

CHANGES IN EMOTIONALLY DETERMINED BEHAVIOR OF RATS INDUCED BY SUBPEPTIDE FRAGMENT GLLDLK OF DIAZEPAM BINDING INHIBITOR PROTEIN

I. V. Zhdanova, R. N. Kordzadze, and Yu. G. Plyashkevich

UDC 612.821.3.06:612.822.1.015.348:/
612.822.014.467:615.214.22/.064

KEY WORDS: anxiety state, neuropeptide, DBI.

The isolation and study of endogenous regulators of emotional states is an important theoretical and practical problem. The evident functional complexity of the emotional system, and the multiplicity and diversity of feedback in it make research in this direction an attractive but difficult process.

Much progress in this direction has been achieved by Costa and co-workers, who have succeeded in isolating a protein (DBI), capable of inhibiting binding of ^3H -diazepam with brain benzodiazepine receptors, initially from the rat brain, but later from the human brain [1, 2]. It was later shown that activity of DBI and some of its peptide fragments in Vogel's test can be assessed as anxiogenic. The minimal fragment, preserving some of the physiological activity of DBI, is a hexapeptide with the structure GLLDLK [3, 4]. When injected into the third ventricle, the hexapeptide GLLDLK potentiated the investigative activity of rats in the open field test and inhibited interspecific aggression of rats induced by social isolation [5].

The aim of this investigation was to determine the spectrum of biological activity of GLLDLK on a model of complex social behavior in rats.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats and on male maze-bright Trayon rats weighing 200-250 g [8]. Investigation of behavior with a partner/victim, by the "emotional resonance" method [6] involved the choice of one of two emotionally negative competitive motivations by the animal: staying in a lit compartment (90 lx), which is ecologically not preferred by rats ("extroversive" behavior), or in the preferred dark (30 lx) compartment, but accompanied by the cry of the victim rat ("introversive" behavior). In the course of 10 daily experiments the total time spent in the dark compartment and the number of times the rat entered it in 5 min were recorded. Based on the results of behavior in this situation the animals were divided into four groups with eight in each group: group 1) marked "extroverts" with complete choice of one dominant motivation (the animals spent the whole experiment in the lit compartment); group 2) "extroverts" with incomplete choice of dominant motivation (the rats spent much time in the lit compartment) but visited the dark compartment several times in the course of the experiment); group 3) "introverts" with incomplete choice (they spent most of the time in the dark compartment but visited the lit compartment several times); group 4) "introverts" with complete choice (the rats visited the dark compartment once and spent virtually the whole time of the experiment in it). The animals were next given a suboccipital injection, under brief ether anesthesia (2 min), of 100 μg GLLDLK in 40 ml physiological saline. The behavior of the recipients was observed in an experimental chamber 2 h after the injection and thereafter daily for 5 days. Control experiments were carried out on 32 male rats, with suboccipital injection of 40 μl physiological saline.

I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad. All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. Research Center for Medical Diagnosis, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. E. Vartanyan). Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 111, No. 2, pp. 165-168, February, 1991. Original article submitted March 5, 1980.

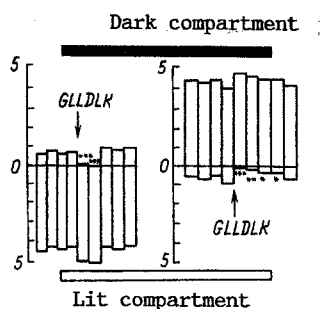


Fig. 1

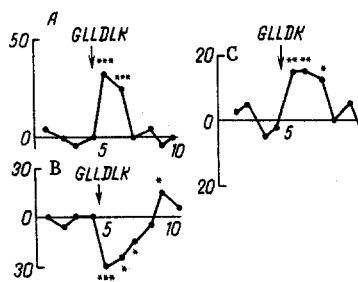


Fig. 2

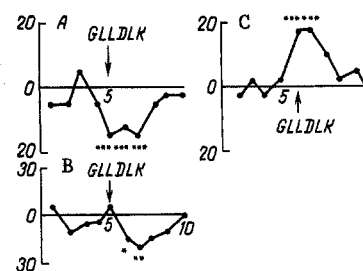


Fig. 3

Fig. 1. Changes in emotional resonance reaction in groups with incomplete choice of dominant motivation after suboccipital injection of 100 μ g GLLDLK. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 2. Changes in behavior of dominant-recipient after suboccipital injection of 100 μ g GLLDLK. A) Number of rearings during 1 h, B) number of active social contacts during 1 h, C) duration of grooming during 1 h (excess over control group). Remainder of legend as to Fig. 1.

Fig. 3. Changes in behavior of subordinate-recipient after suboccipital injection of 100 μ g GLLDLK. Legend as to Figs. 1 and 2.

The rats' behavior in the situation of social hierarchy was studied in seven groups, consisting of three males (weighing 220-250 g) and two females. The males were first kept in isolation for 2 weeks. Animals of each group were transferred simultaneously into a wire cage (550 \times 420 \times 400 mm), and were given food and water ad lib. For 3 days observations were made on the process of formation and stabilization of mutual relations in the group; aggressive, sexual, and feeding behavior, the number of rearings, the time spent on grooming, and the total number of intrasocial contacts were recorded; among the latter were distinguished active social contacts, i.e., how many times contact took place on the initiative of the given animal (sexual, aggressive, grooming of the partner, sniffing). On the 4th day, an unfamiliar mature, strange male rat was introduced into the group and behavior of members of the group in the presence of a highly meaningful social stimulus was observed. The combined results of the observations were used to determine the hierarchic ranks of the males of the community. A linear hierarchy (dominant, subordinate) and a despotic hierarchy (dominant and two subordinates) were distinguished, the dominant pursuing the other two males of the group for a long time and highly aggressively. On the 5th day, GLLDLK was injected by the method described above either into the dominant or into the subordinate in the group, and the behavioral parameters listed above were recorded for 5 days.

Control animals (seven groups) were given a suboccipital injection of 40 μ l physiological saline.

EXPERIMENTAL RESULTS

Changes in behavior were observed by the "emotional resonance" method only in rats of groups 2 and 3 with incomplete choice of dominant motivation (Fig. 1). In these animals the process of choice was complete in character. The intensity of the "emotional resonance" reaction accordingly became more complete. Animals of group 2 ceased to visit the dark compartment, whereas rats of group 3 ceased to leave the lit compartment during the experiment. The effect lasted 2 days. In groups 1 and 4 and also in the control groups no such behavioral changes were observed. Injection of GLLDLK caused no fundamental changes in the hierarchic organization of the community, by the "social hierarchy" method, but clear disturbances of the recipient's individual and social behavior were observed. Injection of GLLDLK into the dominant member of the group (Fig. 2) caused enhancement of general motor activity, the number of rearings was increased, and the duration of grooming was sharply increased. Meanwhile, the number of active social contacts decreased and sexual activity disappeared. This type of behavior was replaced by periods of an immobile state. The effect lasted 1-3 days. In two groups, in which the recipient had previously been either dominant or despotic, the change in its state led to the identification of the subdominant male, i.e., the hierarchy became linear in character. Injection of GLLDLK into the subordinate member of the group (Fig. 3) did not lead to so marked an increase in general activity, and the animals spent most of the time in the corner of the cage, without making active social

contacts, and they squeaked when other members of the group approached and made contact, and sharply increased the duration of grooming.

The results indicate modification of emotionally determined behavior in rats after injection of GLLDLK. The spectrum of these changes in a social hierarchy situation imposed a state of anxiety and depression on animals of different ranks. The polar change of the "emotional resonance" reaction in rats with an incomplete choice of dominant motivation is an interesting fact. The conditions of the investigation presupposed a negative emotional reaction to both competitive stimuli — light and a cry. Judging by the behavior of animals with an incomplete choice, one of the stimuli carried a high negative charge, and the probability of its avoidance by the given rat was higher. For animals with an "extroversive" type of behavior (group 2), the victim's cry was this stimulus, whereas for rats of group 3, with an "introversive" type of behavior, it was a bright light. The fact that animals which changed their behavior after receiving an injection of GLLDLK began persistently to avoid a previously more meaningful stimulus suggests that the dominant motivation became stronger, as a result of which the emotional response became more definite. It is not surprising that it was animals with incomplete choice between two competitive motivations that proved to be sensitive to this procedure. Several investigations [7, 8] have indicated that the low stress resistance and high behavioral lability of this group of rats. We showed previously that these animals reacted for a long time to injection of peptide fractions of the cerebrospinal fluid of patients with symptoms of manic-depression [9, 10]. Under these circumstances, however, the character of behavioral change during the first few days depended only on the affective quality of the state of the patient-donor (manic and depressive), in different groups of rats (2 and 3), each of the CSF samples caused unilateral changes of behavior, irrespective of the intensity of the "emotional resonance" reaction (the character of the change of reaction was determined by the sign of the emotional state). In this particular experimental study, the stimulus applied was uniform, but the behavioral result was polar. This suggests that the primary cause of the effect was not the character of the animal's new emotional state, but the intensity of the dominant motivation, potentiation of which led to uniformity of action.

It can thus be concluded from these results that suboccipital injection of GLLDLK leads to long-term (1-3 days after injection) modification of emotionally determined behavior of rats, consisting of the greater intensity of different patterns of behavior, and with an imposed overlay of anxiety and depression. Activity of the hexapeptide fragment GLLDLK, in the test systems used, differs from activity of peptide fractions isolated from the CSF of patients with manic-depressive symptoms.

LITERATURE CITED

1. M. G. Corda, M. Ferrari, A. Guidotti, et al., *Neurosci. Lett.*, **47**, 319 (1984).
2. P. Ferrero, E. Costa, B. Conti-Tronconi, et al., *Brain Res.*, **399**, 136 (1986).
3. P. Ferrero, M. R. Santi, B. Conti-Tronconi, et al., *Proc. Nat. Acad. Sci. USA*, **83**, 827 (1986).
4. P. Ferrero, A. Guidotti, B. Conti-Tronconi, et al., *Neuropharmacol.*, **23**, 1359 (1984).
5. G. A. Vartanyan, I. V. Zhdanova, and E. S. Petrov, *Zh. Vyssh. Nerv. Deyat.*, **36**, No. 1, 176 (1986).
6. A. V. Zukhar', Author's Abstract of Dissertation, Moscow (1985).
7. I. V. Zhdanova, *Zh. Vyssh. Nerv. Deyat.*, **35**, No. 4, 770 (1985).
8. R. N. Kordzadze, I. V. Zhdanova, Yu. G. Plyashkevich, et al., in: *Physiology of Peptides [in Russian]*, Leningrad (1988), pp. 99-100.
9. P. V. Simonov, *The Emotional Brain [in Russian]*, Nauka, Moscow (1980).
10. N. M. Khonicheva and I. Vil'yar, *Zh. Vyssh. Nerv. Deyat.*, **31**, No. 5, 975 (1981).