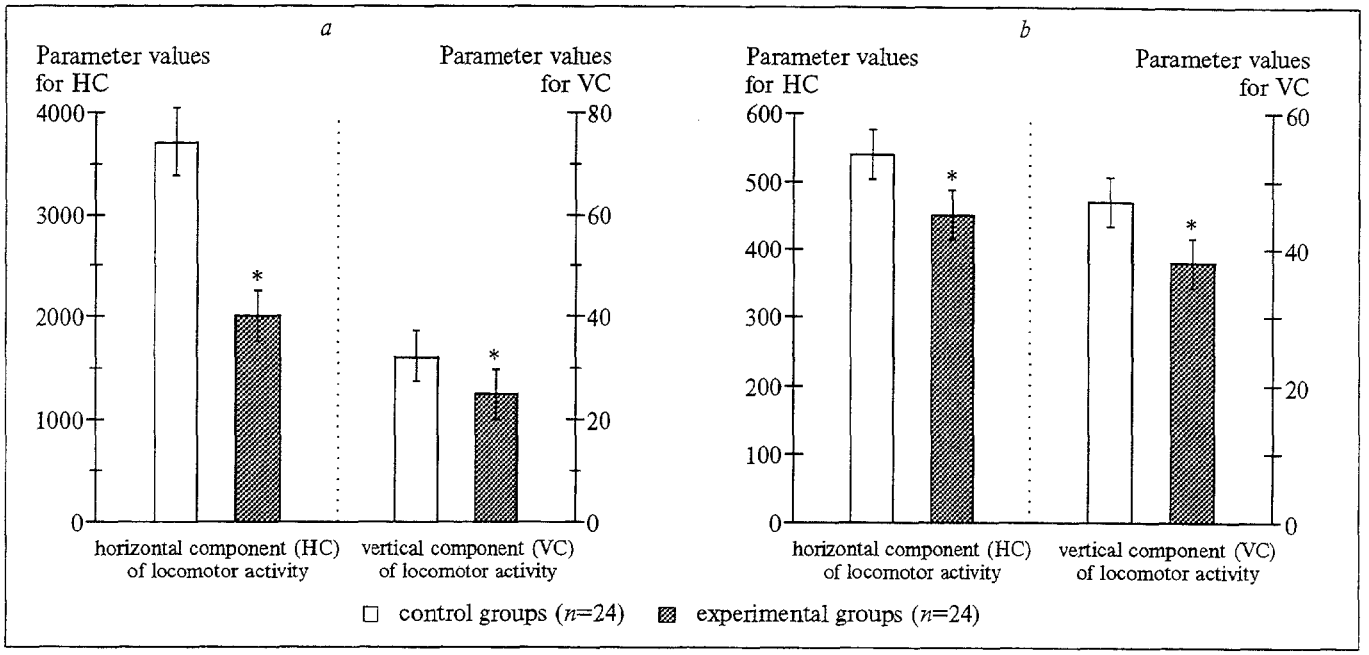

Fig. 1. Effect of β-K-7 (intraperitoneal administration; 20 mg/kg) on locomotor activity of experimental animals in Opto-Varimex (*a*) and RODEO-2 (*b*) tests. Designations: here and in Fig. 2, Asterisk: significant differences between experimental and control groups ($p<0.05$).

Meanwhile, elucidation of the peculiarities of opioid effects on spontaneous behavior and the exploratory reaction is a necessary stage in the investigation of the physiological effects of any newly discovered or synthesized opioid peptide.

The first data regarding casomorphins, opioids of alimentary origin, were published more than 10 years ago. Casomorphins are of major importance in the early ontogenesis of mammals. The most typical representative of this family is the heptapeptide Tyr-Pro-Phe-Pro-Gly-Pro-Ile (known as beta-casomorphin-7, or β-K-7) corresponding to the 60th-66th fragment of β-casein [6]. Although more than 20 natural derivatives and synthetic analogs of β-casomorphins are now known, their physiological effects are poorly understood. It is noteworthy that β-K-7 is characterized by a high affinity to opioid receptors of the μ type and a somewhat lower affinity to receptors of the Δ- and κ- types [7]. It is probable that interaction with opioid receptors accounts for the effects of the peptide and its derivatives on nociception and other functions of the organism, including behavior. The present study was aimed at investigating the effects of β-K-7 and its des-Tyr-analogs on locomotor activity and the exploratory reaction under different experimental conditions.

MATERIALS AND METHODS

β-K-7 and its des-Tyr-analogs β-K-6 (Pro-Phe-Pro-Gly-Pro-Ile) and β-K-4 (Phe-Pro-Gly-Pro) were synthesized in the Laboratory of Regulatory Peptides, Institute of Molecular Genetics, Russian Academy of Sciences. The experiments were performed on 639 male nonpedigree albino rats weighing 150-250 g. Aqueous peptide solutions were administered intraperitoneally in a volume of 0.2 ml. Distilled water was injected into the control animals. The testing was carried out 5 min after peptide administration.

Locomotor activity of rats during spontaneous behavior was registered using Opto-Varimex (Columbus Instruments, USA) and RODEO-2 (Russian Academy of Medical Sciences) apparatus, which make it possible to record separately the horizontal (HC) and vertical (VC) components of locomotor activity and, in the latter case, the number of holes in the chamber floor nose-poked by the animals (NH). Chamber size was 40×40×25 and 47×47×27 cm, respectively, NH=16. The recordings were performed in silence and darkness during 15 min. Parameters were evaluated in arbitrary units.

In addition, the animals were tested in the "open field" (in a round arena with a diameter of 80 cm). A rat was placed in the center of the arena and HC, VC, the grooming reaction, and the number of boluses were assessed during 2 min. A 500 W lamp, an electrical bell, and a red lamp (15 W) were fixed at a height of 80 cm above the arena. Two modifications of the experiment were created: under "stress-free" conditions (i.e., in silence and under red illumination) and "stress" conditions (with both lamps and the bell switched on).

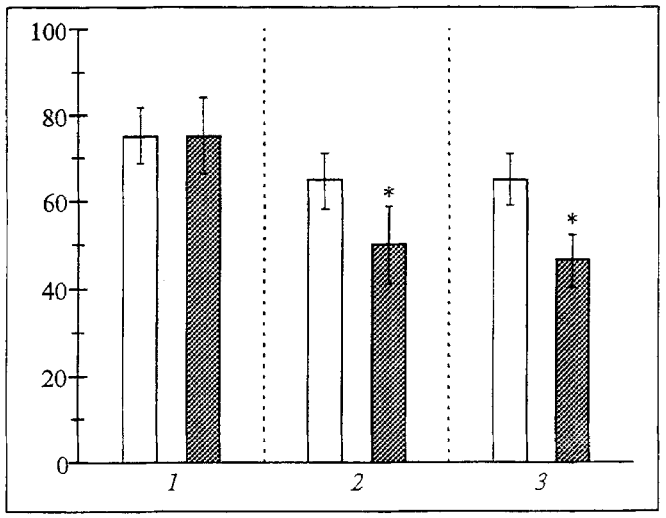

Fig. 2. Effect of β-K-7 (*1*) and its des-Tyr-analogs (β-K-6 (*2*) and β-K-4) (*3*) on number of holes in chamber floor nose-poked by animals in RODEO-2 test (intraperitoneal administration; 20 mg/kg).

The data obtained were processed using ANOVA methods of statistical analysis (STATGRAF software).

RESULTS

Administration of β-K-7 in doses of 1 and 5 mg/kg did not affect spontaneous behavior of the experimental animals in any of the tests used. A decrease in HC value to 78% compared to the control ($p<0.05$) and of VC to 81.1% ($p=0.11$) was recorded in the Opto-Varimex test after administration of β-K-7 in a dose of 20 mg/kg (Fig. 1, *a*). The differences were most significant 6-10 min after the beginning of recording (50.8% for HC and 51.6% for VC). During the 1st-5th and 11th-15th min of the experiment reliable differences between the experimental and control animals were not detected.

In the RODEO-2 test β-K-7 in a dose of 20 mg/kg induced a similar decrease in HC and VC values to 83.8 and 77.8% compared to the control, respectively (Fig. 1, *b*). The most significant differences were also observed during the 6th-10th min of recording (74.3 and 65.8% for HC and VC, respectively). However, we did not detect any reliable differences in NH between the experimental and control groups (Fig. 2).

As for des-Tyr-analogs of β-K-7, the character of their influence on locomotion was similar to the prototype effects. Administration of the substances in doses of 1 and 5 mg/kg did not affect the locomotor activity of the rats (RODEO-2 test), while a dose of 20 mg/kg led to a certain decrease in its value. In the case of β-K-6 this decrease was significant in the 6th-10th min of recording and constituted 60.4% compared to the control, while in the case of β-K-4 it was significant in the 11th-15th min and constituted 59.6%.

In contrast to β-K-7, administration of β-K-6 and β-K-4 in a dose of 20 mg/kg led to a reliable decrease in NH to 76.0 and 73.7%, respectively, compared to the control (Fig. 2). These

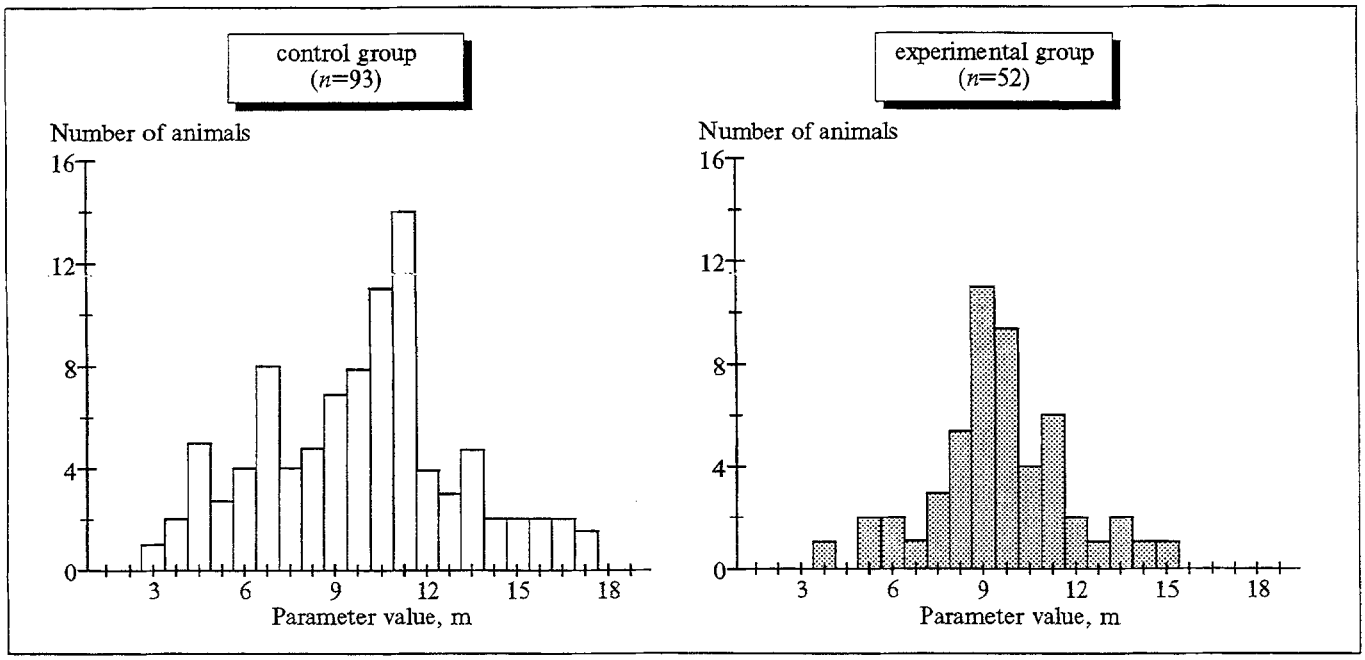

Fig. 3. Effect of β-K-7 (intraperitoneal administration; 1 mg/kg) on distribution of HC values in the "open field" test under stress conditions.

differences were significant in the 1st-5th, 6th-10th, and 11th-15th min of recording.

In the "open field" test (1 and 5 mg/kg β-K-7) under stress-free conditions a decrease in the number of boluses (40-60% compared to the control) was the only significant effect of peptide administration. Under stress conditions, for a common constant mathematical average value of the HC parameter, β-K-7 caused a reduction of its standard square deviation to 69.6% (1 mg/kg, $p<0.01$ according to the Fisher test, Fig. 3) and to 81.3% (5 mg/kg, $p<0.05$) in comparison with the control. In other words, in the experimental groups there were fewer animals which reacted by freezing or nonstop running to the switching on of the stress agents (especially in the 1st min). A decrease in the number of boluses was also observed but did not reach a significant level.

A large body of experimental data has now been accumulated on the behavioral effects of opioid peptides. For instance, intraventricular administration of β-endorphin induces a prolonged psychomotor excitation accompanied by hyperactivity, head twitching, and tremor in cats [15]. Injection of μ- and Δ-opioid agonists produces either a stimulating or an inhibitory effect on locomotion depending on the doses used [12]. Intraventricular injection of D-Ala-D-Leu-enkephalin produces a biphasic effect on locomotor activity, first decreasing and then increasing it [17]. For the systemic mode of administration the effect of μ-opioid agonists is also dose-dependent: low doses of morphine (1-2 mg/kg) induce hyperactivity [10], whereas high doses 20 mg/kg) reduce motor activity to the point of catalepsy [5]. Dermorphin in a wide dose range (0.5-5.0 mg/kg) also decreases motor activity of experimental animals [2]. Selective κ-agonists (U50488H, PD117302) lead to a suppression of motor activity only in high doses; in low doses (0.1 mg/kg) they stimulate grooming, reduce VC, and do not affect HC of locomotor activity [11].

The results obtained support the idea of a predominantly suppressive effect of high doses of opioids on locomotion and the cataleptic effects of β-K-7 derivatives [13]. The mechanisms of these effects are most probably connected with the ability of β-K-7 to interact with peripheral opioid receptors [7]. In additional experiments a specific antagonist of μ receptors, naloxone (1 mg/kg) was administered 15 min prior to injection of the peptide (or water in the control). It turned out that in the groups with combined administration of naloxone and β-K-7 the decrease in HC and VC values was more pronounced than in the groups with administration of either β-K-7 or naloxone only (RODEO-2). This potentiating effect was significant for β-K-7 doses of 20 and 5 mg/kg. It is reasonable to assume that the locomotor effects of β-K-7 are mediated, at least partly, by opioid receptors different from receptors of the μ type. Under normal conditions, the population of μ opioid receptors may carry out a sort of buffer function and prevent the lessening locomotor activity.

The absence of N-terminal tyrosine in β-K-6 and β-K-4 and, as a result, a reduced affinity to opioid receptors may probably account for their more feeble action on spontaneous locomotor activity in comparison with the prototype. At the same time, the selective effect of these peptides on the number of holes nose-poked by the animals together with the data of the antidepressive activity of des-Tyr-analogs of β-K-7 [9] allow us to postulate multiple pathways of the development of the peptide effects.

The decrease in the number of boluses in the "open field" test is probably connected with a direct influence of β-K-7 on μ receptors of the intestine [8]. In our opinion, the smaller scatter in horizontal activity values under stress conditions is a consequence of the antistress effect of the peptide. Similar effects revealed for some other opioids are a consequence of their inhibitory action on different transmitter systems of the brain [1]. It is noteworthy that the antistress activity of β-K-7 manifests itself in doses lower than those required in order to affect spontaneous behavior and pain sensitivity [3]. It seems likely that β-K-7 and its derivatives in these doses can display a number of other neurotropic effects.

Thus, in our opinion, the opioid peptide β-K-7, of interest as an "alimentary" opioid, should be further investigated in view of its possible therapeutic applications as an antistress agent.

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Effect of Enkephalins on the Hydroionic Exchange in Rat Ontogenesis

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The accumulated data on the diverse effects of opioid peptides on the functioning of many, if not all, organs and systems of the human and animal organism [14] underscore the need to study their influence on one of the most important systems involved in the maintenance of homeostasis, namely the regulatory system of hydroionic balance, with the kidney being the central organ in this system. Taking into account the age-dependent changes occurring in this system [1,2,4-7,10], we thought it interesting to compare the effect of opioid peptides on hydroionic exchange in different age groups of animals. Since the specific share of enkephalins and endorphins manifesting their modulating effect on the organism's functions [13] increases under stress conditions [3,9,12-14], it is reasonable to assume that under conditions of hyperhydration, administration of enkephalins will promote a more rational, economical, and optimal return of the organism to the initial state of hydroionic equilibrium.

The objective of the present study was to study renal function in rats of three age groups under the action of an arginine-containing synthetic analog of Leu-enkephalin (SAE).

MATERIALS AND METHODS

The experiments were performed on albino Wistar rats of three age groups: group 1) 25-30-day-old rats (n=102); group 2) 45-50-day-old rats (n=62), and group 3) adult rats (older than 180 days, n=115). After preliminary weighing, the experimental animals were placed in special cages for 1-2 h for the collection of background samples of urine. Subsequently, their stomachs were loaded with water (5 ml/100 g body weight) via a polyethylene probe. At the same time, the animals received an intraperitoneal injection of a synthetic analog of Leu-enkephalin in a dose of 100 μg/kg and a volume of 0.5 ml/100 g. Samples of urine and plasma were collected 1, 3, and 6 h after

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