ETHOLOGIC ANALYSIS OF THE ACTION OF ENKEPHALINS ON INTRASPECIFIC BEHAVIOR

V. P. Poshivalov, Yu. D. Ignatov, and M. I. Titov

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The role of enkephalins and their analogs in the regulation of mechanisms of memory, analgesia, certain motor acts, and autonomic reactions has been demonstrated recently [4, 6-8]. However, the spectra of their action on intraspecific (zoosocial) behavior have not been analyzed.

The aim of the present investigation was to analyze the spectrum of action of met- and leu-enkephalins and of the tetrapeptide Tyr-D-Ala-Gly-Phe-NH_2 on various forms of intraspecific behavior of isolated aggressive mice and to compare it with the analgesic activity of these peptides. Identification and analysis of effects of this sort are important in order to understand the role of endogenous peptides and their synthetic analogs in the regulation of complex forms of behavior.

EXPERIMENTAL METHOD

Experiments were carried out on 30 male CC57W mice kept in single cages for 12 weeks. Free interaction of an aggressive isolant with a standard partner from the group was recorded by means of a special combination of Ethograph and EC 1022 computer [3]. The frequency and order of appearance of 25 different behavioral acts and postures of the isolated animal were recorded. All acts and postures were classified in motivation categories and coded on a binary code for computer analysis. The behavioral acts were subdivided into categories characterizing zoosocial behavior (intraspecific sociality), aggression, ambivalent (double motivational) behavior, defense, and individual behavior (Table 1). The degree of analgesia was estimated in points on the basis of behavioral criteria [1, 2]. Met- and leu-enkephalins and the tetrapeptide were injected intraperitoneally in a volume of 0.1 ml/10 g body weight. Met-enkephalin also was injected into the lateral ventricle in doses of 50 μg. The effects were assessed 5 and 30 min after injection of the preparations. The significance of the results was estimated by means of Wilcoxon's nonparametric criterion for conjugate sets.

EXPERIMENTAL RESULTS

Met-enkephalin in a dose of 50 mg/kg increased the likelihood of an aggressive attack on the partner 5 min after systemic injection (Table 1), reduced intraspecific sociality, potentiated ambivalent forms of behavior, and reduced the likelihood of individual forms of behavior. In the above-mentioned doses, met-enkephalin did not cause analgesia, as assessed by behavioral criteria ($n = 6$, $T^{\Delta} = 0$; $P < 0.05$). After 30 min the frequency of overt aggressive acts was close to the control values, but threatening manifestations were increased, as also was vertical motor activity (standing up on the hind limbs).

Leu-enkephalin, in a dose of 50 mg/kg, reduced intraspecific sociality after 5 min (Table 1) and potentiated aggressive behavior. Unlike met-enkephalin, leu-enkephalin induced defensive behavior in the mice, which was not observed in the control. Leu-enkephalin depressed dynamic and intensified static (sitting) forms of individual behavior. After 30 min

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TABLE 1. Changes in Averaged Probabilities of Appearance of Individual Behavioral Acts in Isolated Mice under the Influence of Met-Enkephalin (m-enk), Leu-Enkephalin (l-enk), and the Tetrapeptide (Tyr-D-Ala-Gly-Phe-NH_2)

Type of behavior	Control	m-enk, 50 mg/kg, intraperitoneally and into lateral ventricle		Control	l-enk, 50 mg/kg, intraperitoneally		Tetrapeptide analog, 25 mg/kg intraperitoneally	
		5 min	30 min		5 min	30 min	5 min	15 min
Intraspecific sociality:								
sniffing the body	0,032	0,006	0,022	0,026	0,000	0,016	0,038	0,030
sniffing the nose	0,018	0,001	0,005	0,005	0,000	0,006	0,007	0,016
sniffing the genitalia	0,018	0,013	0,049	0,035	0,002	0,006	0,038	0,016
grooming the body	0,000	0,000	0,000	0,004	0,000	0,000	0,000	0,000
Aggression:								
attack	0,209	0,338	0,258	0,175	0,390	0,221	0,035	0,026
pursuit	0,000	0,000	0,000	0,000	0,000	0,004	0,000	0,000
threatening	0,081	0,077	0,095	0,127	0,100	0,087	0,046	0,015
Ambivalent behavior:								
shaking the tail	0,050	0,113	0,073	0,041	0,028	0,033	0,035	0,008
circulation	0,007	0,013	0,005	0,000	0,000	0,000	0,003	0,000
Defense:								
standing vertically	0,000	0,000	0,000	0,000	0,004	0,009	0,003	0,003
standing sideways	0,000	0,000	0,000	0,000	0,021	0,000	0,000	0,001
Individual behavior:								
locomotion	0,139	1,101	0,117	0,166	0,203	0,058	0,148	0,217	0,120
standing up on hind limbs	0,084	0,084	0,103	0,042	0,132	0,080	0,158	0,062	0,038
sitting	0,204	0,154	0,170	0,298	0,194	0,219	0,228	0,447	0,471
cleaning	0,106	0,049	0,068	0,144	0,031	0,079	0,034	0,042	0,231

intraspecific aggression was reduced, but not below the initial levels. Leu-enkephalin, injected intraperitoneally, did not cause analgesia ($n = 6$, $T^{\Delta} = 0$; $P < 0.05$).

Met-enkephalin in a dose of 50 μg reduced the likelihood of appearance of attacking and threatening the partner 5 min after injection into the lateral ventricle (Table 1), it activated only certain forms of intraspecific sociality, such as sexual investigation of the partner and grooming the partner's body, and also increased individual motor activity of the animals (locomotion, cleaning). On intraventricular injection, met-enkephalin produced an analgesic effect, manifested as depression of highly integral components [5] of the nociceptive behavioral response (biting, locomotor excitation in response to pain).

The tetrapeptide, in a dose of 25 mg/kg, on systemic injection reduced aggressive behavior and manifestations of threatening, reduced the frequency of ambivalent forms of behavior, but did not significantly change intraspecific sociality. The tetrapeptide depressed vertical forms of individual activity and intensified static forms of individual behavior (locomotion, standing up on the hind limbs), but sharply activated grooming of the animal's own body. The tetrapeptide depressed some behavioral manifestations of the nociceptive response, such as biting the forceps ($n = 6$, $T^{\Delta} = 0$; $P < 0.05$), but had little effect on vocalization in response to pain.

After systemic injection of met- and leu-enkephalins changes were thus found in the structure of intraspecific behavior, in the absence of analgesia, i.e., dissociation of behavioral and analgesic effects. These results are in agreement with data [7, 8] on the effect of enkephalins on some forms of learning in animals in the absence of an analgesic effect. The peptides themselves or their metabolic products evidently induce a central effect when administered by the systemic route [8]. In animals protected by analogs of the enkephalins and, in particular, Tyr-D-Ala-Gly-Phe-NH_2, the emotional effect was distinctly marked (depression of aggression) and it was accompanied by some degree of general depression of behavior. After intraventricular injection correlation was observed between the depression of intraspecific aggression and of affective behavioral responses to pain (in particular, a generalized emotional response, and biting). Depression of intraspecific aggression by met-enkephalin was associated not with general depression of dynamic forms of behavior and predominance of static, but with activation of competitive ("mixed," as understood in ethology) forms of activity, such as grooming and cleaning. The onset of "mixed" forms of activity of grooming type is characteristic not only of enkephalins, but also of morphine [2] and opiates as a whole [8], and is evidence that the animals are in a "comfortable" state. Enkephalins evidently participate in the control not only of specific nociceptive responses, but also of affective responses forming part of the structure of intraspecific (zoosocial) behavior.

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MECHANISM OF THE ANTIHYPOXIC EFFECT OF VALPROATE

R. U. Ostrovskaya

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Many GABA-ergic substances are known to increase the resistance of the organism to hypoxia. Sodium hydroxybutyrate has been studied in the greatest detail in this respect [4, 6]. The antihypoxic acitivity of fenibut* [3] also has been described; ability to increase the survival rate during exposure to hypoxia and to restore brain functions, disturbed by hypoxia, to normal are characteristic features of piracetam, a compound which, from the chemical point of view, is a cyclic derivative of GABA [5, 10]. The benzodiazepine tranquilizers, whose action in the modern view is due largely to potentiation of GABA-ergic inhibition also possess antihypoxic activity [1, 2, 13].

The object of the present investigation was to study the effect on resistance to hypoxia of the antiepileptic agent valproate, the anticonvulsant properties of which are associated by most workers with the accumulation of GABA because of inhibition of α-ketoglutarate-GABA transaminase (GABA-T) [14], and to compare the action of valproate and of some GABA derivatives or of pharmacologic agents interfering with the course of different stages of the "GABA shunt."

EXPERIMENTAL METHOD

To estimate the effect of the various substances tested the following parameters were used: the length of survival of mice exposed to hypoxic normobaric hypoxia, the concentrations of lactate and pyruvate in brain and heart tissues, the calculated value of the hypoxic lactate excess (after Huckabee [11]); the dynamics of changes in the ECG of the rats during exposure to hypoxic hypoxia. Hypoxic normobaric hypoxia was induced in mice by placing them (one at a time) in airtight containers with a capacity of 250 ml, in which the initial O_2 concentration in the inspired air was 8 vol. %. To reproduce the conditions of hypobaric hypoxic hypoxia, mice (12 animals at the same time, six control and six receiving the preparation) were placed in a pressure chamber, and raised to an "altitude" of 10,500 m at the rate of 1000 m/min (exposure 10 min, "descent" in the course of 3 min). The concentrations of lactate [7] and pyruvate [9] in the brain and heart tissues were determined in mice in four series of experiments: series I) the mice were kept under conditions of normal respiration and received no drugs, II) the mice were exposed to normobaric hypoxic hypoxia and

*β-Phenyl-γ-aminobutyric acid.

Laboratory of Pharmacology of the Nervous System, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 2, pp. 42-44, February, 1982. Original article submitted June 19, 1981.

