LITERATURE CITED

1. M. D. Mashkovskii, A. N. Grinev, N. I. Andreeva, et al., Khim.-farm. Zh., No. 3, 60-63 (1974).
2. F. A. Trofimov, N. G. Tsyshkova, and A. N. Grinev, Khim. Geterotsikl. Soedin., No. 3, 308-310 (1973).
3. V. I. Shvedov, L. B. Altukhova, and A. N. Grinev, Khim.-farm. Zh., No. 3, 25-31 (1967).
4. V. I. Shvedov, L. B. Altukhova, N. I. Andreeva, et al., Khim.-farm. Zh., No. 10, 14-17 (1972).
5. V. I. Shvedov, L. B. Altukhova, E. S. Krichevskii, et al., Khim.-farm. Zh., No. 4, 22-24 (1976).
6. V. I. Shvedov, L. B. Altukhova, and A. N. Grinev, Transactions of Scientific Research Pharmaceutical Institute [in Russian], No. 9, Moscow (1982), pp. 55-62.
7. USSR Inventors Certificate 349686; Byull. Izobr., No. 26 (1972).
8. P. A. Martorana and R. E. Nitz, Arzneimittel Forsch., 29, 946-949 (1979).
9. B. Rubni, M. H. Malone, et al., J. Pharm. Exp. Ther., 120, 125-136 (1957).

ANTI-AMNESTIC AND ANTIDEPRESSANT ACTIVITY OF MELANOSTATIN ANALOGS

T. A. Voronina, N. V. Markina,
T. S. Kalinina, V. M. Kabanov,
A. A. Mazurov, and S. A. Andronati

UDC 615.357:577.175

The anti-amnestic and antidepressant activity of melanostatin analogs, obtained by changing the second and third amino acid residues has been determined. Pro-Gln-Gly-NH_2 (II) has an anti-amnestic activity comparable with piracetam, but in a dose lower by a factor of 60.

The hypothalamus hormone melanostatin (MIF, Pro-Leu-Gly-NH_2) has a wide spectrum of biological activity [1], which explains the never lessening interest with respect to this compound of both chemists and pharmacologists. However, among the numerous synthetic analogs of this tripeptide only a few compounds are known with a high psychotropic activity [2, 3], whereby attempts to exchange the leucine residue for another natural amino acid in the molecule of melanostatin led to interesting results from the standpoint of biological activity [2, 4, 5]. We have previously shown that analogs of melanostatin containing the residues of phenylalanine and glutamine at the 2-position [Pro-Phe-Gly-NH_2 (I) and Pro-Gln-Gly-NH_2 (II)] have psychostimulating activity during central administration [6]. In the present work we report the results obtained in the study of the anti-amnestic and antidepressant activity of these compounds, and also of analogs of melanostatin in which the glycine residue was exchanged by the conformationally more labile and biologically active residue of γ-aminobutyric acid (H-Pro-Leu-Abu-NH_2) (III) and the amide group was replaced by introducing the biologically active phenamine (H-Pro-Leu-Gly-NHCH(CH_3)$CH_2C_6H_5$, IV) and the hydrazide groups (H-Pro-Leu-Gly-$NHNH_2$, V).

The synthesis of Boc-Pro-Leu-OH, melanostatin, analogs I and II has been described in [6]. Peptides III-V were synthesized by the carbodiimide method. Condensation of dipeptide Boc-Pro-Leu-OH with γ-aminobutyramide, N-glycylphenylamine and glycine methyl ester gave the N-substituted peptides VI, VII, IX. Hydrazyl VIII was obtained by the hydrazinolysis of Boc-Pro-Leu-Gly-OMe (VII). Peptides VI, VIII, IX were deblocked by 4 N HCl in dioxane.

$$\left.\begin{matrix}\text{Boc-Pro-Leu-OH}\\ \text{H-X-R}\end{matrix}\right\rbrace \rightarrow \text{Boc-Pro-Leu-X-R}$$
(VI, VII, IX),

where

VI: X=Gly, R=NHCH(CH_3)$CH_2C_6H_5$, VII: X=Gly, R=OMe, IX: X=Abu, R=NH_2.

Physicochemical Institute, Ukrainian Academy of Sciences. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, Nos. 9-10, pp. 72-74, September-October, 1992. Original article submitted August 26, 1991.

0009-150X/92/0910-0753$12.50

$$VII + N_2H_4 \cdot H_2O \rightarrow Boc\text{-}Pro\text{-}Leu\text{-}Gly\text{-}NHNH_2 \text{ (VIII)}.$$

$$Boc\text{-}Pro\text{-}Leu\text{-}X\text{-}R \rightarrow HCl \cdot H\text{-}Pro\text{-}Leu\text{-}X\text{-}R$$
(III—V, VI, VIII, IX),

where

III: X=Abu, R=NH_2, [illegible]=Gly, R=$NHCH(CH_3)CH_2C_6H_5$, V: X=Gly, R=$NHNH_2$, VI: X=Gly, R=$NHCH(CH_3)CH_2C_6H_5$, VIII: X=Gly, R=$NHNH_2$, IX: X=Abu, R=NH_2.

EXPERIMENTAL (CHEMICAL)

The purity of the compounds was controlled by TLC on Silufol plates (Kavalier, Czechoslovakia) in systems of solvents – A: chloroform–ethyl acetate–methanol, 9:3:2; B: ethanol–ammonia, 9:1; C: benzene–acetone–acetic acid, 100:50:1; D: ethanol–ammonia, 4:1. The chromatographic plates were developed by chlorotoluidine and ninhydrin reagents. The amino acid analysis was carried out on a T-339 amino acid analyzer (Mikrotechna, Czechoslovakia) with preliminary hydrolysis of the peptides in 6 N HCl at 110°C for 24 h. The specific rotation of the compounds was determined on a Perkin-Elmer 24IMC spectropolarimeter (Germany). For the synthesis of the pepetides, amino acids and their derivatives with L-configuration from the firms Fluka (Switzerland) and Reanal (Hungary) were used. The abbreviations of the amino acids used correspond to the recommendations of the Commission on Biochemical Nomenclature (IUPAC-IUB) [7]. Other abbreviations: Abu) aminobutyric acid; Boc) tert-butyloxycarbonyl; DCCI) dicyclohexylcarbodiimide; DCCU) dicyclohexylurea; Et_3N) triethylamine; SuOH) N-hydroxysuccinimide; DMFA) dimethylformamide; EA) ethyl acetate.

Boc-Gly-NHCH(CH_3)$CH_2C_6H_5$. A 1.75 g portion (10 mmoles) of Boc-Gly-OH in 10 ml of absolute DMFA was cooled to –10°C, 1.265 g (11 mmoles) of SuOH and 2.27 g (11 mmoles) of DCCI were added, and the mixture was stirred at room temperature for 2 h. Dicyclohexylurea was then filtered off and 1.72 g (10 mmoles) of phenamine hydrochloride and 1.4 ml (10 mmoles) Et_3N in 10 ml of DMFA was added. After 16 h, the solution was filtered off, and to the filtrate 20 ml of a saturated aqueous solution of NaCl was added, and the mixture was extracted with EA (3 × 50 ml). The organic fractions were washed successively with 1 N HCl (2 × 200 ml), a saturated aqueous solution of NaCl (200 ml), 5% solution of $NaHCO_3$ (2 × 200 ml) and a saturated solution of NaCl (200 ml). The ethyl acetate solution was dried over $MgSO_4$, the solvent was evaporated, and the residue was rubbed with hexane and recrystallized from anhydrous ether. Yield, 2.57 g (87.9%) of Boc-Gly-NHCH(CH_3)$CH_2C_6H_5$. Mp 168-170°C, R_f 0.7 (A).

HCl·H-Pro-Leu-Gly-NHCH(CH_3)$CH_2C_6H_5$ (IV). Preparation of solution A: A 0.53 g portion (1.62 mmole) of Boc-Pro-Leu-OH in 10 ml of EA was cooled to –10°C and 0.2 g (1.78 mmole) of SuOH and 0.37 g (1.78 mmole) of DCCI were added. The solution was stirred for 2 h and then was filtered. Preparation of solution B: a 0.47 g portion (1.62 mmole) of Boc-Gly-NHCH(CH_3)·$CH_2C_6H_5$ was dissolved in 5 ml of EA, and 2 ml of 4 N HCl in dioxane was added. After 20 min the solvents were evaporated, the residue was dissolved again in 5 ml of EA, and 0.23 ml of Et_3N was added. After 20 min, solution B was filtered off and combined with solution A. After 16 h, the reaction mixture was filtered, the filtrate was diluted with EA to 30 ml and washed successively with 1 N HCl (3 × 30 ml), a NaCl solution (30 ml), a 5% solution of $NaHCO_3$ (3 × 30) and a NaCl solution. The solution was dried over $MgSO_4$, evaporated in vacuo and peptide VI formed was deblocked with 3 ml of 4 N HCl in dioxane. After 20 min, dioxane was evaporated in vacuo, and the residue was recrystallized from anhydrous ether. Yield 0.3 g (42.9%) of IV. Mp 121-123°C, $[\alpha]_{578}^{20} = -23.8$ (c 1.2, methanol), R_f 0.8 (B), amino acid analysis: Pro:Leu:Gly - 1:1.01:1.05.

Boc-Pro-Leu-Gly-OMe (VII). A 5.75 g portion (17.5 mmoles) of Boc-Pro-Leu-OH in 20 ml of DMFA was cooled to –10°C and 2.214 g (19.5 mmoles) of SuOH and 3.97 g (19.5 mmoles) of DCCI were added. The solution was stirred at room temperature for 2 h and then a solution of 2.2 g (17.5 mmoles) of HCl·H-Gly-OMe and 2.45 ml (17.5 mmoles) of Et_3N in 10 ml of DMFA was added. After 16 h of stirring at room temperature, DCCU was filtered off, 20 ml of a saturated aqueous solution of NaCl was added to the filtrate, and the mixture was extracted with EA (3 × 50 ml). The organic solution was treated as in the separation of VI. After evaporation, the residue was rubbed with hexane and dried in vacuo. Yield, 5.2 g (71.6%) of VII, mp 122-123°C, $[\alpha]_{589}^{21} = -12.5°$ (c 1.1, methanol), R_f 0.55 (C).

Boc-Pro-Leu-Gly-$NHNH_2$ (VIII). A 2.1 g portion (5.3 mmoles) of VII was dissolved in 5 ml of methanol, and 5 ml of tert-butanol, 1.06 ml (21.2 mmoles) of 75% $N_2H_4 \cdot H_2O$ were added.

TABLE 1. Anti-amnestic (M) and Antidepressant (A) Activity of the Peptides

Peptide	M (%)			A (%)	
	300 mg/kg	5 mg/kg	1 mg/kg	5 mg/kg	1 mg/kg
MIF		37,0		7,28	15,02
I		14,2		19,7	32,37*
II		68,2†		24,22	34,45*
III		35,2			
IV		37,5†	—33,1		
V		—21,9	—59,9		
Piracetam	72,75†				
Imipramine				39,4	

*$p < 0.01$.
†$p < 0.05$.

The solution was held for 3 h on a water bath (50°C), the solvents were evaporated and the residue was dissolved in 30 ml of EA. The solution was washed with water (3 × 30 ml) and dried over $MgSO_4$. After evaporation, 2.1 g (99%) of VIII was obtained, oil, $[\alpha]_D^{20} = -61.8°$ (c 4.0, methanol), R_f 0.15 (C).

HCl·H-Pro-Leu-Gly-$NHNH_2$ (V). A 2 g portion (5 mmoles) of VIII was treated with 3.5 ml of 4 N HCl in dioxane. After 30 min the precipitate was separated, and dried in vacuo to yield 1.68 g (100%) of V. Mp 190-192°C, $[\alpha]_{578}^{20} = -28.8°$ (c 1.2, methanol). R_f 0.72 (D). Amino acid analysis: Pro:Leu:Gly = 1:1.05:1.06.

HCl·H-Pro-Leu-Abu-NH_2 (III). A 20 ml portion of DMFA and 0.69 ml (4.9 mmoles) of Et_3N were added to 0.68 g (4.9 mmoles) of HCl H-Abu-NH_2. After 20 min the solution was filtered, 1.613 g (4.9 mmoles) of Boc-Pro-Leu-OH was added to the filtrate, and the mixture was cooled to −10°C. Then 0.62 g (5.4 mmoles) of SuOH and 1.11 g (5.4 mmoles) of DCCI was added. After 16 h the reaction mixture was filtered, DMFA was evaporated in vacuo (50°C) and the residue was chromatographed on a column (70 × 1.5 cm) with silica gel. Systems of solvents: chloroform:hexane, 4:1; chloroform; chloroform:methanol, 9:1. Compound IX that has been separated was treated with 3 ml of 4 N HCl in dioxane. Yield, 0.7 g (40.8%) of III, oil, $[\alpha]_{578}^{16} = -27.1°$ (c 0.5, methanol). R_f 0.65 (D). Amino acid analysis: Pro:Leu:Gly = 1.04:1.07:1.

EXPERIMENTAL (PHARMACOLOGICAL)

The pharmacological activity of the peptides was studied on white nonpedigree male mice, each weighing 16-23 g. As the reference preparation we have used the classical nootrop piracetam and the classical antidepressant imipramine. All the peptides were administered subcutaneously 7 min before the experiment.

The anti-amnestic activity of the compounds was studied using the conditional reflex of passive escape (CRPE) according to a method described in [8] with a subsequent application of a maximal electroshock (MES) as an amnesizing factor. The test for the reaction retention was carried out 24 h after the conditioning, using the latent time of entry of the mice into a dark compartment of the chamber as the criterion of the reaction retention. The MES caused an amnesia in mice.

The amnestic activity (M) was considered positive, and the reverse effect – negative in comparison with control in accordance with the formula proposed in [9] and modified by us:

$$M = \frac{t_c - t}{t_c} 100\ \%,$$

where t is the latent time of entry of mice to which the compound studied was administered into the dark compartment of the chamber (sec), t_c – the same time for the control group animals.

For studying the antidepressant activity the "swimming test" was used according to the method of Porsolt [10], and the time of immobilization of the animals in glass cylinders during 10 min of observation was evaluated. The antidepressant action (A) was expressed in percent according to a formula similar to that cited above

$$A = \frac{t_c - t}{t_c} 100\ \%$$

From the results presented in Table 1 it is seen that the compounds II and IV displayed anti-amnestic activity.

Pro-Gln-Gly-NH_2 (II) has an anti-amnestic activity comparable with piracetam, but in a dose smaller by a factor of 60. In analog V, a tendency is observed (statistically unreliable) to a reverse effect.

Replacement of leucine by glutamine led to increase in the anti-amnestic and antidepressant activity. At the same time introduction of a phenamine residue into the molecule of melanostatin and exchange of glycine by γ-aminobutyric acid practically did not change the anti-amnestic effect.

The time of immobilization in the Porsolt test was reliably shortened under the influence of peptides I and II in a dose of 1 mg/kg. According to the extent of the effect, the activity of these peptides is similar to the activity of imipramine in a dose of 5 mg/kg. In melanostatin a tendency was observed for the manifestation of this effect (statistically unreliable). It should be noted that with increase in the dose, the activity of the peptides studied increases.

At the same time, in tests on the reaction with 5-hydroxytryptophan, phenamine and reserpine, melanostatin and its analogs do not display any noticeable biological effect.

LITERATURE CITED

1. V. E. Klusha, Peptides – Regulators of the Brain Function [in Russian], Riga (1984).
2. H. N. Bhargava, Life Sci., 29, No. 1, 45-51 (1981).
3. E. D. Nicolaides, F. J. Tinney, J. S. Kalfeubroun, et al., J. Med. Chem., 29, No. 6, 959-971 (1986).
4. K.-L. Yu. G. Rajakuma, K. Labitet, et al., J. Med. Chem., 53, No. 7, 1430-1436 (1988).
5. G. Valle, M. Crisme, K.-L. Yu., et al., Coll. Czech Chem. Commun., 63, No. 1, 2863-2876 (1988).
6. A. A. Mazurov, S. A. Andronati, B. A. Lobasyuk, et al., Khim.-farm. Zh., No. 2, 155-157 (1989).
7. IUPAC-IUB Joint Commission on Biochemical Nomenclature, Pure Appl. Chem., 56, 595-624 (1984).
8. I. Bures and O. Buresova, Techniques and Experiments for the Study of Brain and Behavior, Elsevier, North Holland Biomedical Press (1976), p. 91.
9. T. A. Gudasheva, R. U. Ostrovskaya, S. S. Trofimov, et al., Khim.-farm. Zh., No. 11, 1322-1328 (1985).
10. R. D. Porsolt, M. Le Richon, and M. Galfre, Nature, 266, 730 (1977).