EFFECT OF REPEATED COLD SWIM STRESS ON NOCICEPTIVE RESPONSES AND BINDING OF ^{3}H-NALOXONE TO HYPOTHALAMIC AND MIDBRAIN OPIATE RECEPTORS IN RATS

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Several investigations have shown that swimming, like other forms of stress, induces the development of antinociceptive responses in laboratory animals [1-3, 5, 10]. Indirect pharmacological and physiological investigations using opioid antagonists and agonists have shown that this type of stress-induced analgesia is largely due to activation of the opiatergic systems of the body [4-6]. It has been shown [7, 8] that analgesia as a result of a single episode of swimming by animals is accompanied by a change in binding of opioid substances with the brain opiate receptors (OR). The possibility cannot be ruled out that during chronic exposure, activation of opioid systems will also be observed in brain centers responsible for nociceptive information processing, which include in the first place structures of the midbrain and hypothalamus.

The aim of the present investigation was to study correlation between the state of nociceptive sensitivity in rats during chronic (repeated) swimming in cold water and the character of changes in activity of opiate μ-receptors in the midbrain and hypothalamus.

EXPERIMENTAL METHOD

Experiments were carried out on 83 albino rats weighing 200-220 g. Daily for 3 min the animals swam in cold (4°C) water. The control rats were not subjected to any procedure and were kept under the same conditions as the experimental rats. On the 1st, 3rd, 5th, and 15th days before swimming, the nociceptive sensitivity of the animals was estimated according to the duration of the latent periods (LP) of the tail withdrawal reflex (TWR) and the paw licking reflex (PLR), by the method described previously [1, 2]. The animals (n = 40) were decapitated 3, 5, and 15 days later and the midbrain and hypothalamus were isolated and used to determine parameters of receptor binding: the dissociation constant K_d and the number of binding sites (B_{max}) with ^{3}H-naloxone. The source of receptors was the coarse synaptosomal fraction obtained by homogenization of brain tissue followed by centrifugation of the cellular homogenate.

For this purpose the isolated brain structures (separately from each animal) were subjected successively to homogenization (by means of a mechanical tissue disintegrator, West Germany), washing twice in 50 volumes of cold 0.05 M Tris-HCl buffer, pH 7.4, and incubation for 40 min at 37°C in the same buffer (centrifugation in all manipulations at 14,000g for 15-20 min at 2°C). The residue obtained as a result of the final centrifugation (a preparation of synaptic membranes) was resuspended in working buffer at the rate of 5 mg tissue to 1 ml buffer. The incubation samples for determination of specific binding in a total volume of 1 ml contained 100 μl of the membrane preparation (0.16-0.3 mg protein/ml, determined by the method in [9]) and 0.25-10 nM ^{3}H-naloxone ("Amersham," England; specific radioactivity 58-60 Ci/mmole) in 0.05 M Tris-HCl buffer, pH 7.4, with 0.1 M NaCl. Samples for determination of nonspecific binding contained, besides the above-mentioned components, 5 μM of nonradioactive naloxone. Incubation proceeded for 60 min at 22°C. The unbound ligand was separated by filtration of the samples through Whatman GF/B filters and washing with cold working buffer. The radioactivity of the samples was determined on a RackBeta liquid scintillation counter ("LKB-Wallac," Finland). All samples were set up in two

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TABLE 1. Changes (in %) in Kinetic Parameters of Binding of ^{3}H-Naloxone with OR of Midbrain and Hypothalamus of Rats After Swimming in Cold Water Compared with Control ($B_{max} = 10^{-15}$ mole/kg protein; $K_d = 10^{-9}$ M; $M \pm m$)

Days	Midbrain		Hypothalamus			
	B_{max}	K_d	B'_{max}	K'_d	B''_{max}	K''_d
Control	266,3±22,9	1,09±0,2	132,1±17,5	0,49±0,19	283,9±51,3	2,48±0,61
3-rd	38,6±11,3	62,0±11,5	2,96±15,3	—25,8±31,8	4,3±11,1	—3,6±27,7
5-th	—7,5±9,0	—20,3±2,5*	63,4±31,6	46,7±37,2	34,2±32,8	77,3±29,2
p_{3-5}	<0,01	<0,001	<0,05	—	—	<0,05
15-th	—8,8±2,5*	—15,2±1,6*	—24,5±8,7*	—48,7±24,5	—7,2±13,2	—27,6±6,2*
p_{5-15}	—	—	<0,05	<0,05	—	<0,05
p_{3-5}	<0,01	<0,001	—	—	—	—

Legend. Significant ($p < 0.05$) differences compared with control (Student's test); indices denote significant differences between values of parameters at different times of experiment (Duncan's test).

or three repetitions and the standard error of counting did not exceed 5%. After analysis the results were presented in the form of Scatchard plots: dependence of B/F on B, where B stands for the quantity of bound ligand (in fmoles/mg protein) and F for the concentration of free ligand (in nM). The linear regression method was used to determine K_d and B_{max}.

The results of the biochemical and physiological experiments were subjected to statistical analysis by Student's, Dunnett's, and Duncan's tests.

EXPERIMENTAL RESULTS

The results described reflect changes in the background LP (measured immediately before a routine swimming session), TWR, and PLR of the experimental animals. Initial values of both parameters were identical in the control and experimental groups, namely: LP of PLR 14.6 ± 0.5 sec, LP of TWR 2.8 ± 0.1 sec. On measurement of LP of PLR in rats of the control group, a decrease in its value was observed toward the end of the 3rd, 5th, and 15th days of the experiment: to 14.4 ± 0.8, 10.9 ± 1.2 ($p < 0.05$), and 11.4 ± 0.9 sec respectively. Changes of a similar type were found in the values of nociceptive sensitivity for LP of TWR also. For instance, after 3 days of the experiment LP of TWR fell to 2.6 ± 0.1, after 5 days to 1.8 ± 0.2 ($p < 0.05$), and after 15 days to 2.0 ± 0.2 sec ($p < 0.05$). In the experimental group of animals, on the other hand, an increase was observed in LP of PLR and TWR compared with their initial values. By the end of 3, 5, and 15 days LP of PLR was increased significantly ($p < 0.05$) to 24.6 ± 1.4, 20.0 ± 2.4, and 21.1 ± 1.6 sec, whereas LP of TWR was increased to 3.6 ± 0.2, 3.1 ± 0.1, and 4.0 ± 0.1 sec respectively. Comparison of LP of the control rats showed that LP of PLR and TWR of the swimming rats was significantly higher at all times of the experiment. Thus repeated swimming in cold water by rats led to an increase in LP of PLR and TWR compared with both the initial and the control values. This state of nociceptive sensitivity in rats can be interpreted as evidence of increased tone of the antinociceptive systems during frequent swimming in cold water.

Percentage changes in the parameters of binding of ^{3}H-naloxone to OR in the midbrain and hypothalamus of the experimental rats compared with the controls at different stages of the experiment are given in Table 1.

Analysis of the Scatchard plots shows that ^{3}H-naloxone binding sites of one type are present in tissues of the midbrain, and were characterized in the control animals by the following values of the parameters: $B_{max} = (266.3 \pm 22.9) \cdot 10^{-15}$ mole/mg protein, and $K_d = (1.09 \pm 0.2) \cdot 10^{-9}$ M. After three swimming sessions B_{max} was increased, but not significantly, by 38%, after five sessions it was 7.5% lower, and after 15 sessions it was significantly reduced by 8.8% ($p < 0.05$) compared with the control values. Statistical analysis by Duncan's test showed that significant differences were present in the values of B_{max} between different times of the experiment: between the 3rd and 5th ($p < 0.01$) and between the 3rd and 15th ($p < 0.01$) sessions. Changes of a qualitatively similar character were found also for K_d. For instance, after 3 days of swimming K_d, rose by more than 1.5 times although not significantly, whereas after the 5th and 15th days, it was significantly lowered by 20% ($p < 0.02$) and 15% ($p < 0.02$) respectively compared with the control. Incidentally, values of K_d of midbrain receptors in the rats were significantly lower after 5 ($p < 0.001$) and 15 ($p < 0.001$) sessions compared with the 3rd day of the experiment. In the hypothalamic tissues ^{3}H-naloxone had two types of binding sites: with high and low affinity, with binding parameters in the control rats of: $B'_{max} = (132.1 \pm 17.5) \cdot 10^{-15}$ mole/mg protein; $K'_d = (0.49 \pm 0.19) \cdot 10^{-9}$ M and $B''_{max} = 283.9 \pm 51.3) \cdot 10^{-51}$ mole/mg protein; $K''_d = (2.48 \pm 0.61) \cdot 10^{-9}$ M respectively.

The study of the parameters of ^{3}H-naloxone binding with hypothalamic receptors in rats of the experimental group gave the following results (Table 1). After 3 days of swimming the number of high-affinity binding sites was virtually unchanged. After five sessions this parameter was increased (not significantly) by about 1.5 times, but by the end of the experiment it was significantly reduced by 24.5% ($p < 0.05$) compared with the control. Comparative analysis at different times revealed a significant ($p < 0.05$) increase in the values of B'_{max} in the rats after five sessions of swimming compared with the rest. Evaluation of K'_d showed a decrease (not significant) after three and 15 sessions (by 25.8 and 48.7% respectively) and an increase (by 46.7%) after the 5th day. However, comparison of K'_d at different times of the experiment revealed significant tendencies for K'_d to differ between the 5th and 15th sessions ($p < 0.05$). So far as the number of low-affinity binding sites is concerned, changes in this parameter were qualitatively similar but were less marked. For instance, B''_{max} rose by 4.3% after 3 days and by 34.2% after the 5th day, whereas after 15 days it fell by 7.2%. The differences in the fluctuations between different times in this case were not significant. Changes in K''_d were more marked. After the 3rd and 15th swimming sessions K''_{max} showed a decrease of 3.6 and 27.6% respectively ($p < 0.05$), whereas after 5 days it rose sharply, and was almost doubled ($p < 0.05$). Under these circumstances the value obtained after five swimming sessions was significantly ($p < 0.05$) higher than at other times of the experiment.

The changes in the two kinetic parameters of ^{3}H-naloxone binding by synaptosomal fractions of tissues of the midbrain and hypothalamus described above are evidence of changes in activity of the μ-receptor apparatus of these structures under the influence of repeated swimming in cold water. It is important to note that the dynamics of changes in μ-OR activity in the midbrain and hypothalamus were not equivalent in character. This suggests that chronic swimming in cold water is accompanied by dynamic reorganization of the opioid receptor apparatus in the different brain structures. It is perfectly probable that changes in integrated behavioral manifestations, such as strengthening of the background antinociceptive responses, may be connected with changes in relative activity of opioid receptors in the midbrain and hypothalamus.

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