MODIFICATION OF MSH RELEASE-INHIBITING HORMONE

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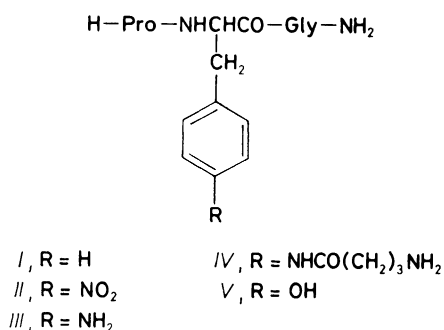
[Phe[2]]MIF (*I*) and the ring substituted analogues (*II*–*V*) have been synthesized. Their antidepressant activity was estimated by Porsolt's test.

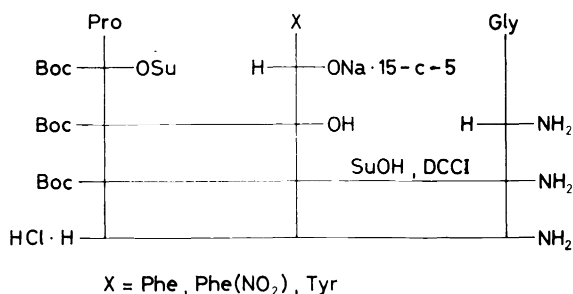
Hypothalamic hormone MSH release-inhibiting hormone (MIF, Pro-Leu-Gly-NH_2) possesses a wide spectrum of neurotropic activities[1]. The majority of analogues described in literature are the peptides where the amino acids are substituted by other natural amino acids. This method of peptide modification is the most simple procedure. The replacement of the second amino acid in MIF molecule causes a sharp alteration of the psychotropic effect up to its disappearance[2–4].

We have modified MIF using the principle of obligatory similarity of the amino acids. Each natural amino acid is coded by the triplets of nucleotides – codones. According to the Crick "wobbling" hypothesis[5], the first two codone basis (obligatory nucleotides) make the most significant contribution into the specific coding as compared with the third base (facultative nucleotide). Thus, leucine (coded by UUA or UUG) in MIF can be exchanged for the obligatory similar Phe (coded by UUU or UUC).

The obtained [Phe[2]]MIF was more efficient than MIF after intraventricular administration[6]. Structural alteration of the natural amino acids of the biologically active peptide resulting in an analogue allows to increase its resistance towards enzymatic degradation and to influence the spectrum of its activity. Thus, we have synthesized the peptides *I*–*IV* with substituted phenyl ring. Tyrosine was used to obtain peptide *V* as a structural phenylalanine-analogue, containing OH group in a *p*-position.

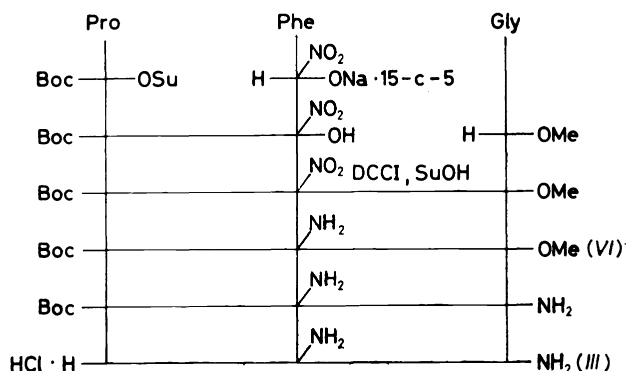


Peptides *I*, *II*, *V* were synthesized using an activated ester method (Scheme 1) from the complexes of alkali salts of amino acids with crown ethers[7], these complexes



SCHEME 1

being solved in organic solvents. The readily purified ester *VI* prepared according to Scheme 2 was more suitable for the synthesis of compounds *III*, *IV*, than *II*.



SCHEME 2

Addition of 4-aminobutyric acid to peptide *VI* has been carried out by means of the carbodiimide method, followed by aminolysis and acidolytic cleavage of the blocking groups.

Antidepressant activity of the synthesized peptides has been estimated by the Porsolt test[8], generally employed for the screening of typical and atypical antidepressants. The compound activity is expressed as a percentage in terms of the control group according to the formula:

$$\Delta A = \frac{\sum \Delta t_{im}^{contr} - \sum \Delta t_{im}^{exp}}{\sum \Delta t_{im}^{exp}} \times 100\%,$$

where Δt_{im} is time of mice immobilization in s. Table I shows the results of the experiment. After the subcutaneous administration, the activity of peptide *I* at the dose 5 mg/kg was higher than that of MIF. Introduction of the electronacceptor nitro group into *p*-position of the phenylalanine phenyl ring reduced the activity of peptide *II* as compared with *I*, while the presence of electronodonor substituents at the same position resulted in an almost complete loss of activity of compounds *III*–*V*. The compound *IV* reduced at the dose 5 mg/kg the period of the animal immobilization by more than 30%. The reverse effect of peptide *V* at the dose 1 mg/kg – prolongation of the period of immobilization by 20% should be noted. The same effect is shown for peptides *II* and *IV*, when decreasing doses to 0·001 mg/kg (Table I).

Thus, leucine substitution for the obligatory similar phenylalanine in MIF does not change its antidepressant activity. Simultaneously, introduction of the substi-

TABLE I

Antidepressant activity of MIF and its analogues at variable doses

Compound	ΔA, %				
	5·0 mg/kg	1·0 mg/kg	0·1 mg/kg	0·01 mg/kg	0·001 mg/kg
MIF	7·2	14·8**	19·3**	29·6*	−1·8
I	38·3**	32·0*	21·3*	23·0*	−10·6
II	19·4	8·9	22·7*	16·4*	−25·2*
III	−7·9	2·9	15·1	−6·3	−1·6
IV	31·3*	1·6	−1·1	1·6	−17·3*
V	4·0	−18·2*	8·9	−1·0	8·2

* $p < 0.01$; ** $p < 0.05$.

tuents into *p*-position of the phenylalanine phenyl ring results in decrease or loss of the antidepressant activity of MIF derivatives.

EXPERIMENTAL

^{1}H NMR spectra were obtained on Bruker WM 250 instrument in $(CD_3)_2SO$ using Me_4Si as an internal standard. Chemical shifts are listed in the Table II. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. For TLC on precoated plates (Silufol, Kavalier) we used the following solvent systems: (A) benzene–acetone–acetic acid, 100 : 50 : 1; (B) ethanol–25% ammonia, 4 : 1; (C) ethyl acetate–pyridine–acetic acid–water, 5 : 5 : 1 : 3. Column chromatography was carried out on silica gel L (0·10—0·25 mm). For amino acid analysis the peptides were hydrolyzed in 6M HCl for 24 h at 110°C and the hydrolyzates were analyzed on Mikrotechna T 339 amino acid analyzer. Amino acid analysis results confirmed the chemical composition of the peptides. MIF and *I* were prepared according to[6], and *p*-nitrophenylalanine according to[9].

N-t-Butyloxycarbonylprolyl-*p*-nitrophenylalanine

To a cooled (—10°C) solution of 2·15 g (10 mmol) Boc-Pro-OH in 10 ml of DMFA, 1·26 g (11 mmol) of N-hydroxysuccinimide and 2·27 g (11 mmol) of N,N-dicyclohexylcarbodiimide (DCCI) were added. The mixture was stirred for 2 h (solution A). To a solution of 2·1 g (10 mmol) H-Phe(NO_2)-OH in 10 ml of 1M KOH, 2·2 g (10 mmol) of 15-crown-5 and 15 ml ot DMFA were added. The water was evaporated under reduced pressure (50°C). The solution A was filtered and added to this solution and the mixture was stirred for 16 h. Then it was filtered, and 0·6 ml (10 mmol) AcOH, 30 ml of ethyl acetate and 60 ml of the saturated aqueous solution of NaCl were added. The organic phase was separated and washed with saturated aqueous solution of NaCl (3 × 30 ml). Organic solution was dried over $MgSO_4$, evaporated, and the residue crystallized from ether to yield 2·65 g (65%) of Boc-Pro-Phe(*p*-NO_2)-OH: m.p. 180—182°C; $[\alpha]_{578}^{20}$ —9·0° (*c* 1·3, MeOH); R_F(A) 0·41. For $C_{18}H_{23}N_3O_7$ (393·18) calculated: 35·3% C, 45·1% H, 5·9% N, 13·7% O; found: 35·5% C, 44·9% H, 5·8% N, 13·8% O.

N-t-Butyloxycarbonylprolyl-tyrosine

This compound was prepared in a similar manner. The substance was isolated on a column (70 × 3 cm) of silica gel, eluted with chloroform–methanol (1 : 0, 19 : 1, 10 : 1) to afford 76% of Boc-Pro-Tyr-OH: oil, $[\alpha]_{578}^{20}$ —27·0° (*c* 1·6, MeOH); R_F(A) 0·31. For $C_{18}H_{24}N_2O_6$ (364·18) calculated: 36·0% C, 48·0% H, 4·0% N; found: 36·3% C, 47·9% H, 4·0% N.

Prolyl-*p*-nitrophenylalanyl-glycinamide Hydrochloride (*II*)

To a solution of 0·3 g (2·8 mmol) of glycinamide hydrochloride in 10 ml of DMFA, 0·4 ml (2·8 mmol) of Et_3N and 1·13 g (2·8 mmol) of Boc-Pro-Phe(*p*-NO_2)-OH were added. Then to the cooled (—10°C) mixture 0·35 g (3·1 mmol) of N-hydroxysuccinimide and 0·78 g (3·1 mmol) of N,N-dicyclohexylcarbodiimide were added. The mixture was filtered after mixing for 16 h and 20 ml of ethyl acetate and 30 ml of saturated aqueous solution of NaCl were added to the filtrate, the organic phase was separated, the aqueous phase was extracted with ethyl acetate (30 ml). Ethyl acetate solution was washed with 1M HCl (3 × 50 ml), saturated aqueous solution of NaCl (3 × 50 ml), 5% solution of $NaHCO_3$ (3 × 50 ml) and saturated aqueous solution of NaCl (3 × 50 ml). The residue was chromatographed on a column (70 × 1·5 cm) of silica gel using elution with chloroform–methanol (1 : 0, 19 : 1, 14 : 1). Deblocking of Boc-Pro-Phe(*p*-NO_2)-

TABLE II

Chemical shifts of the peptide protons

Peptide	Amino acid	δ, ppm						
		$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	Ar	NH	NH_2
MIF	Pro	3·75	2·30; 1·87	1·87	3·18		10·19; 8·50[a]	
	Leu	4·32[b]	1·51	1·64	0·94		8·82	
	Gly	3·63					8·21	7·20; 7·41
I	Pro	4·14	2·28; 1·89	1·82	3·14		10·17; 8·43[a]	
	Phe	4·55	3·08; 2·85			7	8·98	
	Gly	3·63					8·47	5·70
		3·71						
II	Pro	4·13	2·27; 1·83	1·82	3·15		10·16; 8·41[a]	
	Phe(p-NO_2)	4·66	3·25; 2·97			7·60; 8·13	9·04	
	Gly	3·68					8·57	7·17; 7·40
III	Pro	4·11	2·28; 1·81	1·81	3·13		10·04; 8·45[a]	
	Phe(p-NH_2)	4·57	2·87			7·32; 7·44[c]	8·98	10·65
	Gly	3·61					8·68	[d]
IV	Pro	4·15	2·28; 1·85	1·85	3·16		10·06; 8·44[a]	
	Phe	4·51	3·03; 2·78			7·20; 7·53	8·92	
	γ-Abu	2·44	1·89	2·82			10·28	8·25
	Gly	3·71					8·39	7·09; 7·31
V	Pro	4·13	2·27; 1·83	1·83	3·15		10·06; 8·44[a]	
	Tyr	4·45	2·96; 2·71			6·66; 7·06	8·86	
	Gly	3·63					8·37	7·23; 7·43

[a] N-protonated peptide. [b] Chemical shifts were identified by 2D NMR SECSY[11]. [c] Theoretical calculation of the chemical shifts of aromatic protons according to the additive scheme by Beeby–Sternhell[12] shows the exchange of HCl proton between both amino moieties. It is confirmed by the broad common peaks of both amino moieties. [d] The chemical shifts of this signals are similar to chemical shifts of aromatic protons.

-Gly-NH_2 with 4M HCl/dioxane yielded 0·77 g (69%) of the desired product, m.p. 148–150°C, $[\alpha]_{578}^{21}$ −11·0° (*c* 1·6, MeOH), R_F(B) 0·70. For $C_{15}H_{18}ClN_5O_5$ (383·60) calculated: 34·1% C, 40·9% H, 2·2% Cl, 11·4% N; found: 34·0% C, 40·6% H, 2·4% Cl, 11·5% N.

Prolyl-tyrosyl-glycinamide Hydrochloride (*V*)

The peptide was prepared in a similar manner. Yield 49%, m.p. 143–145°C, $[\alpha]_{572}^{20}$ +72·5° (*c* 1·2, MeOH), R_F(B) 0·67. For $C_{15}H_{20}ClN_4O_4$ (355·60) calculated: 34·1% C, 45·5% H, 2·2% Cl, 9·1% N; found: 34·3% C, 45·3% H, 2·1% Cl, 9·0% N.

Methyl N-t-Butyloxycarbonylprolyl-*p*-nitrophenylalanyl-glycinate

This compounds was prepared from Boc-Pro-Phe(NO_2)-OH and H-Gly-OMe in a similar manner. Yield 75%, oil, $[\alpha]_{578}^{20}$ −54·0° (*c* 1·1, MeOH), R_F(A) 0·42. For $C_{21}H_{25}N_4O_8$ (461·21) calculated: 36·2% C, 43·1% H, 6·9% N; found: 36·4% C, 43·0% H, 7·0% N.

Prolyl-*p*-aminophenylalanyl-glycinamide Dihydrochloride (*III*)

A solution of 1·6 g (3·5 mmol) Boc-Pro-Phe(NO_2)-Gly-OMe in 10 ml of methanol was stirred under hydrogen in the presence of 0·1 g of 10% Pd/C; then the solution was filtered and the solvent was evaporated. The solution of the residue in 150 ml of methanol was saturated with dried NH_3 at 0°C and allowed to stand for 8 h at room temperature. The solution was filtered and evaporated under reduced pressure. Boc-Pro-Phe(NH_2)-Gly-NH_2 was isolated on a column (70 × 2 cm) of silica gel using chloroform–methanol mixture (19 : 1, 17 : 1, 15 : 1). Compound *III* was obtained after deblocking with 4M HCl/dioxane. Yield 1·13 g (87%), oil, $[\alpha]_{578}^{20}$ +37·0° (*c* 1·5, MeOH), R_F(B) 0·45. For $C_{15}H_{21}ClN_5O_3$ (354·60) calculated: 33·3% C, 46·7% H, 2·2% Cl, 11·1% N; found: 33·5% C, 46·4% H, 2·2% Cl, 11·3% N.

Prolyl-*p*-(γ-aminobutyrylamino)phenylalanyl-glycinamide Dihydrochloride (*IV*)

To a solution of 0·81 g (4 mmol) Boc-Abu-OH[10] and 1·79 g (4 mmol) of Boc-Pro-Phe(NH_2)-Gly-OMe in 15 ml of ethyl acetate, 0·51 g (4 mmol) of N-hydroxysuccinimide and 0·91 g (4·4 mmol) DCCI were added at −10°C and the mixture was stirred for 24 h. The precipitate was filtered off, the filtrate was washed with 1M HCl (3 × 20 ml), saturated aqueous solution of NaCl (3 × 20 ml), 5% $NaHCO_3$ (3 × 20 ml) and saturated aqueous solution of NaCl (3 × 20 ml). The organic phase was dried over $MgSO_4$ and evaporated. A solution of the residue in 150 ml of methanol was saturated with dried NH_3 at 0°C and allowed to stand for 72 h at room temperature. The solution was evaporated under reduced pressure, and the residue was chromatographed on column (70 × 2 cm) of silica gel, using chloroform–methanol (1 : 0, 19 : 1, 15 : 1). Compound *IV* was obtained after deblocking with 4M HCl/dioxane to give 0·92 g (48%), $[\alpha]_{578}^{20}$ +6·5° (*c* 1·4, MeOH), R_F(C) 0·33. For $C_{19}H_{30}Cl_2N_6O_4$ (477·09) calculated: 31·1% C, 49·2% H, 3·3% Cl, 9·8% N; found: 31·3% C, 49·0% H, 3·4% Cl, 9·6% N.

Pharmacological Methods

The antidepressant activity of the analogues was determined according to the method of Porsolt[8] using mice weighing 18–20 g. A single experimental animal was placed into a narrow beaker (diameter 10 cm, height 25 cm), filled with water to 1/3 of the volume at 23°C. The activity of the peptides was estimated from the duration of immobilization of mices in water with respect to the control. Animals were observed for 6·5 min the record began in the 2nd min. All mice received

5–0·001 mg/kg of the hypodermic dose of the tested compounds dissolved in saline 5 min before a test. The results were calculated by Wilkinson–Mann–Witney aparametric criterion[13].

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