METHODS

Reactions between β-Casomorphins-7 and 5-HT$_2$-Serotonin Receptors

O. Yu. Sokolov, N. A. Pryanikova*, N. V. Kost, Yu. A. Zolotarev, E. N. Ryukert*, and A. A. Zozulya**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 11, pp. 595-597, November, 2005
Original article submitted March 5, 2005

Radioreceptor analysis showed that human β-casomorphin-7 displaced ^{3}H-spiperone from 5-HT$_2$-serotonin receptors of the rat cerebral frontal cortex: EC$_{50}$ 8±1 μM. Human and bovine β-casomorphin-7 dose-dependently blocked serotonin-induced human platelet aggregation: IC$_{50}$ 5±1 and 20±4 μM, respectively. It was proved that β-casomorphins-7 act as 5-HT$_2$-serotonin receptor antagonists; one of the mechanisms of their biological effects is presumably associated with modulation of the serotoninergic system.

Key Words: *human β-casomorphin-7; bovine β-casomorphin-7; 5-HT$_2$-receptors; spiperone; platelet aggregation*

Casomorphins are exogenous bioactive peptides forming during proteolytic degradation of human and animal milk casein [4]. They modulate nociception [2,6], regulate maternal and child behavior [1], produce anxiolytic [3] and cardiotropic [12] effects. However, the mechanisms of action of these peptide bioregulators remain unclear. Casomorphins interact with μ- and δ-opioid receptors [10], but the wide spectrum of their biological activity can hardly be explained by relationships with the opioid system alone. It was found that casomorphins reduced hyperlocomotion in rats induced by injection of apomorphine (dopamine receptor agonist) [13]; the role of casomorphins in the pathogenesis of postpartum psychosis is now intensively studied [11].

We studied the interactions of β-casomorphins-7 with receptor binding sites of spiperone (antipsychotic, serotonin and dopamine receptor antagonist) and its capacity to modify serotonin-induced aggregation of human platelets.

Research Center of Mental Health, Russian Academy of Medical Sciences; *Russian State Medical University, Ministry of Health of the Russian Federation; **Institute of Molecular Genetics, Russian Academy of Sciences, Moscow. ***Address for correspondence:*** sokolov@rcmh.msk.ru. O. Yu. Sokolov

MATERIALS AND METHODS

Human β-casomorphin-7 (Tyr-Pro-Phe-Val-Glu-Pro-Ile) affinity for serotonin and dopamine receptors was evaluated *in vitro* by their competition with tritiated spiperone for binding to the membrane fraction receptors of rat cerebral frontal cortex and striatum, characterized by high density of 5-HT$_2$-serotonin and D$_2$-dopamine receptors, respectively.

Radioreceptor analysis was carried out using the method developed by Bymaster *et al.* [7]. The reaction mixture for studies of spiperone binding to receptors in rat cerebral frontal cortex (final volume 300 μl) contained 50 mM Tris-HCl buffer (pH 7.4), membrane fraction of the frontal cortex (0.15 mg protein/ml), 50 μg/ml bacitracin, ascorbic acid (0.1 mg/ml), 10 μM pargyline, 5 nM ^{3}H-spiperone (120 Ci/mmol, Amersham), and human β-casomorphin-7 in concentrations of 0.1-100.0 μM.

0007-4888/05/1405-0582

The reaction mixture for the analysis of spiperone binding with striatum receptors (final volume 300 μl) contained 50 mM Tris-HCl buffer (pH 7.4), 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, membrane fraction of the striatum (0.15 mg protein/ml), 50 μg/ml bacitracin, ascorbic acid (0.1 mg/ml), 10 μM pargyline, 0.7 nM ^{3}H-spiperone, and human β-casomorphin in concentrations of 0.1-100.0 μM. The mixture was incubated at 25°C for 40 min. Bound and free label were separated on a Skatron harvester on GF-B fiberglass filters (Whatman) pre-impregnated in 0.1% polyethylenimine. Ketanserin, sulpiride, and unlabeled spiperone in concentrations of 0.1 nM to 1 μM were used for plotting the control displacement calibration curve. Each point was determined for three parallels in three independent experiments. Protein in samples was measured by the method of Lowry.

The effects of human and bovine (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) β-casomorphins on serotonin-induced human platelet aggregation were studied *in vitro* on a Biola two-channel optical aggregometer by the method proposed by Borne and O'Bryan [9]. Venous blood was collected from the ulnar veins of 5 healthy volunteers and put into 3.8% Na citrate (pH 6.5) in 9:1 ratio. Platelet-rich plasma (PRP) was prepared by blood centrifugation at 200*g* for 10 min. Platelet-free plasma was prepared from the supernatant by repeated centrifugation at 600*g* for 20 min. Changes in PRP light transmission were analyzed at different concentrations of the inductor. Changes in the mean radius of platelet aggregates were recorded simultaneously with light transmission. PRP was analyzed within 2 h after preparation of the suspension. Spontaneous and induced aggregation was evaluated at 37°C. Serotonin (Sigma) in the final concentration of 10 μM served as the inductor. Ketanserin (0.01-1.00 μM) or studied peptides (0.1-100.0 μM) were added into incubation medium 2 min before serotonin.

The results were processed using Prism 3.0 software (GraphPad).

RESULTS

Specific binding (SB) of ^{3}H-spiperone with cortical and striatal receptors, determined as the difference between total (TB) and nonspecific binding in the presence of unlabeled spiperone excess, was 60-70% TB.

Relationship between ^{3}H-spiperone SB to rat cerebral frontal cortical receptors and concentrations of ketanserin and human β-casomorphin-7 in the incubation medium is presented in Fig. 1. The curve of displacement with ketanserin (selective ligand of 5-HT_2 receptor) reached a plateau corresponding to 35% TB. Spiperone can bind not only 5-HT_2-, but also 5-HT_1-serotonin, dopamine (D_2, D_3, and D_4), σ-opioid receptors, and α_1-adrenoreceptors [5,8]. This can account for incomplete displacement of ^{3}H-spiperone from the rat cerebral frontal cortical receptors by ketanserin. The difference between TB and binding in the presence of high concentrations of ketanserin can be regarded as a 5-HT_2 component of ^{3}H-spiperone SB.

Starting from the concentration of 500 nM, human β-casomorphin-7 displaced ^{3}H-spiperone from rat cerebral frontal cortical receptors (Fig. 1). Curves presenting displacement of the studied peptide and ketanserin are parallel and reach the same plateau, which attests to competitive interactions of these ligands with spiperone binding sites. It can be hypothesized that human β-casomorphin-7 also reacts with 5-HT_2-receptors. Therefore, for estimation of EC_{50} values for β-casomorphin-7 we used 5-HT_2-component of spiperone SB. EC_{50} for human β-casomorphin-7 was 8±1 μM, which was 15-fold higher than EC_{50} for ketanserin (0.5±0.1 μM). No interactions of human β-casomorphin-7 with striatal receptors were detected (EC_{50}>80 μM). It was previously shown that β-casomorphins-7 can interact with μ- and δ-opioid receptors. Inhibition constants (K_i) for β-casomorphins-7 during interactions with opioid receptors varied from 5 μM for μ- to 20 μM for δ-receptors [10]. The affinity of human β-casomorphin-7 for 5-HT_2-receptors varied within the same range (Fig. 1).

In order to confirm the capacity of β-casomorphins-7 to interact with 5-HT_2-serotonin receptors and identify the agonist/antagonistic type of this

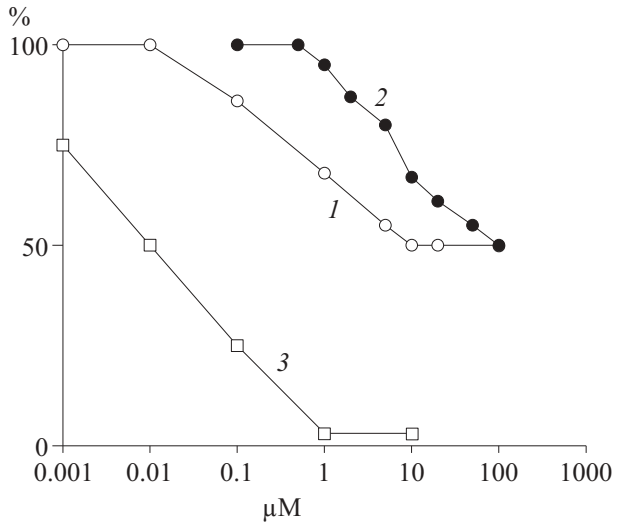

Fig. 1. Effects of ketanserin (*1*) and human β-casomorphin-7 (*2*) on specific binding of ^{3}H-spiperone (*3*) to rat cerebral frontal cortical receptors.

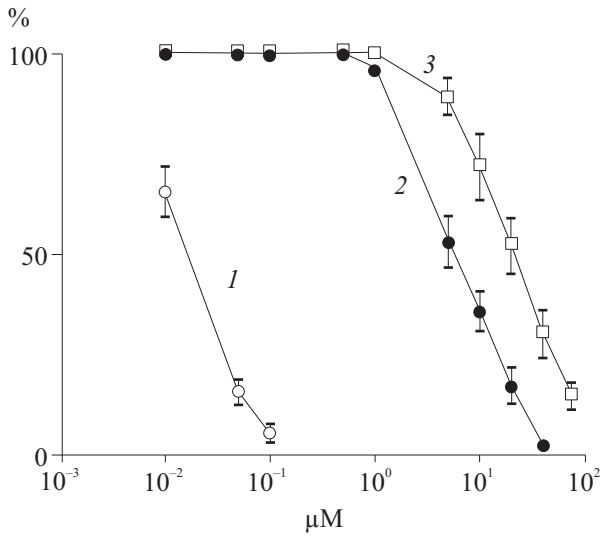

Fig. 2. Effects of human and bovine β-casomorphins-7 on serotonin-induced human platelet aggregation. *1*) ketanserin; *2*) human β-casomorphine; *3*) bovine β-casomorphin-7.

interaction, we studied the effects of human and bovine β-casomorphins-7 on human platelet aggregation. None of the peptides in the selected concentration range induced platelet aggregation, *i.e.* they were not serotonin receptor agonists. However, both peptides dose-dependently blocked serotonin-induced platelet aggregation (Fig. 2), *i.e.* acted as 5-HT_2-serotonin receptor antagonists. IC_{50} values for human and bovine β-casomorphins-7 in this test were 5±1 and 20±4 μM, respectively. The inhibitory activity of peptides was much lower than that of ketanserin (IC_{50} 10±2 nM).

Hence, the capacity of β-casomorphines-7 to react with 5-HT_2-serotonin receptors was demonstrated by two independent methods. Hence, one of the mechanisms of central and/or peripheral effects of β-casomorphines-7 can be due to their effects not only on the opioid, but also on the serotoninergic system.

REFERENCES

1. V. A. Dubynin, Yu. A. Ivleva, I. V Malinovskaya, *et al.*, *Zh. Vyssh. Nervn. Deyat.*, **51**, No. 3, 386-389 (2001).
2. V. A. Dubynin, A. S. Maklakova, L. Yu. Alfeeva, *et al.*, *Byull. Eksp. Biol. Med.*, **114**, No. 9, 284-286 (1992).
3. A. S. Maklakova, V. A. Dubynin, N. G. Levitskaya, *et al.*, *Ibid.*, **116**, No. 8, 155-158 (1993).
4. I. G. Mikheeva, O. B. Kurasova, T. G. Vereshchagina, *et al.*, *Pediatriya*, No. 5, 96-99 (2003).
5. F. Amenta, E. Bronzetti, L. Felici, *et al.*, *J. Auton. Pharmacol.*, **19**, 151-159 (1999).
6. E. M. Blass and J. Blom, *Pediatr. Res.*, **39**, Nos. 2-3, 199-203 (1996).
7. F. Bymaster, D. Calligaro, J. Falcone, *et al.*, *Neuropsychopharmacology*, **37**, No. 2, 87-96 (1996).
8. T. Coccini, L. Manzo, and L. Costa, *Immunopharmacol.*, **22**, 93-106 (1991).
9. Z. A. Gabbasov, E. G. Popov, I. Yu. Gavrilov, and E. Ya. Pozin, *Thromb. Res.*, **54**, No. 3, 215-223 (1989).
10. G. Koch and V. Brantl, *Beta-Casomorphines and Related Peptides*, Uppsala (1990), pp. 43-52.
11. L. Lindstrum, S. Lyrends, F. Nyberg, and L. Terenius, *Ibid.*, pp. 157-162.
12. P. Mentz, K. Neubert, C. Liebmann, *et al.*, *Ibid.*, pp. 133-138.
13. H. L. Ruthrich, G. Grecksch, and H. Matthies, *Pharmacol. Biochim. Behav.*, **44**, No. 1, 227-231 (1993).