SYNTHESIS AND STUDY OF THE OPIOID ACTIVITIES OF ANALOGUES OF β-CASOMORPHIN-5

M. M. Sobirov, Sh. Kh. Khalikov, S. V. Zaitsev,
S. S. Saidov, and S. D. Varfolomeev

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β-Casomorphin-5 (I) is a μ-selective ligand of the opioid receptors. In studying structural-functional relationships we have synthesized five new β-casomorphin-5 analogues (II-VI). These compounds differ from β-casomorphin-5 by a greater length of the molecule, achieved as the result of the addition to the C-end of the initial peptide (I) of a chain consisting of one or two glycine residues and an ethylenediamine residue ($-NH-CH_2-CH_2-NH \leftarrow$ Gly or $-NH-CH_2-CH_2-NH \leftarrow$ Gly ← Gly). This modification, in particular, leads to peptides that have two free amino groups at the ends and no carboxyl and therefore possess basic properties (II and III). Further modification was carried out in the direction of decreasing the basicity of these compounds by the monoacetylation (IV) and monosuccinylation (V) of the extended parts of the molecule. In addition, a dimeric analogue of β-casomorphin-5 (VI) was obtained.

The synthesis of the peptides was effected by the classical method in solution using esters and the carbodiimide method and a combination of the stepwise growth of the peptide chain with block synthesis. Selective elimination of the benzyloxycarbonyl (Z) and t-butoxycarbonyl (Boc) protective groups was used, which made it possible to grow the peptide chain from either end. The final products were purified by HPLC on an Ultrosphere-ODS column (10 mm × 25 cm). The structures of the compounds were confirmed by the results of elementary and amino acid analyses.

The new analogues of β-casomorphin-5 (I) so obtained were investigated for opioid activity by the radioreceptor method of analysis on lyophilized rat brain membranes [2], using tritium-labeled Tyr-D Ala-Gly-MePhe-Gly-ol (DAGO) and Tyr-D Ala-Gly-Phe-D Leu (DADLE) as μ- and δ-ligands, respectively. Opioid activity was determined from the 50% inhibition of the binding of the radioactive ligands by compounds (II-VI) (IC_{50}). All the peptides exhibited opioid activity with different degrees of change in the affinity for the binding centers of the opioid receptors. The introduction into the β-casomorphin-5 molecule of an additional element in the form of $-NH-CH_2-CH_2-NH \leftarrow$ Gly (II) or $-NH-CH_2-CH_2-NH \leftarrow$ Gly ← Gly (III) somewhat lowered the affinity of the ligand.

The decrease in basicity in compound (IV) as compared with (III) scarcely changed the μ-affinity. However, the appearance of a $-COOH$ group in compound (V) led to a marked enhancement of affinity. This was apparently connected with the appearance of a quasi-cyclic structure [3] arising as the result of the interaction of the terminal ionogenic groups. Such a structure is probably more complementary to the opioid centers of binding of the receptors. The dimerization of β-casomorphin-5 (VI) led to some increase in affinity for the μ-receptors.

TABLE 1. Table of the Opioid Activities of the Peptides

Peptide	IC_{50}, nmole	
	μ	δ
I. Tyr-Pro-Phe-Pro-Gly	296	10 000
II. Tyr-Pro-Phe-Pro-Gly—NH—$(CH_2)_2$—NH←Gly	900	10 000
III. Tyr-Pro-Phe-Pro-Gly-NH—$(CH_2)_2$—NH←Gly←Gly	1 000	10 000
IV. Tyr-Pro-Phe-Pro-Gly—NH$(CH_2)_2$—NH←Gly←Gly-$COCH_3$	12 000	10 000
V. Tyr-Pro-Phe-Pro-Gly—NH$(CH_2)_2$—NH←Gly← ←Gly-CO-$(CH_2)_2$-COOH	48	200
VI. Tyr-Pro-Phe-Pro-Gly—NH$(CH_2)_2$—NH←Gly←Gly←Pro← ←Phe←Pro←Tyr	191	10 000

V. I. Lenin Tadzhik State University, Dushanbe. M. V. Lomonosov Moscow State University. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 565-567, July-August, 1990. Original article submitted July 28, 1989; revision submitted February 2, 1990.

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STUDY OF THE LIGNIN OF THE COTTON PLANT OF VARIETY C-4880 BY THE METHOD OF ACIDOLYTIC CLEAVAGE

V. E. Madzhidova, B. Kh. Pulatov,
and Kh. A. Abduazimov

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Continuing a study of the lignin of cotton plants of the variety C-4880 [1], we have used the method of acidolytic cleavage of the lignin. The comminuted (0.25 mm) mature stems of the cotton plant were extracted with ethanol—benzene (1:2) and with hot water. Then the residue was subjected to the acidolysis reaction by Adler's method [2]. After the end of the reaction, the solution was brought to pH 3 with a 0.4 N solution of $NaHCO_3$ and the low-molecular-mass fraction of the acidolysis products was extracted with chloroform. The yield of the products of acidolytic cleavage was 7.6% of the initial material (30% on the Komarov lignin). In order to facilitate the identification of the components of the low-molecular-mass products with the aid of GLC, the products of the acidolysis reaction were reduced with the use of Raney nickel and lithium tetrahydroaluminate.

The reduction of the total acidolysis products with Raney nickel was carried out in the following way: Raney nickel catalyst freshly prepared from 5 g of nickel—aluminum alloy was added to a solution of 2.28 g of the total acidolysis products in 30 ml of 8% NaOH. Then, with constant stirring, the reaction mixture was boiled for 8 h under a current of nitrogen, after which it was acidified to pH 8, and the low-molecular-mass products were extracted with ether; the extract was evaporated and the residue was dried in vacuum over P_2O_5. The yield of reduced products was 0.66% of the initial raw material (3.19% on the Komarov lignin).

The reduction of the low-molecular-mass acidolysis products by lithium tetrahydroaluminate was performed in the following way: 4.5 g of lithium tetrahydroaluminate was suspended in absolute dioxane in a three-necked flask fitted with a condenser plus calcium chloride tube, a dropping funnel, and a stirrer, and, with constant stirring, 1 g of the total acidolysis products, dissolved in 10 ml of absolute dioxane, was added over 1 h. Stirring was continued for 1.5 h, and then, with ice cooling, a mixture of 10 ml of distilled water and 15 ml of glacial acetic acid was added. The solid matter was separated off by centrifugation, and the pH of the reaction mixture was brought to 6-7 with 5% NaOH solution, and it was extracted with ether. The extract was evaporated, and the residue was dried in vacuum over P_2O_5. The yield of lithium-tetrahydroaluminate-reduced material from acidolytic cleavage was 2.0% of the initial raw material (9.2% on the Komarov lignin).

The compositions of the products obtained were investigated by the GLC method on a Chrom 41 instrument with a stainless steel column (0.3 × 370 cm) filled with 15% of Apiezon on Chromaton NAW-DMCS (0.200-0.250 mm) at a rate of flow of carrier gas (helium) of 40 ml/min and a thermostat temperature of 205°C.

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