Neurotropic Effects of a Luliberin Analog Administered Intraventricularly to Rats with Different Sensitivities to Ethanol

L. A. Sever'yanova and I. I. Bobyntsev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, No. 2, pp. 129-132, February, 1995
Original article submitted March 14, 1994

In experiments with random-bred male rats separated into short and long sleepers according to the duration of ethanol-induced sleep (narcosis), a synthetic luliberin analog (Surfagon) administered into a brain ventricle was found to reduce pain sensitivity and affective aggressiveness in response to unavoidable painful electrostimulation, and to increase convulsive activity. Short-sleeping rats differed from long-sleeping ones in showing greater behavioral excitability and aggressiveness and in being more responsive to Surfagon, which lowered both these parameters in the former rats to a greater extent than in the latter before castration as well as after it. Mechanisms of the observed behavioral effects of Surfagon are discussed, and it is concluded that they are not mediated by sex steroids, and that the major factor in the mechanisms of its action is the accessibility of limbic structures and of the central gray substance in the midbrain.

Key Words: *luliberin; Surfagon; stress; aggressive/defensive behavior; alcohol motivation*

Our previous study [1] demonstrated direct neurotropic effects of the synthetic luliberin analog Surfagon following systemic (intraperitoneal) administration to rats differing in sensitivity to ethanol. In particular, Surfagon was shown to activate their aggressive/defensive behavior in response to unavoidable painful stimulation. In an attempt to disclose the mechanisms of these effects and to gain insight into the physiological role of luliberin, we examined in this study the neurotropic actions of the peptide Surfagon administered into a brain ventricle.

MATERIALS AND METHODS

A total of 132 random-bred male rats weighing 180-210 g were used, separated, on the basis of the "lateral position" test using ethanol, into short sleepers (mean sleep duration 71±6 min after ethanol intake) which are believed to be predisposed to ethanol consumption, and long sleepers (mean sleep duration 211±14 min) which show no predilection for alcohol [2]. With these rats, two series of tests were staged to examine the effects of ethanol on the behavior of intact (series I) and castrated (series II) short-sleeping (SS) and long-sleeping (LS) animals.

Five days prior to behavior testing, a cannula was implanted stereotaxically into the right lateral ventricle [12]. Ten minutes before the tests, Surfagon (synthesized at the Institute of Experimental Cardiology at the Cardiology Research Center of the Russian Academy of Medical Sciences) was slowly injected into the ventricle through the cannula from a microdispenser in a dose of 3 or 30 ng in 3 μl of physiological saline. After the tests, the cannula's position was checked and found to be correct.

Department of Pathological Physiology, State Medical Institute, Kursk. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0002-0120$12.50

Castration was performed under Hexenal anesthesia using a median incision through the scrotum, and the animals were tested for behavior 12 days later. Pain stress was produced by stimulating rats, housed in pairs in chambers having an electrified floor, with incremental electric current (from 1 V per second to 70 V). This stimulation gave rise to an aggressive/defensive behavior which was evaluated by recording thresholds for its sequential displays (startling, squeaking, rearing up, running, and fighting) and the incidence of fights. With each pair of rats, two tests were run in sequence at a 1-minute interval. Also evaluated were the vertical component of orienting activity (by noting the number of upright postures per min in a round glass vial 35 cm in diameter), emotionality (by noting the number of fecal pellets discharged), and grooming and urination rates. The significance of differences between the test results was evaluated by Student's test.

RESULTS

In control tests, intact SS rats were observed to startle at 20.6±0.7 V, squeak at 27.2±0.6 V, rear up on the hind legs at 31.33±0.7 V, run at 38.9± ±0.9 V, and fight at 47.7±1.3 V, the incidence of fighting being 100%. In the group of LS rats, thresholds for the indicated behavioral displays were 20.6±0.5, 31.28±1.0, 33.7±0.7, 53.1±0.56, and 61.8±0.7 V, respectively, and the incidence of fighting was 90%. Thus, the intact SS rats were characterized by lower thresholds of most of the behavioral displays under study ($p<0.05$) and, consequently, by higher behavioral excitability and aggressivity under pain stress. Their orienting activity was also less strongly marked than that of LS rats (by 51%; $p<0.05$).

Surfagon administration to SS rats in the dose of 3 ng led to 11-17% increases in thresholds for all displays of aggressive/defensive behavior ($p<0.05$) (Fig. 1), and to a 52% rise in vertical activity ($p<0.05$). After the dose of 30 ng, the incidence of fights fell sharply, while that of convulsions in response to electric current rose (to 84%).

Among the LS rats, no instances of fighting behavior were observed within the range of stimuli used, despite the absence of significant alterations in the thresholds of defensive and pain components of behavior. Convulsive activity increased, but the incidence of convulsions was lower than in the group of SS rats. Other effects of the peptide in LS rats included increased grooming rates (by 6.4-7.6 times; $p<0.01$) and decreased vertical activity (by 25-26%). Among the SS rats, such changes were only noted after the 30 ng dose.

Castration led (Fig. 2) to significant rises in thresholds of aggressive/defensive displays in both groups, but particularly in the SS rats, so that the differences observed between the two groups before castration virtually disappeared, except that vertical activity was higher in the SS group (by 29%; $p<0.05$).

Surfagon affected the aggressive/defensive behavior of castrated SS and LS rats in the same way as it did that of intact animals, but its effects were even somewhat more pronounced. The differences between castrated SS and LS rats were similar to those between their intact counterparts. Thus, the SS rats showed elevated thresholds of all aggressive/defensive displays after the lower dose of the peptide than did the LS rats. It should also

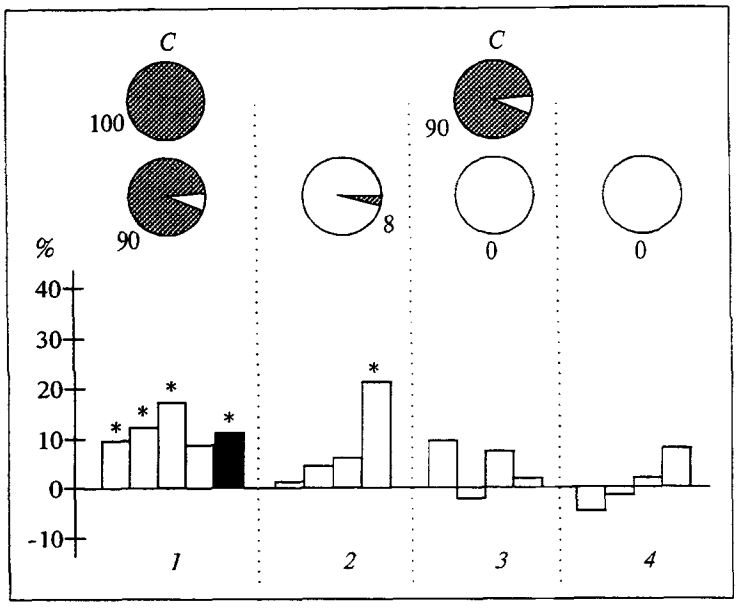

Fig. 1. Aggressive/defensive behavior of short-sleeping (*1* and *2*) and long-sleeping (*3* and *4*) rats given 3 ng (*1* and *3*) and 30 ng (*2* and *4*) of Surfagon. Here and in Fig. 2, alterations in thresholds of startling, squeaking, rearing up, and running are represented by the white bars and that of fighting, by the black bar, all expressed in percent relative to control tests (before Surfagon administration). Hatched portions of the circles and numbers indicate the number of fights, expressed in percent of the number of tests. *C*: control tests. The asterisk designates a significant difference from the control tests ($p<0.05-0.001$).

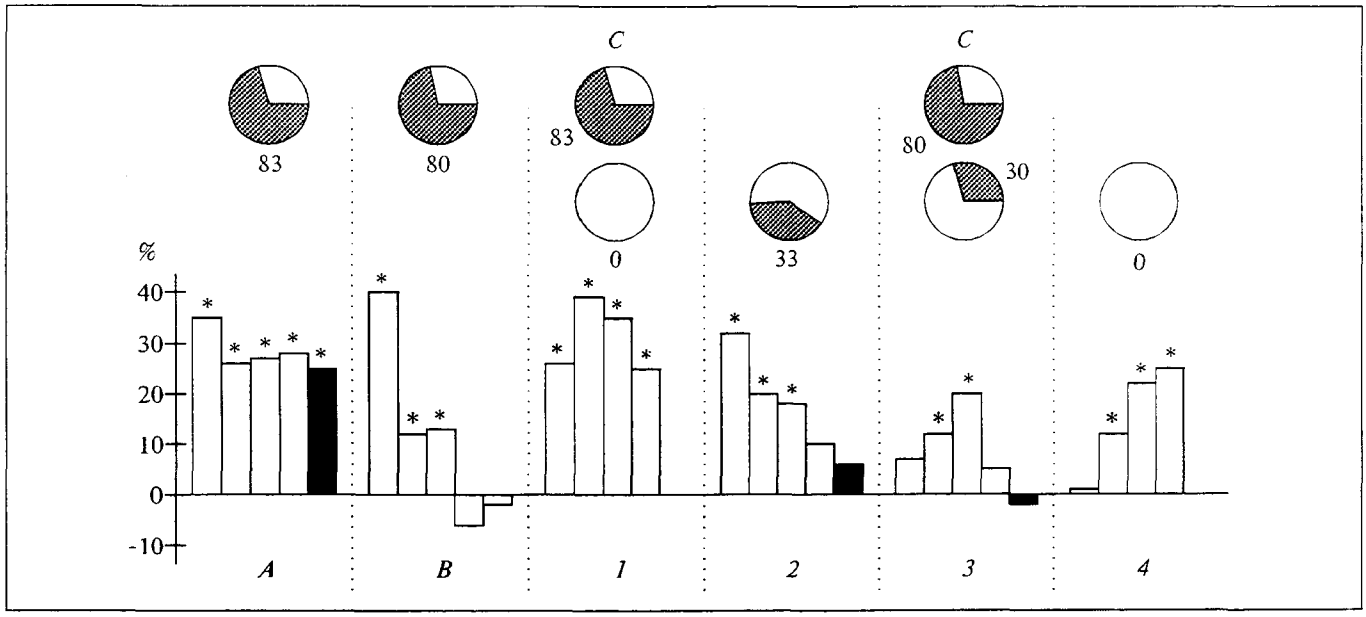

Fig. 2. Aggressive/defensive behavior of castrated short-sleeping (*A*) and long-sleeping (*B*) rats given 3 ng (*1* and *3*) and 30 ng (*2* and *4*) of Surfagon. *C*: control tests. For other designations see Fig. 1.

be noted that Surfagon significantly reduced vertical activity in both groups (by 46-65%; $p<0.01$).

The results of this study suggest that the major effects from intraventricularly administered Surfagon are diminished pain sensitivity and affective aggressivity in response to unavoidable painful electrostimulation. The occurrence of these effects after castration indicates that they are not mediated by sex steroids. The behavioral effects of Surfagon administered by this route were thus opposite to those observed in our previous study, where the peptide was injected intraperitoneally. These differences are determined by specific characteristics of the complex process by which Surfagon binds to receptors in various brain structures and which depends on how the drug enters the central nervous system and is distributed there.

With systemic (intraperitoneal) administration, the main sites of Surfagon penetration into the central nervous system appear to be the more permeable parts of the blood-brain barrier, notably the hypothalamic region, so that there is considerable activation predominantly of the mediobasal hypothalamus, where specific binding sites for luliberin are located [8]. Activation of this nucleus, which is largely responsible for reactions of rage and aggression, should result in intensified aggressive/defensive behavior in response to unavoidable painful electrostimulation. And this was indeed the case under conditions of such electrostimulation in our experiments with intraperitoneal injection of Surfagon. These effects of the peptide may be supplemented by algetic and aggression-promoting actions of adrenocorticotropic hormone (ACTH) [3], whose increased release by pituitary cells under the influence of luliberin has been demonstrated in *in vitro* tests [5]. Augmented ACTH release may also occur consequent to activation of the mediobasal hypothalamus.

With intraventricular administration, the analgesic and antiaggression effects of Surfagon are most likely to be due to its direct interaction with specific receptors in the limbic system and mesencephalon. Indeed, as found in studies using autoradiography, luliberin binds with a high receptor density in the hippocampus and amygdaloid complex [6,9,10,14], which are known to be involved in the regulation of behavior. These structures have been associated with the neurotransmitter action of luliberin, manifested, in particular, by a dose-dependent serotonin release from hippocampal synaptosomes under its influence [7]. This peptidergic effect may bring about activation of the descending serotoninergic system, which inhibits nociceptive responses [11].

A second mechanism through which intraventricularly delivered Surfagon exerts analgesic and antiaggression effects may be its action on the central gray substance which adjoins the ventricles and where widely distributed luliberin-containing fibers have been found and neurons responding to this peptide applied iontophoretically have been identified [14]. As this brain region is the central component of the antinociceptive system, its activation by Surfagon should result in decreased pain sensitivity. The latter effect may also involve a modulating action of enkephalins on the serotoninergic system [13]. Since there is experimental evi-

dence implicating the endogenous opioid system as a possible mediator of convulsive activity [4], the markedly increased convulsive responses to painful electrostimulation seen in our study suggest that enkephalins may be released in considerable amounts following intraventricular Surfagon administration. Supporting this suggestion is the enhanced grooming displayed by rats, which reflects a state of comfortableness. The possibility that the effects of Surfagon are brought on by the mechanisms described above is strengthened by the reported similarities in binding patterns of the naturally occurring peptide and its agonistic analogs in brain structures [8].

The observed greater behavioral excitability and aggressivity of SS rats during pain stress as compared to their LS counterparts is fully compatible with the previously discovered typological distinctions between these two groups of animals [1]. It should also be noted that the peptidergic effects with both intraventricular and intraperitoneal routes of Surfagon delivery were more pronounced in SS rats, indicating a higher sensitivity of brain structures to the peptide in these rats.

REFERENCES

1. I. I. Bobyntsev, L. A. Sever'yanova, and Yu. D. Lyashev, *Byull. Eksp. Biol. Med.*, **112**, № 12, 612-615 (1991).
2. Yu. V. Burov and N. N. Vedernikova, *Neurochemistry and Pharmacology of Alcoholism* [in Russian], Moscow (1985).
3. L. A. Sever'yanova, *Influence of Adaptive Hormones on the Integrative Activity of the Brain* [in Russian], Moscow (1988).
4. B. J. Crain, K. J. Chang, and J. W. McNamara, *Brain Res.*, **412**, № 2, 343-351 (1987).
5. M. Gambacciani, S. S. C. Yen, and D. D. Rasmussen, *Life Sci.*, **43**, № 9, 755-760 (1988).
6. F. Havour, M. Dussaillant, P.. Leblanc, and W. Rostene, *C.R. Acad. Sci.* (Paris), (Ser. 3), **305**, № 2, 41-44 (1987).
7. J.-F. Heurtier, E. Ban, M. Crumeyrolle, and F. Havour, *Neuroendocrinology*, **52**, Suppl. 1, 80 (1990).
8. L. Jennes, *Gen. Comp. Endocrinol.*, **74**, № 2, 288-289 (1989).
9. P. Leblanc, M. Crumeyrolle, L. Jacquelle, *et al.*, *Neuroendocrinology*, **49**, № 5, 482-488 (1988).
10. J. Merchenthaller, G. Setalo, P. Patrusz, *et al.*, *Exp. Clin. Endocrinol.*, **94**, № 1-2, 133-140 (1989).
11. A. Oliverio, C. Costellano, and S. Puglisi-Allegra, *Int. Rev. Neurobiol.*, **25**, 277-337 (1984).
12. L. J. Pellegrino, A. S. Pellegrino, and A. J. Cushman, *A Stereotaxic Atlas of the Rat Brain*, New York - London (1979).
13. M. Sheard, *Aggres. Behav.*, 7, № 1, 41-49 (1981).