Comparative Analysis of Neurotropic Activity of Exorphines, Derivatives of Dietary Proteins

V. A. Dubynin, L. S. Asmakova, N. Yu. Sokhanenkova, Zh. D. Bespalova, V. N. Nezavibat'ko, A. A. Kamenskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 2, pp. 153-157, February, 1998
Original article submitted December 26, 1996

The effects of wheat gluten fragments, hemoglobin, and milk β-caseins (exorphine C, hemorphine-6, and β-casomorphine-7) on nociceptive sensitivity and behavior were studied in albino rats. Hemorphine-6 and exorphine C induced hyperalgesia and increased anxiety; β-casomorphine-7 decreased anxiety and nociceptive sensitivity. All peptides partially decreased motor activity and the orientative-exploring reaction. In contrast to β-casomorphine-7, exorphine C and hemorphine-6 did not exhibit neurotropic activity intrinsic to opioids and can be characterized as functional antagonists of endogenous opioid peptides.

Key Words: *exorphines; β-casomorphine-7; nociceptive sensitivity; motor activity; anxiety*

The particular group of opioid peptides, exorphines, was discovered in the 1980s. They are characterized by the ability to enter the organism with dietary proteins. β-Casomorphine-7, a fragment of milk protein β-casein, is a typical exorphine studied in details. This peptide enters the blood from the intestine [14] and affects opiate μ- and δ-receptors [11]. β-Casomorphine-7 is highly resistant to enzymatic degradation. It possesses neurotropic and analgesic potencies [6], affects the release of some enzymes [13], inhibits peristalsis of the gastrointestinal tract [8], etc.

Release of exorphines may accompany utilization of wheat gluten, hemoglobin, and a number of other peptides [9, 10]. The ability of these peptides to bind with opiate receptors of various types was revealed in the mouse deferent duct preparations [15]. However, physiological properties of wheat gluten and hemoglobin fragments referred to as exorphine C and hemorphine were virtually unknown. Our aim was to assess neurotropic activities of these peptides in comparison with physiological activity of β-casomorphine-7.

Moscow State University; Russian Cardiology Center, Ministry of Health; Institute of Molecular Genetics, Russian Academy of Sciences, Moscow;

MATERIALS AND METHODS

Exorphine C (Tyr-Pro-Ile-Ser-Leu) and hemorphine-6 (Tyr-Pro-Trp-Thr-Gln-Arg) were synthesized at the Cardiology Research Center; β-casomorphine-7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) at the Institute of Molecular Genetics, Russian Academy of Sciences. The study was carried out on 365 randomly bred male albino rats weighing 200 g. The rats were subdivided into groups of 10-15 animals. Aqueous solutions of the peptides (5 and 20 mg/kg) were injected intraperitoneally (0.2 ml). Control rats received an equivalent volume of distilled water.

The threshold of nociceptive sensitivity was determined in the standard tail-flick and hot plate (55±1°C) tests.

Unrestrained motor activity was recorded for 30 min with an Opto-Varimex apparatus (Columbus Instruments), which records horizontal (HC) and vertical (VC) components of motor activity.

The orientative-exploring reaction was assessed in a "hole chamber" test. Motor activity, grooming,

0007-4888/98/0002-0131$20.00 ©1998 Plenum Publishing Corporation

and the number of examined holes were recorded during 2 min.

Evaluation of anxiety was performed using several tests [5]. Durations of the following events were recorded: descent from elevation, passing through a hole in chamber's wall, and going out from the "house". The complex included also a 5 min testing in the "open field". In this test a 3-min bright illumination was followed by 1 min of red light, thereafter bright illumination was restored. The assessed phenomena were motor activity, the number of visits to the center of arena, and grooming. After this test, the animal reaction to experimenter's hand was determined.

The data were statistically analyzed by the standard methods. Significance of differences was assessed by the standard nonparametric tests.

RESULTS

β-Casomorphine-7 (20 mg/kg) decreases nociceptive sensitivity in rats 10-70 min after intraperitoneal injection [1]. In this work we studied analgesic effect of the peptides hemorphine-6 and exorphine C. There were no differencies between control and test groups after administration of hemorphine-6 (5 and 20 mg/kg) concerning the nociceptive sensitivity in the tail-flick test. In the hot plate test, a persistent decrease in latency of the avoidance behavior was observed after a dose of 20 mg/kg. Significant differences from the control level were detected 40-150 min postinjection (Fig. 1, *a*, *b*). Exorphine C caused no differences between control and test animals in the hot plate test. However, higher doses of the pep-

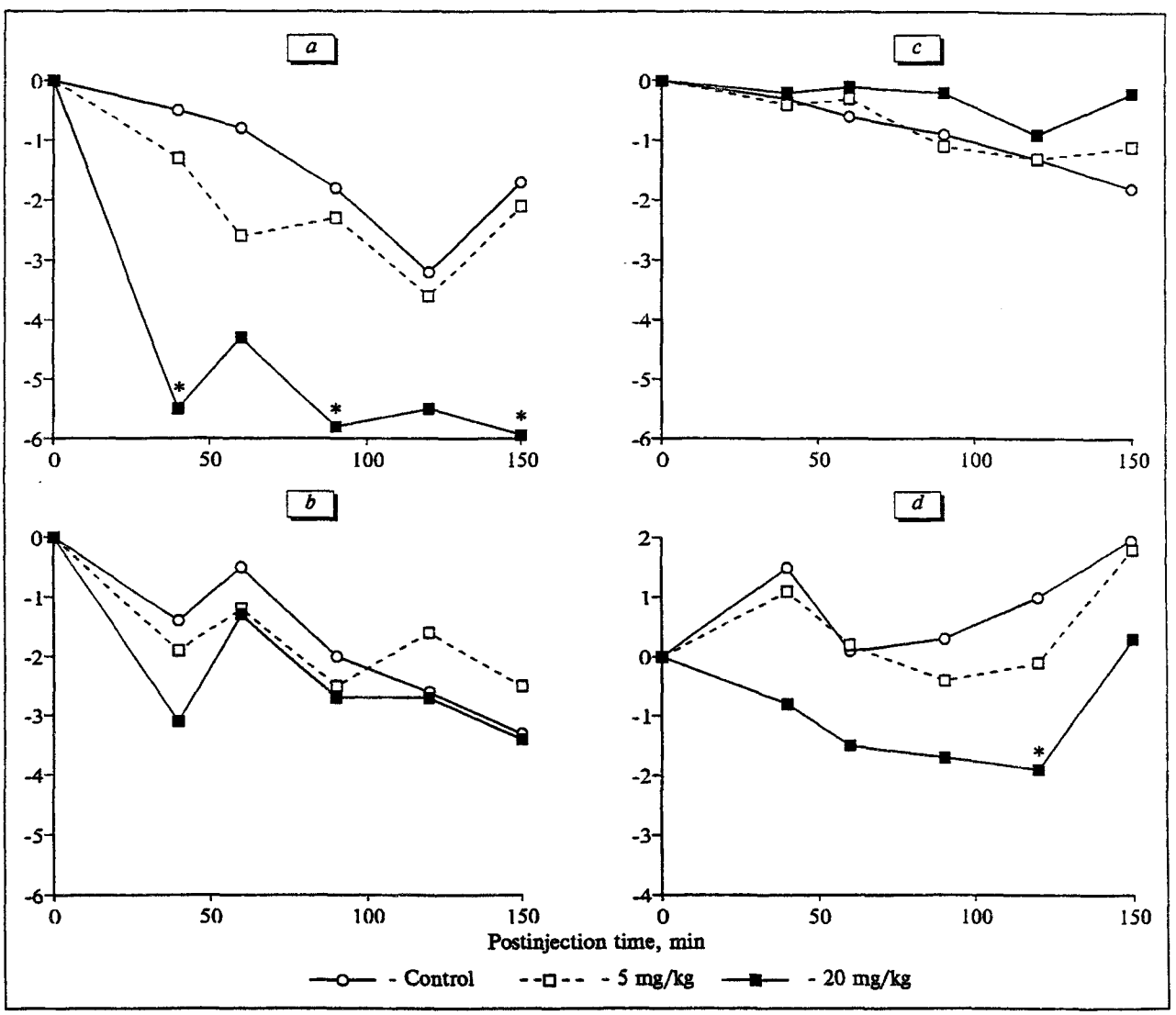

Fig. 1. Effects of hemorphine-6 and exorphine C on nociceptive sensitivity in rats. Ordinate: changes in the latency against the background values (sec). Effect of (*a*) hemorphine-6 and (*b*) exorphine C in the hot plate test; effect of (*c*) hemorphine-6 and (*d*) exorphine C in the tail-flick test. Here and in Fig. 2: *$p<0.05$ in comparison with the control group.

tides induced a marked decrease in the tail flick latency, which was significant 120 min postinjection (Fig. 1, *b, d*). Thus, the effect of exorphine C and to a greater extent hemorphine-6 on nociceptive sensitivity in rats was opposite to the effect of β-casomorphine-7 and can be characterized as hyperalgetic.

In the model of unrestrained behavior in an Opto-Varimex apparatus it was previously shown that both HC and VC decreased 5 min after injection of β-casomorphine-7 [4].

This test also revealed a decrease in HC and VC after injection of hemorphine-6 (5 and 20 mg/kg). However, this effect was observed only for 3 min at the beginning of recording. No significant differences from the control were revealed for both doses of exorphine C.

The orientative-exploring reaction was studied in the "hole chamber" 5 min after injection of hemorphine-6 and exorphine C. Injections of higher doses of the peptides led to a decrease in the run and the number of sets, which was most pronounced on the 2nd min of observation (Fig. 2). In addition, exorphine C led to a decrease in the number of examined holes on the 2nd min of testing for the dose of 5 mg/kg and in total for both minutes for the dose of 20 mg/kg.

Hemorphine-6 and exorphine C did not affect the duration of descent from an elevation, the period of passing through a hole in the chamber's wall, and the period necessary to leave the "house". However, in the open field test, these peptides decreased the run, the number of sets, and the number of visits to the center (Fig. 3). In addition, hemorphine-6 (20 mg/kg) induced spontaneous backward motion and a 4-fold increase in the number of vocalization in response to experimenter's hand compared with the control.

β-Casomorphine-7 (5 mg/kg) increased the value of run and the number of visits to center on the 4th min, i.e., after turning the bright illumination off, the

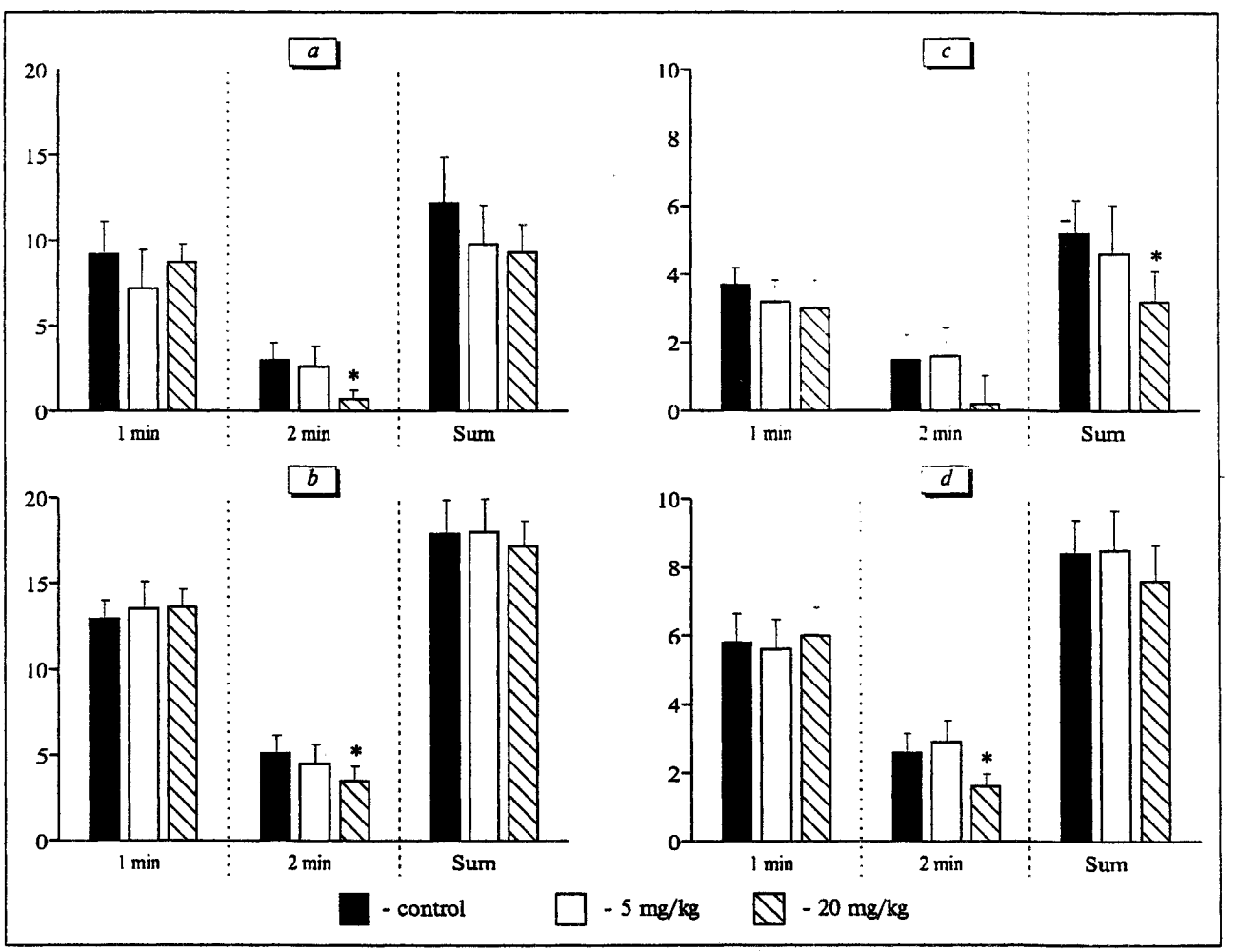

Fig. 2. Effects of hemorphine-6 and exorphine C injected 5 min prior to testing on the animal behavior in the "hole chamber" test. Ordinate: the indices in arbitrary units. The effect of (*a*) hemorphine-6 and (*b*) exorphine C on the value of a run; the effect of (*c*) hemorphine-6 and (*d*) exorphine C on the number of sets.

Peptides	Time, min														
	run					visits to the center					sets				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Hemorphine-6, 5 mg/kg					↓										↓
Hemorphin-6, 20 mg/kg									↓			↓			
Exorphine C, 5 mg/kg		↓	↓					↓	⇩			↓			
Exorphine C, 20 mg/kg						⇩									
β-casomorphine-7, 5 mg/kg				↑					↑						

Fig. 3. Effects of hemorphine-6, exorphine C, and β-casomorphine-7 on rat behavior in the "open field" test. Dark downward arrows show a decrease of the parameter relative to the control value ($p<0.10$); dark upward arrows indicate an increase of the parameter ($p<0.05$).

test rats adapted to new conditions more rapidly than the control rats. In addition, there was a decrease in grooming on the 3rd min of testing. β-Casomorphine-7 (5 mg/kg) seems to produce some decrease in the anxiety level. This agrees with the data on anxiolytic ability of endogenous opioids [7].

From our results it can be concluded that notwithstanding the revealed ability of hemorphine-6 and exorphine C to bind with opioid receptors [15], these peptides have no neurotropic properties characteristic of opioids. In contrast to other opioid peptides, β-casomorphine-7 included, hemorphine-6 and exorphine C provoke hyperalgesia and increase anxiety in rats. Considering the possible reasons of these phenomena, it should be pointed out that these peptides lack phenylalanine in the third position. It can be assumed that the binding of hemorphine-6 and exorphine C to opioid receptors does not activate them, because these peptides (or their fragments resulting from enzymatic degradation) act as blockers of opioid receptors and at least partially as antagonists of the exogenous opioids. A similar situation, when a shorter or modified peptide is an antagonist of its precursor, has been described for the substance P fragments and endogenous immunopotentiator tuftcin, as well as for tripeptide lu-Arg-Pro, an analog of ACTH(5-7) and functional antagonists of ACTH(4-7) [3].

A decrease in motor activity and in orientative-exploring reaction under the action of hemorphine-6 and exorphine C can result from moderation of anxiety, the more so that this decrease occurs in another period that in the case of β-casomorphine-7 injection. Anxiolytic effect of the peptides seems to be at least a partial reason of their hyperalgetic action. There are data on significant decrease in latency of nociceptive reaction accompanied by increase in anxiety in animals [2].

The anxiogenic effect of exorphine C can be attributed to a similarity of its structure (Tyr-Pro-Ile-Ser-Leu) with that of the peptide Pro-Pro-Ile, a fragment of corticoliberin, which is known to increase anxiety [12].

The multidirectional neurotropic activity of exorphines may account for some "unexpected" physiological effects of dietary peptide fragments. The ability of these fragments to affect nociceptive sensitivity, emotional status, and memory should be taken into account when absorption of the peptide in the gastrointestinal tract is augmented by any reason (in neonates, under stress, upon mucosa damage, *etc.*).

This work was supported by the Russian Foundation for Basic Research, project No. 97-04-48166.

REFERENCES

1. V. A. Dubynin, A. S. Maklakova, V. N. Nezavibat'ko, *et al.*, *Byull. Eksp. Biol. Med.*, **114**, No. 9, 284-286 (1992).
2. L. V. Kalyuzhnyi, *Physiological Mechanisms of Pain Sensitivity Regulation* [in Russian], Moscow (1984).
3. N. G. Levitskaya, M. G. Uranova, O. G. Voskresenskaya, *et al.*, *Vestn. Mosk. Gos. Univ., Ser. Biol.*, No. 1, 13-19 (1995).
4. A. S. Maklakova, V. A. Dubynin, N. G. Levitskaya, *et al.*, *Byull. Eksp. Biol. Med.*, **116**, No. 8, 155-158 (1993).
5. V. I. Rodina, N. A. Krupina, G. N. Kryzhanovskii, *et al.*, *Zh. Vyssh. Nerv. Deyat.*, **43**, No. 5, 1006-1017 (1993).
6. V. Brantl, A. Pfeiffer, A. Herz, *et al.*, *Peptides*, **3**, 793-797 (1990).
7. J. L. Carrasco and Y. Saiz, *Rev. Psiquiat. Fac. Med. Barcelona*, **17**, 303-312 (1990).
8. H. Daniel, M. Vonhwinkel, and G. Renher, *J. Nutr.*, **120**, 252-257 (1990).
9. S. Fukudome and M. Yoshikava, *FEBS Lett.*, **296**, 107-111 (1992).
10. E. Glamsta, B. Meyerson, J. Silberring, *et al.*, *Biochem. Biophys. Res. Commun.*, **184**, 1060-1066 (1992).
11. G. Koch and V. Brantl, in: *β-Casomorphines and Related Peptides*, Uppsala, (1994), pp. 21-26.
12. A. A. Martyanov, O. F. Selofonova, J. A. Khamidova, *et al.*, *Neuropeptides*, **26**, 50-52 (1994).
13. V. Shacoori, J. Guerin, A. Girre, *et al.*, *Life Sci.*, **51**, 899-907 (1992).
14. D. Tome, A. Dumontier, M. Hautefeulle, *et al.*, *Am. J. Physiol.*, **253**, G737-G744 (1987).
15. C. Zioudrou, R. Streaty, and W. Klee, *J. Biol. Chem.*, **254**, 2446-2449 (1979).