NEUROTROPIC EFFECTS OF A LULIBERIN (LH-RH) ANALOG IN RATS DIFFERING IN SENSITIVITY TO ETHANOL

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Considering, on the one hand, the possibility of peptidergic effects of LH-RH [8], and on the other hand, the disturbance of coordination of the hypothalamo-hypophyseo-gonadal system in alcoholism [1], it was decided to study the neurotropic effects of surfagon in rats differing in their predisposition to ethanol consumption, the study of which is important when an attempt is made to elucidate the principles governing the formation of alcohol motivation.

The aim of this investigation was to demonstrate and to define the direction of the neurotropic effects of surfagon (S), which is an analog of natural LH-RH and which, it is intended, may be used as a therapeutic preparation [7].

EXPERIMENTAL METHOD

Experiments were carried out on 240 noninbred male rats weighing 180-220 g, divided on the basis of the "side position" test into short-sleepers (SS), with a duration of sleep of 76 ± 11 min, and considered to be predisposed to ethanol consumption, and long-sleepers (LS), not so predisposed, with a duration of sleep of 198 ± 17 min [3]. Altogether 1000 rats were tested The selected animals were divided into groups with 10-12 rats in each group, and on which three series of experiments were carried out. In series I (80 rats) the effect of S was studied on the behavior of intact SS and LS rats, in series II, on the behavior of castrated rats, and in series III on the behavior of hypophysectomized rats. The S was synthesized at the Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, and it is an analog of LH-RH, highly resistant to the action of peptidases, and possessing high biological activity. The peptide was injected intraperitoneally in doses of 0.15, 5.0, and 50.0 μg/kg body weight 10-15 min before the beginning of the investigation Animals receiving equivalent doses of physiological saline (0.2 ml/100 g body weight) served as the control. Castration was undertaken under hexobarbital anesthesia, through a midline incision of the scrotum; hypophysectomy was performed through a parapharyngeal approach. The animals took part in the experiments 12 days after the operation. At the end of the experiments the completeness of hypophysectomy was verified. The rats' behavior was studied by means of a combination of tests Pain stress was created by gradually increasing (IB into IC) stimulation of pairs of rats in a chamber with an electrified floor Under these circumstances an aggressive-defensive reaction appeared and was assessed in thresholds of its successive components (quivering, vocalization, standing up, running, and fighting), and also of the number of fights (as a percentage of the number of tests). Two tests were carried out in succession,with an interval of 1 min. In all rats the following parameters also were studied: the vertical component of orienting activity (based on the number of standings per minute in a round glass jar 35 cm in diameter), emotional behavior (the number of defecations), the frequency of grooming and urination under these same conditions, and also responses of the rats (approaching, sniffing, avoidance, squeaking) in relation to an unexpectedly moving object (a stick), or to contact with or grasping by a hand. The results were subjected to statistical analysis by Student's t-test.

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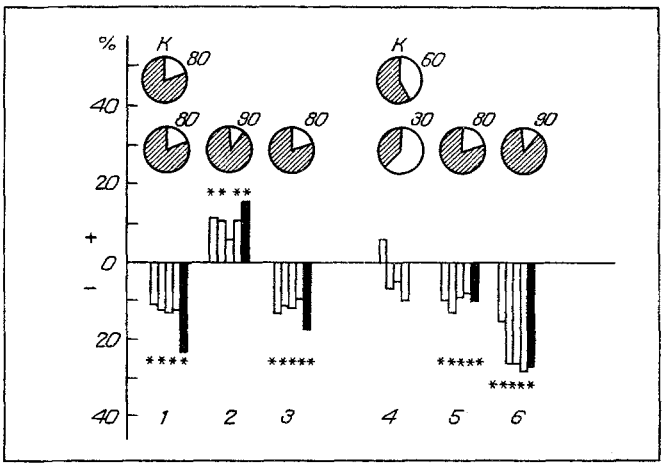

Fig. 1. Changes in parameters of aggressive-defensive response after injection of surfagon into SS (1, 2, 3) and LS (4, 5, 6) rats in doses of 0.15 (1, 4); 5.0 (2, 5), and 50 (3, 6) μg/kg body weight. Columns: change in thresholds of reactions of quivering, squeaking, standing, running (unshaded) and fighting (shaded black) in percent of their values in control animals (scale on the left). Obliquely shaded parts of diagrams and numbers relate to number of fights (as a percent of number of tests). C) Control conditions. *) Results differing significantly from controls (at $p < 0.05$-0.001 level).

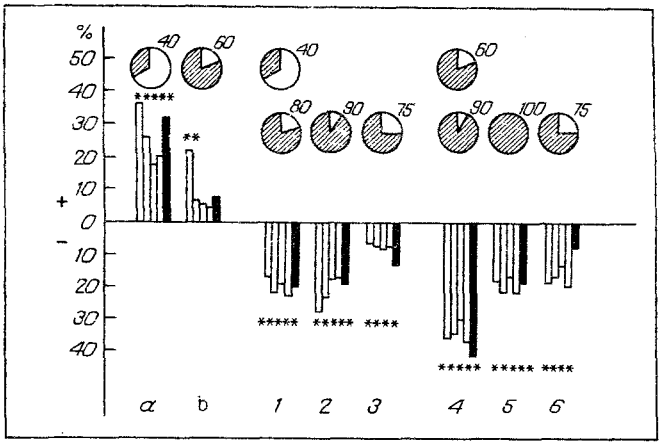

Fig. 2. Changes in parameters of aggressive-defensive behavior after castration of SS (a) and LS (b) rats and injection of surfagon into castrated SS and LS (1-6) Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

In the control, quivering of the SS rats was observed at a voltage of 20.6 ± 0.4 V, squeaking at 27.8 ± 0.3 V, standing at 33.2 ± 0.6 V, running at 42.9 ± 0.8 V, and fighting at 48.8 ± 1.0 V, the frequency of fights being 80%. In the LS rats the thresholds of these same responses were: 23.0 ± 0.5, 33.9 ± 0.7, 38 ± 0.5, 51.1 ± 1.6, and 58.8 ± 1.5 V; the frequency of fighting was 60%. Thus intact SS rats are characterized by lower thresholds of all components of aggressive-defensive behavior (at the $p < 0.1$-0.001 level) and by a greater number of fights. They are also distinguished by the greater intensity (two- to threefold) of vertical activity and grooming, and also the larger number of investigations and the smaller number of avoidances in relation to provocative activity.

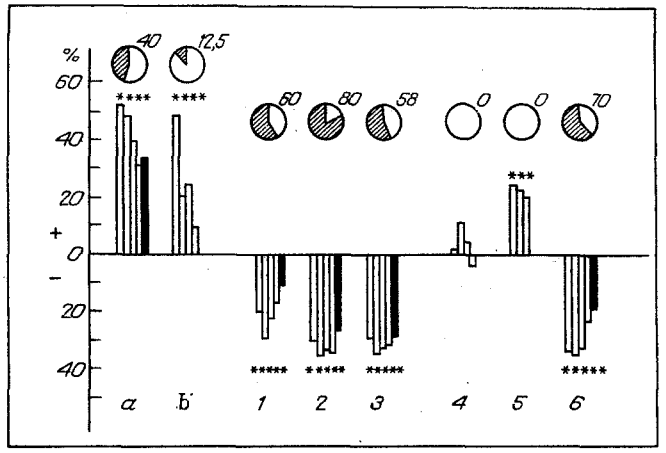

Fig. 3. Changes in parameters of aggressive-defensive behavior of rats after hypophysectomy on SS (a) and LS (b) rats and injection of surfagon into hypophysectomized SS and LS rats (1-6). Remainder of legend as to Fig. 1.

In the SS rats lowering of the thresholds of all components of aggressive-defensive behavior was observed after injection of S in doses of 0.15 and 50 μg, whereas in a dose of 5 μg the increase became statistically significant, so that the direction of the effects of the peptide is V-shaped in character (Fig. 1). However, the level of aggression remained high in all doses used. In the LS rats, against the background of the action of S lowering of the thresholds of the nociceptive and fighting reactions was observed and was most marked when the peptide was injected in a dose of 50 μg, together with an increase in the number of fights when doses of 5 and 50 μg were used.

Reduction of vertical activity (by 32-55%) and of grooming (21-62%) in the SS rats and, conversely, intensification of grooming (two- to threefold) in LS rats under the influence of S must be noted.

As will be clear from Fig. 2, castration evoked a statistically significant decline in reactivity relative to nociceptive stimulation and weakening of affective aggressiveness, more marked in the SS rats, in rats of both groups. However, the lower frequency of grooming (by 89%, $p < 0.001$) and emotional defecation (by 64%, $p < 0.05$) must be noted in the castrated SS rats compared with the LS rats.

Against the background of the action of S, a statistically significant increase in pain sensitivity and in the number of fights, was observed in the castrated rats of both groups, and, consequently, preservation of its peptidergic effects was established. Under these circumstances, it is worth noting that after injection of the peptide in doses of 0.15 and 50 μg, its effect on nociceptive sensitivity in the LS rats was more marked than in SS. The effect of S on vertical activity and grooming, however, preserved the same direction as in intact animals in both groups of rats after castration.

Hypophysectomy led to statistically significant elevation of the thresholds of all components of aggressive-defensive behavior and to a significant reduction (two- to fivefold) in the number of fights (Fig. 3); the degree of reduction of reactivity to nociceptive stimulation, moreover, was more marked in SS rats, whereas weakening of aggressiveness was more marked in LS rats. The increased predisposition to convulsions in response to electrical stimulation in hypophysectomized rats (20-25% of seizures within the voltage range used for producing electric shocks), must be emphasized. An increase in orienting activity (by 15-50%) and in the frequency of grooming also was noted.

It will be clear from Fig. 3 that the activating action of S on aggressive-defensive behavior, evoked by unavoidable nociceptive stimulation, persisted in the SS rats after hypophysectomy. An increase in the frequency of fights also was observed. In the LS rats injection of S in doses of 0.15 and 5 μg was accompanied by an inhibitory effect, with cessation of fighting, whereas in a dose of 50 μg, however, it was accompanied by activation. In both groups an increase in the frequency of convulsions was observed, especially after injection of the peptide in a dose of 50 μg.

Against the background of the action of S, a decrease in the intensity of grooming also was observed (by 20-100%), especially in the LS rats, together with a decrease in the frequency of emotional defecations (by 25-63%) and of investigative reactions in the provocation test.

As the results show, the predominant effect of systemic injection of S in intact, castrated, and hypophysectomized rats is an increase in nociceptive sensitivity and aggressiveness in response to unavoidable electrical stimulation. Since this form of behavior is considered to be motivated by fear, it can be concluded that S intensifies the emotionally negative state of animals This conclusion is supported by the intensification of emotional defecation and weakening of orienting behavior and grooming observed under the influence of the peptide. Grooming, from the etiological point of view, is interpreted as a mixed form of activity, indicative of a state of comfort of animals [5]. However, in certain cases, under conditions of high activation, grooming may be potentiated as a manifestation of frustration, and may reflect enhancement of emotionally negative reactivity [2]. It is possible that the intensification of grooming following administration of S to intact LS rats may have the same explanation.

Preservation of the activating effect of S on aggressive-defensive behavior in castrated and hypophysectomized rats with pain stress is evidence that the drug has a direct neurotropic action.

As the results showed, intact SS rats differ from LS in their higher behavioral excitability and aggressiveness under conditions of pain stress, the more marked lowering of their reactivity to nociceptive stimulation, and weakening of aggressiveness after castration and hypophysectomy, as well as certain particular features of the peptidergic effects of S.

Since no statistically significant differences in the intensity of steroid production or secretion of luteinizing hormone have been found in rats subdivided according to sensitivity to ethanol [1], it can be tentatively suggested that the difference in the magnitude of the results of the tests to which the animals were subjected was due to differences in the features of activity of the nervous centers involved in the formation of behavior. This hypothesis is confirmed by data on the activating effect of natural LH-RH on the CNS obtained by the evoked potentials method and, in particular, the hypothalamus and structures of the limbic system [7], and also data on the higher reactivity of the system for regulation of functions, established in animals sensitive to ethanol according to the level of responses of their hypothalamo-hypophyseo-adrenal and thyroid systems [4, 8, 9].

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