NOVEL ANALOGS OF THYROLIBERIN

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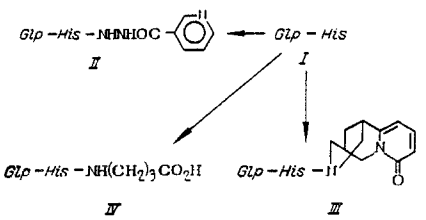
UDC 615.214.32:577.175.829+615.357:577.175.829].017:615.214.32

Since the discovery of psychotropic activity in thyroliberin (Glp-His-Pro-NH_2, TRH) [6], numerous derivatives have been prepared. The structure of this neuropeptide was modified with a view to separating and increasing its antidepressant and hormonal effects, and to increasing its resistance to enzymic degradation.

We have obtained analogs of thyroliberin in which the proline amide moiety is replaced by the acyclic and heterocyclic fragments of biologically active compounds. Compounds (II) and (III) were obtained by the carbodiimidazole method in the presence of N-hydroxysuccinimide, by coupling pyroglutamylhistidine (I) [1] with nicotinic acid hydrazide and cytisine



Compound (IV) was synthesized by the "salt condensation" method previously described by us, in an anhydrous medium [1]. Acylation of pyroglutamylhistidine hydrazide (VI), readily obtained from the methyl ester (V) [3] in the presence of catalytic amounts of butanol, with arachidonic acid gave (VII):

$$\text{Glp-His-OMe} \xrightarrow{} \underset{\text{V}}{\text{Glp-His-NHNH}_2} \xrightarrow{} \underset{\text{VI}}{\text{Glp-His-NHNHCO(CH}_2)_3\text{(CH=CHCH}_2)_4\text{(CH}_2)_3\text{CH}_3}.$$
$$\text{VII}$$

The hormonal activity of thyroliberin (stimulation of the secretion of thyrotropin) is known to restrict the use of TRH as a psychotropic drug. Replacement of histidine in thyroliberin by phenylalanine has little effect on its anticataleptogenic effect, but reduces its hormonal activity by a factor of ten [7]. Consequently, in order to obtain psychotropic compounds devoid of hormonal activity we have prepared compounds (XII) and (XIII), which are derivatives of the hydrazide (VI):

$$\underset{\text{VIII}}{\text{Glp}} + \underset{\text{IX}}{\text{DL-Phe-OMe}} \xrightarrow{} \underset{\text{X}}{\text{Glp-DL-Phe-OMe}} \xrightarrow{} \underset{\text{XI}}{\text{Glp-DL-Phe-NHNH}_2} \xrightarrow{}$$
$$\xrightarrow{} \underset{\text{XII}}{\text{Glp-DL-Phe-NHNHCO(CH}_2)_2\text{CO}_2\text{H}} \xrightarrow{} \underset{\text{XIII}}{\text{Glp-DL-Phe-NHNHCO(CH}_2)_2\text{NHCO}_3\text{C}_6\text{H}_2\text{(OMe)}_3\text{-3, 4, 5}}$$

The condensation of pyroglutamic acid (VIII) with phenylalanine methyl ester (IX) was effected in acetonitrile by the carbodiimide method. The hydrazimide (XI) obtained from the ester (X) was acylated with succinic anhydride to give the succinoyl derivative (XII), which on reaction with mescalin gave the pseudopeptide (XIII).

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 0091-150X/87/2111-0760$12.50

The products were purified by column chromatography on silica gel, and by recrystallization. The purity of the compounds was checked by TLC, and their composition and structure by elemental and amino acid analysis, IR spectroscopy, and mass spectrometry.

EXPERIMENTAL (CHEMISTRY)

IR spectra were recorded on a Specord IR spectrophotometer (East Germany) in chloroform, KBr disks, and Nujol. Mass spectra were obtained on a Varian MAT-112 spectrometer (Switzerland), ionizing electron energy 50-70 eV. The compounds were chromatographed on Silufol plates (Kavalier, Czech SSR) in the solvent systems: (A) ethanol—water (7:3), (B) ethyl acetate—pyridine—acetic acid—water (5:5:1:3), (C) benzene—acetone—acetic acid (100:50:1), (D) butanol—acetic acid—water—ethanol—ammonia (4:1:1:12:3), (E) benzene—acetone—acetic acid—butanol—water (100:50:2:4:1), and (F) butanol—acetic acid—water (4:1:1). The chromatographic plates were developed with chlorotoluidine reagent. Amino acid analyses were carried out on a Hitachi amino acid analyser (Japan) with prior hydrolysis of the peptides in 6 N hydrochloric acid at 110°C for 24 h. The amino acid and elemental analyses were in good agreement with the calculated values. The elemental analyses are shown in Table 1. The specific rotations of the compounds were measured on a Perkin-Elmer 141 spectropolarimeter (West Germany). The amino acids used for the synthesis of the peptides were of the L-configuration, with the exception of DL-phenylalanine, obtained from Merck (Switzerland) and Reanal (Hungary). The abbreviations used in the text are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [5].

N-(Pyroglutamylhistidyl)-N'-nicotinoylhydrazine(II). To 532 mg (2 mole) of (I) in 15 ml of DMF was added 274 mg (2 mmole) of nicotinic acid hydrazide, 253 mg (2.2 mmole) of N-hydroxysuccinimide, and at 0°C 453 mg (2.2 mmole) of dicylohexylcarbodiimide. The mixture was stirred at room temperature for 24 h, filtered, the solid washed with 5 ml of DMF, and the filtrate evaporated under reduced pressure at 40-50°C. The residue was recrystallized from a mixture of methanol and ethyl acetate (4:1) to give 330 mg (43%) of (II), mp 250-255°C (decomp.), R_f 0.52 (B). IR spectrum, ν_{max}, cm^{-1} (KBr): 3260, 1660, 1630, 1533, 700. Mass spectrum, m/z 385. Compounds (III) and (VII) were obtained similarly.

N-(Pyroglutamylhistidyl)cytisine (III), yield 32%, mp 100-105°C (decomp.), R_f 0.45 (B). IR spectrum, ν_{max}, cm^{-1} (KBr): 3210, 1650, 1620, 1520, 1415. $[\alpha]_{578}^{20} = -130.2°$ (c 1.3, methanol).

N-(Pyroglutamylhistidyl)-N'-(cis-5-cis-8-cis-11-cis-14-eicosanetetraenoyl)-hydrazine (VII), yield 28%, mp 225-227°C, R_f 0.77 (C). IR spectrum, ν_{max}, cm^{-1} (KBr): 3320, 1630, 1570, 1425. Mass spectrum, m/z 566.

N-(Pyroglutamylhistidyl)-4-aminobutyric Acid (IV). 4-Aminobutyric acid (206 mg, 2 mmole) was dissolved in 1 ml of 2 N NaOH, and 0.44 g (2 mmole) of 15-crown-5 and 20 ml of DMF added. Water and 1-2 ml of DMF were evaporated under reduced pressure [2], and the residue treated with a mixture of 532 mg (2 mmole) of the peptide (I), 263 mg (2.2 mmole) of N-hydroxysuccinimide, and 453 mg (2.2 mmole) of dicyclohexylcarbodiimide in 20 ml of DMF. The mixture was stirred at room temperature for 24 h, 2 ml of 1 N HCl added, filtered, the solid washed with 5 ml of DMF, and the filtrate evaporated under reduced pressure at 40-50°C. The residue was chromatographed on silica gel (70× 1.5 cm column), and successively eluted with the solvent systems chloroform—methanol (3:2, 2:3, and 1:4) to give 210 mg (30%) of (IV), mp 225-230°C (with charring), R_f 0.44 (A). IR spectrum, ν_{max}, cm^{-1} (KBr): 3330, 1650, 1640, 1535.

Pyroglutamyl-DL-phenylalanine Methyl Ester (X). To 2.15 g (10 mmole) of DL-phenylalanine methyl ester hydrochloride (IX) and 1.29 g (10 mmole) of pyroglutamic acid (VIII) in 40 ml of acetonitrile were added 1.5 ml (10 mmole) of triethylamine, 1.26 g (11 mmole) of N-hydroxysuccinimide, and at 0°C 2.26 g (11 mmole) of dicyclohexylcarbodiimide. The mixture was stirred at room temperature for 24 h, filtered, the solid washed with 10 ml of acetonitrile, and the filtrate evaporated under reduced pressure. The residue was dissolved in ethyl acetate, the organic layer washed with 10 ml of 1 N NaOH, 10 ml of 1 N HCl, and water, dried over $MgSO_4$, the ethyl acetate removed, and the residue recrystallized from a mixture of ethyl acetate and hexane (2:1) to give 1.88 g (65%) of (X), mp 141-143°C, R_f 0.77 (E). IR spectrum, ν_{max}, cm^{-1} (CCl_4): 3415, 1730, 1670, 1590, 1265. Mass spectrum, m/z 290. $[\alpha]_D^{20} = -1.0°$ (c 1.4, methanol).

TABLE 1. Elemental Analyses of Compounds Obtained

Compound	Found, %			Empirical formula	Calculated, %		
	C	H	N		C	H	N
II	53,3	4,9	25,3	$C_{17}H_{18}N_7O_4$	53,1	4,7	25,5
III	63,6	6,1	20,0	$C_{22}H_{25}N_6O_4$	63,3	6,0	20,1
IV	51.4	5,5	20,2	$C_{15}H_{20}N_5O_5$	51,4	5,7	20,0
VII	68,0	8,2	15,0	$C_{31}H_{45}N_6O_4$	68,3	8,3	15,4
X	62,0	6,3	9,8	$C_{15}H_{18}N_2O_4$	62,1	6,2	9,7
XI	57,9	6,6	19,2	$C_{14}H_{18}N_4O_3$	57,9	6,2	19,3
XII	55,8	5,8	14,2	$C_{18}H_{22}N_4O_6$	55,4	5,6	14,4
XIII	62,6	6,5	12,7	$C_{29}H_{37}N_5O_8$	62,3	6,6	12,5

TABLE 2. Effects of Thyroliberin Analogs on the Duration of Hexenal Sleep in Mice

Compound	Number of animals	Duration of sleep (M ± m)
Hexenal, 75 mg/kg	18	110,7±12,7
TRH	17	19,8±3,0*
II	10	86,4±9,07
III	10	105,9±20,2
IV	10	21,7±4,0*
XII	10	42,2±16,5*
XIII	9	39,0±10,4*

Note. TRH and the other compounds were introduced together with hexenal. The asterisk denotes $P < 0.05$ with respect to hexenal.

TABLE 3. Effects of Thyroliberin Analogs on Development of Haloperidol Catalepsy in Mice.

Compound	Number of animals	Duration of catalepsy, with (M ± m)	
		after 1 h	after 3 h
Haloperidol, 10 mg/kg	35	158,5±7,2	158,8±6,4
TRH	35	124,7±7,2*	155,5±6,9
II	10	180,0±0,0*	167,5±13,3
III	10	180,0±0,0*	180,0±0,0*
IV	10	147,2±17,8	163,8±17,5
VII	9	180,0±0,0*	180,0±0,0*
XII	9	155,4±16,5	88,4±21,4*
XIII	10	147,4±17,6	140,5±17,2

Note. TRH and the other compounds were administered together with haloperidol. The asterisk denotes $P < 0.05$ with respect to haloperidol.

Pyroglutamyl-DL-phenylalanine Hydrazide (XI). A mixture of 1.16 g (4 mmole) of (X), 4 ml of methanol, and 2 ml of butanol was heated for 1 h at the boil with 0.66 ml (12 mmole) of 94.5% hydrazine hydrate. The mixture was cooled, and after 24 h the colorless, crystalline solid was filtered off, and washed with methanol and ether to give 1.12 g (96%) of product, mp 178-180°C, R_f 0.57 (E). IR spectrum, ν_{max}, cm^{-1} (KBr): 3260, 1650, 1530. Mass spectrum, m/z 290. $[\alpha]_D^{20} = +2.8°$ (c 1.2, DMF).

Studies of the eye's hydrodynamics in glaucoma patients using rheoophthalmography did not reveal any verifiable changes in the initial value of the rheographic coefficient (RC) within 30 min or 1 h, however, in 2 h after application a verifiable ($P < 0.05$) increase of the RC was noted. As shown by clinical observations, in the overwhelming majority of glaucoma patients the reduction in arterial pressure is temporary, and the initial level returns after about 3 h. At the same time, the hypotensive effect of clofelin on the IOP is sustained for 6-8 h, which is evidently due to the stability of the perfusion pressure of the blood in the vessels of the eye.

The abvisability of prolonged prescription of clofelin is determined by its hypotensive effect and the degree of side effects. Regular use of clofelin causes a stable reduction of the IOP in 70% of glaucoma patients examined. Along with the hypotensive action, a significant reduction in the range of daily IOP oscillations is simultaneously observed. The reduction of IOP is due to a verifiable decrease in the production of ocular fluid and an increase in the discharge [2, 8, 15]. However, in spite of the favorable effect clofelin has on the hydrodynamcis of the eye in some patients, tolerance (tachyphylaxia) to the preparation may develop several months after the initial treatment [48].

In light of the above discussion, during prolonged use of clofelin it is necessary to make regular checks of the preparation's effectiveness, including regular (every 1-3 months) measurements of the intraocular and blood pressures and a visual field examination.

Results of long-term (over the course of 5-9 years) observations of glaucoma patients receiving regular instillations of clofelin with good hypotensive and functional effects were produced by the Helmholtz Scientific Research Institute of Eye Disease (Moscow).

Instillation of 0.25 and 0.5% clofelin solutions can cause such general side effects as dryness of the mouth, which is observed in 60% of patients; however, this effect decreases and sometimes disappears in 2-3 weeks after the initial treatment. Among the more rarely seen complications are weakness, drowsiness, and reduced systemic arterial pressure. Among localized side effects which may arise from time to time are hyperemia of the conjunctiva, which appears 1-2 h after the instillation of clofelin, lasts for 2-3 h, and disappears on its own.

In concluding, the following features of this preparation should be stressed. Clofelin has a pronounced hypotensive activity and therefore is a highly effective treatment for primary wide-angle glaucoma. An advantage of this preparation over other hypotensive compounds is its lack of effect on the pupil, which favorably distinguishes from miotic and sympathomimetic compounds. Also among the positive aspects of this preparation is the possibility of its use in conjunction with pilocarpine and β-adrenergic blockers.

The possibilites of hypotensive glaucoma therapy are significantly broadened due to the use of β-adrenergic blocking compounds (BABC).

Propranolol (Inderal, Obsidan, Anapriline) is the first BABC which has enjoyed widespread clinical application. In a work by Phillips et al. [50] published in 1967, data were presented concerning the reduction of the IOP in glaucoma patients receiving propranolol orally or intravenously at an internal medicine clinic. Later, a form of propranolol was created for optical use: 1 or 2% solutions (Tonum), the use of which produced a pronounced hypotensive effect that was intensified in a series of cases upon prolonged, regular use [26, 28]. According to data of Bietty [27], instillation of propranolol to 300 glaucoma patients led to a marked reduction of the IOP in 77% of them. In some patients, the IOP reduction under the effect of propranolol began only after 3 weeks following the initial treatment. The hypotensive effect of propranolol is intensified when used in combination with pilocarpine.

Among the positive aspects of propranolol are its lack of effect on the pupil width and its lack of general complications in localized use. The hypotensive effect of propranolol is due mainly to its decreasing the production of ocular fluid; the combined use of propranolol and adrenaline improves the discharge of ocular fluid.

A form of anapriline for use in the eye (as a 1% solution) which was used successfully in clinical studies was developed in a joint effort by the faculties of the I. M. Sechenova Moscow Medical Institute and the Helmholtz Scientific Research Institute of Eye Disease (Moscow) and was presented in the Pharmacological Committee of the USSR Minzdrava [11].

N-(Pyroglutamyl-DL-phenylalanyl)-N'-(β-carboxypropionyl)hydrazine (XII). A solution of 488 mg (1.68 mmole) of (XI) in 4 ml of glacial acetic acid was treated with 168 mg (1.68 mmole) of succinic anhydride. The mixture was stirred at room temperature for 20 min, and the solid filtered off, washed with ether, and recrystallized from a mixture of methanol and ether (3:1) to give 518 mg (80%) of product, mp 165°C, R_f 0.55 (F). IR spectrum, γ_{max}, cm^{-1} (CCl_4): 3500, 3370, 1740, 1670, 1570. $[\alpha]_D^{23°} = +4.4°$ (c 1.4, DMF).

N-(Pyroglutamyl-DL-phenylalanyl)-N'-[2-(3,4,5-trimethoxyphenyl)ethyl]-aminosuccinoyl hydrazine (XIII) was obtained by carbodiimide condensation, as for (II), from the hydrazide (XII) and mescalin. After removal of the DMF, the residue was chromatographed on silica gel and eluted with chloroform, yield 25%, mp 190-192°C, R_f 0.75 (F). IR spectrum, γ_{max}, cm^{-1} ($CHCl_3$): 3430, 1650, 1570, 1115.

EXPERIMENTAL (PHARMACOLOGY)

The neurotropic activity of (II-IV), (VII), (XII), and (XIII) was examined in mice, by their ability to change the hexenal sleep time and influence the development of haloperidol catalepsy. The results were compared with the effects of thyroliberin (rifathyroin, Biolar, Riga) in equal doses. In order to asses the effects on the duration of hexenal sleep in mice weighing 15-20 g, the test compounds were administered in a dose of 30 mg/kg together with hexenal in a dose of 75 mg/kg. The sleep time was taken to be the time from the moment of loss of the turnover reflex until its return.

The presence and depth of catalepsy induced by haloperidol was assessed by the ability of the mice to maintain an enforced pose (placing the forepaws of the animal on a cube of height 5 cm) for 180 sec. The animals were tested every 30 min for 3 h following the simultaneous administration of haloperidol in a dose of 10 mg/kg and the test compound in a dose of 1 mg/kg.

Statistical treatment was carried out using Student's t-criterion. The results are shown in Tables 2 and 3.

In the hexenal sleep test, the activity of (IV) was similar to that of thyroliberin, which shortened the duration of hexenal sleep [4]. Compounds (XII) and (XIII), which contain the DL-phenylalanine and succinic acid residues, also showed a certain amount of activity.

In the haloperidol catalepsy test, (XII) is noteworthy in showing anticataleptic activity superior to that of thyroliberin after 3 h. Compounds (II), (III), and (VII), which do not contain the succinic acid moiety, increased the duration of catalepsy.

Hence, replacement of the proline amide moiety in thyroliberin by the 4-aminobutyric acid residue (IV), and replacement of the His-Pro-NH_2 fragment by DL-Phe-NHNHCO (XII) results in retention of neurotropic activity of TRH, but somewhat modifies its effects on the CNS.

LITERATURE CITED

1. S. A. Andronati and A. A. Mazurov, Bioorg. Khim., 10, 1445-1446 (1984).
2. S. K. Ogorodnikov, T. M. Lesteva, and V. B. Kogan, Azeotropic Mixtures [in Russian], Leningrad (1971), p. 62.
3. British Patent No. 1 592 552 (1981); Ref. Zh. Khim., No. 21, 0 20P (1982).
4. G. R. Breese, J. M. Cott, B. R. Cooper, et al., J. Pharmacol. Exp. Ther., 193, No. 1, 11-22 (1975).
5. IUPAC-IUB Joint Commission of Biological Nomenclature, Pure Appl. Chem., 56, No. 5, 595-624 (1984).
6. A. J. Prange, Jr., P. P. Lara, I. C. Wilson, et al., Lancet, 2, 999-1002 (1972).
7. T. Szirtes, L. Kisfaludy, E. Palosi, and L. Szporny, J. Med. Chem., 27, 741-745 (1984).