SYNTHESIS OF STRUCTURAL ANALOGS OF THE 13 – 19 SEQUENCE OF HUMAN GROWTH HORMONE RELEASING FACTOR

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Development of the principles of synthesis of bioregulators responsible for the secretion of natural hormones in the organism is among currently important tasks. One particular basic problem consists in finding previously unknown peptide agents stimulating the secretion of insulin, a hormone playing a key role in the regulation of metabolic processes in higher mammals and humans.

One possible approach to solving this problem is via the synthesis of peptide structures capable of participating in hormone – receptor interactions and enhancing insulin secretion by β cells in Largenhans' islets in the pancreas. A real pathway to such peptide stimulators of insulin secretion can be based on the synthesis of previously unknown structural analogs of the amino end region of somatoliberin, the hypothalamic releasing factor of human growth hormone [1, 2]. Our previous investigations showed that this R-factor is capable of indirectly influencing insulin biosynthesis and secretion by pancreatic islet cells.

However, from the standpoint of pharmacology, a significant disadvantage of using natural somatoliberin as a bioregulator of insulin secretion is its relatively low activity and insufficient selectivity. In addition to weakly stimulating the secretion of insulin, natural somatoliberin enhances (to a much greater extent) production of growth hormone and activates the secretion of glucagon. For this reason, much better prospects in solving the problem under consideration can be related to the amino end region of somatoliberin. This conclusion agrees with the results of computer simulation of hormone – receptor interactions involved in the biosynthesis and secretion of insulin.

In this context, we have undertaken a special investigation aimed at the development of a method for the synthesis of previously unknown structural analogs of the amino end region of somatoliberin, which can be of interest as potential stimulators of insulin secretion. After a series of preliminary experiments and a comparative analysis of the results, it was concluded that the most promising approach is that based on the use of activated esters of N-protected amino acids. This approach leads to a high yield of the target compounds, excludes undesired side reactions, allows effective control over the racemization processes, and is less tedious than the other possible schemes.

As a result, we have developed an effective method for the synthesis of previously unreported structural analogs of the 13 – 19 somatoliberin peptide sequence, which is a part of the amino end of somatoliberin. Distinguishing features of the proposed method are as follows:

(i) use of benzyl esters of oligopeptides as amino components;

(ii) use of *p*-nitrophenyl or pentafluorophenyl esters of N-protected amino acids as carboxy components;

(iii) use of an acid-labile *tert*-butyloxycarbonyl protection for masking α-amino groups in the carboxy components;

(iv) stepwise peptide synthesis in the C → N direction.

An important advantage of the proposed method is the possibility of using protected derivatives of trifunctional amino acids for the synthesis of hydrophobic analogs of somatoliberin.

It was also established that the isolation of target compounds from the reaction mixtures is considerably simplified by conducting the condensation stage in DMF. In this case, the target peptides can be isolated in a crystalline state and purified by recrystallization. The structure of peptides obtained using the proposed scheme is uniquely determined by the scheme of synthesis. The synthetic analogs of the terminal region of somatoliberin can be obtained in an analytically pure form.

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The proposed method was used to obtain previously unreported peptides (I – V), which are structural analogs of the 13 – 19 somatoliberin peptide sequence, representing a part of the amino end of somatoliberin.

The initial compounds in our syntheses were N-*tert*-butyloxycarbonyl-glycine *p*-nitrophenyl ester (VI), N-*tert*-butyloxycarbonyl-*L*-leucine pentafluorophenyl ester (VII), N-*tert*-butyloxycarbonyl-*L*-valine pentafluorophenyl ester (VIII), and the previously synthesized [3] *L*-glutaminyl-*L*-leucyl-O-benzyl-*L*-seryl-*L*-alanine benzyl ester (IX). Particular procedures involved in the synthesis of previously unreported peptides (I – V) are described in detail below.

The synthesis of structural analogs of the 13 – 19 somatoliberin peptide sequence provides the necessary prerequisite for investigation into the biological activity of such compounds, in particular, their influence on the process of insulin secretion.

EXPERIMENTAL PART

The optical activity was measured with a polarimeter of the MA-511-0 type (Hilger Watts). The melting points of the products were determined using a heating stage of the Boetius Analytik type (Germany). TLC analyses were performed on Silufol UV-254 plates as described in [4]. The plates were eluted in the following solvent systems: 1-butanol – acetic acid – water, 4 : 1 : 1 (system 1); ethyl acetate – petroleum ether, 1 : 1 (system 2); chloroform – methanol, 9 : 1 (system 3); and 1-butanol – pyridine – acetic acid – water, 15 : 10 : 3 : 12 (system 4). The sample spots were revealed by treating the TLC plates with ninhydrin or by exposure to iodine vapor.

Prior to amino acid analysis, the peptides were subjected to acid hydrolysis under standard conditions (5.7 N HCl, 110°C, 20 h). The content of amino acids in the hydrolyzate was determined with an automated amino acid analyzer of the TSM type (Technicon). The data of elemental analyses of the synthesized compounds coincided with the results of analytical calculations.

N-*tert*-Butyloxycarbonyl-glycyl-*L*-glutaminyl-*L*-leucyl-O-benzyl-*L*-seryl-*L*-alanine benzyl ester (I). To a solution of 1 g (1.6 mmole) of *L*-glutaminyl-*L*-leucyl-O-benzyl-*L*-seryl-*L*-alanine benzyl ester [3] in 10 ml of DMF, cooled to 0°C, was sequentially added with stirring 0.52 g (1.7 mmole) of N-*tert*-butyloxycarbonylglycine (VI) and 0.2 ml (0.6 mmole) of triethylamine. The mixture was stirred for 1 h at 0°C and then allowed to stand for 20 h at 20°C. The precipitate was separated by filtration. The filtrate was evaporated to dryness in vacuum, and the residue was purified by reprecipitation with water from ethanol to obtain 1.04 g (83%) of pentapeptide I; m.p., 219 – 220°C; $[\alpha]_D^{20}$, –18.5° (*c*, 1.0; DMF); TLC: R_f, 0.68 (system 1), 0.30 (system 3), 0.88 (system 4); $C_{38}H_{54}N_6O_{10}$; amino acid analysis: Gly, 1.03; Glu, 1.01; Leu, 1.00; Ser, 0.79; Ala, 1.02.

Glycyl-*L*-glutaminyl-*L*-leucyl-O-benzyl-*L*-seryl-*L*-alanine benzyl ester (II). To 6 ml of a 10% solution of hydrogen chloride in dioxane was added 0.88 g (1.2 mmole) of compound I and the mixture was kept for 45 min at 20°C. Then, the solvent was evaporated in vacuum and the residue was treated with diethyl ether. The precipitated crystals were separated by filtration and dried in a vacuum desiccator over phosphorus anhydride to obtain 0.85 g (98%) of pentapeptide II in a monohydrochloride form; $C_{33}H_{46}N_6O_8 \cdot HCl$; amino acid analysis: Gly, 1.01; Glu, 0.98; Leu, 1.02; Ser, 0.78; Ala, 1.00.

N-*tert*-Butyloxycarbonyl-*L*-leucyl-glycyl-*L*-glutaminyl-*L*-leucyl-O-benzyl-*L*-seryl-*L*-alanine benzyl ester (III). To a solution of 0.85 g (1.2 mmole) of compound II in 8 ml of DMF, cooled to 0°C, was sequentially added with stirring 0.15 ml (1.2 mmole) of triethylamine and 0.5 g (1.3 mmole) of N-*tert*-butyloxycarbonyl-*L*-leucine pentafluorophenyl ester (VII). The reaction mixture was stirred for 1 h at 0°C and then kept for 18 h at 20°C. The precipitate was separated by filtration, the filtrate was concentrated in vacuum, and the concentrate was treated with ethyl and acetate The crystalline precipitate was separated by filtration and washed sequentially with a 5% aqueous potassium bisulfate solution, water, 5% aqueous potassium bicarbonate solution, and water again. Then, the ethyl acetate solution was dried in a vacuum desiccator over anhydrous pjhosphorus anhydride, purified by reprecipitation with water from methanol, and dried to obtain 0.89 g (91%) of hexapeptide III; m.p., 223 – 224°C; $[\alpha]_D^{20}$, –25.6° (*c*, 1.0; DMF); TLC: R_f, 0.75 (system 1), 0.49 (system 3); 0.86 (system 4); $C_{44}H_{65}N_7O_{11}$; amino acid analysis: Leu, 2.12; Gly, 1.08; Ser, 0.78; Ala, 1.00.

***L*-Leucyl-glycyl-*L*-glutaminyl-*L*-leucyl-O-benzyl-*L*-seryl-*L*-alanine benzyl ester (IV)**. To 8 ml of a 10% solution of hydrogen chloride in dioxane was added 0.62 g (0.7 mmole) of compound III and the mixture was kept for 45 min at 20°C. Then, the solvent was evaporated in vacuum and the residue was triturated with diethyl ether. The obtained crystals were separated by filtration, washed with diethyl ether, and dried in a vacuum desiccator over phosphorus anhydride to obtain 0.60 g (89%) of hexapeptide IV in a monohydrochloride form; $C_{38}H_{57}N_7O_9 \cdot HCl$; amino acid analysis: Leu, 2.08; Gly, 1.01; Glu, 1.04, Ser, 0.86; Ala, 1.00.

N-*tert*-Butyloxycarbonyl-*L*-valyl-*L*-leucyl-glycyl-*L*-glutaminyl-*L*-leucyl-O-benzyl-*L*-seryl-*L*-alanine benzyl ester (V). To a solution of 0.57 g (0.7 mmole) of compound IV in 10 ml of DMF, cooled to 0°C, was added with stirring 0.9 ml (0.7 mmole) of triethylamine and the mixture was stirred for 15 min. To this mass was added with stirring 0.27 g (0.72 mmole) of N-*tert*-butyloxycarbonyl-*L*-valine (VIII), and the mixture was stirred for 1 h at 0°C and then allowed to stand for 20 h at 20°C. The precipitate was separated by filtration, the filtrate was evaporated to dryness in vacuum, and the residue was reprecipitated with water from methanol and dried in vacuum over phosphorus anhydride to obtain 0.62 g (91%) of heptapeptide V; m.p., 233 – 234°C;

$[\alpha]_D^{20}$, –22.5° (*c*, 1.0; DMF); TLC: R_f, 0.87 (system 1), 0.36 (system 3), 0.85 (system 4); $C_{49}H_{74}N_8O_{12}$; amino acid analysis: Val, 1.09; Leu, 2.03; Gly, 1.02; Glu, 1.07; Ser, 0.82; Ala, 1.00.

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