STEREOSELECTIVITY OF THE ANTIDOPAMINE EFFECT OF N-CAPROYLPROLYLTYROSINE METHYLATE – A TRIPEPTIDE NEUROTENSIN ANALOG

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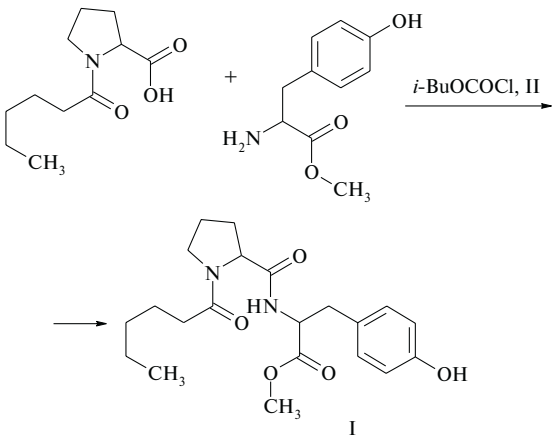
Previously we described the synthesis of a series of N-acylprolyltyrosines showing neuroleptic activity [1]. Prolyltyrosines were initially designed as analogs of sulpiride – an atypical benzamide neuroleptic [2]. It was suggested that both sulpiride and its dipeptide analogs represent exogenous ligands for the receptors of neurotensin – a tridecapeptide possessing neuroleptic-like activity [3, 4].

An analysis of the molecular conformations of sulpiride, Pro-Tyr-NH_2, and neurotensin (Fig. 1) showed that Leu^{13} – a side radical of neurotensin that is important for its binding to receptors – can be modeled by a hydrophobic terminal N-radical of prolyltyrosine. Study of the structure – activity relationship in the series of N-acylprolyltyrosines allowed N-caproylprolyltyrosine methylate (I) to be selected as a promising atypical neuroleptic for extended pharmacological characterization [5].

It was established that this peptide (0.4 – 4.0 mg/kg, i.p.) exhibited activity in dopamine-dependent tests (such as apomorphine-induced verticalization and stereotypy and *L*-DOPA-induced stereotypy and violation of the extrapolation avoidance reflex), decreased the body temperature, and potentiates hexenal action. It is also important to note that, at these doses, the peptide does not affect motor activity, coordination of movements, level of alertness, emotional state, and pain sensitivity threshold. N-Caproylprolyltyrosine methylate (I) is nontoxic and does not produce catalepsy at doses below 500 mg/kg (i.p.) [6].

The purpose of this work was to study stereospecificity of the antidopamine effect of peptide I in order to verify the hypothesis concerning the receptor mechanism of its neuroleptic action and estimate the effect of a diastereomer admixture on the activity.

The diastereomers of compound I were synthesized by the method of mixed anhydrides proceeding from N-caproylproline and tyrosine methylate. The mixed anhydride was obtained from isobutylformate and the tertiary base was represented by N-ethylmorpholine (II):



I

The diastereomer purity of the target peptides (above 98%) was confirmed by ^{1}H NMR (250 MHz). A double set of proton signals from $CH_3(CH_2)_4$, $C^{\beta}H_2$Tyr, NHTyr, $C^{\alpha}H$ Pro, and CH_3O observed in the spectra of all diastereomers is manifested due to the *trans – cis* isomerism relative to the tertiary imide bond –C(O)–N< [7].

The antidopamine activity of the synthesized peptides was determined using the most selective and sensitive behavioral test for neuroleptics – a decrease in the apomorphine-induced verticalization in mice [8]. Investigation of the effect of amino acid configuration in peptide I upon its antidopamine activity showed that substitution of the optical isomers for *L*-proline and *L*-tyrosine leads to the loss or reversal of activity (see Table 1). While the peptide $CH_3(CH_2)_4C(O)$-*L*-Pro-*L*-Tyr-OCH_3, possessing a natural configuration of amino acid residues, suppresses the apomorphine verticalization at a dose of 0.4 and 0.8 mg/kg (by 21 and 30%, respectively), the replacement of any amino acid by its *D*-isomer leads to the loss of this effect: neither $CH_3(CH_2)_4C(O)$-*D*-Pro-*L*-Tyr-OCH_3 nor $CH_3(CH_2)_4C(O)$-*L*-Pro-*D*-Tyr-OCH_3 are ac-

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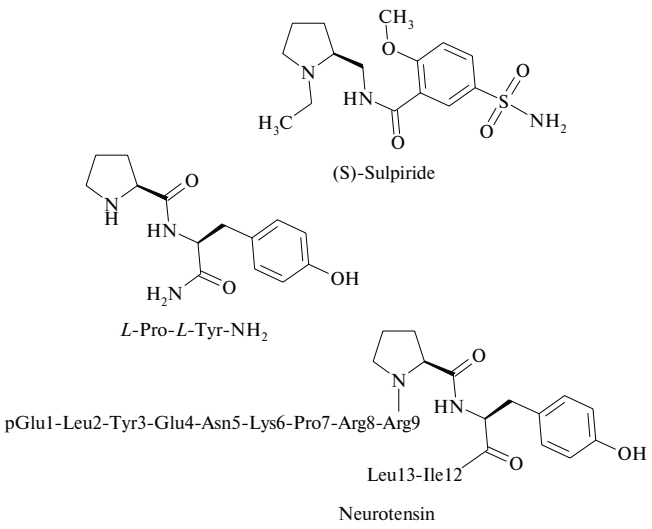

Fig. 1. Structural formulas of sulpiride, *L*-Pro-*L*-Tyr-NH_2, and neurotensin.

tive at these doses. Moreover, the enantiomer $CH_3(CH_2)_4C(O)$-*D*-Pro-*D*-Tyr-OCH_3 at a dose of 0,4 mg/kg inhibits the prodopamine effect (increases the apomorphine verticalization by 50%). The latter fact indicates that N-caproyl-*L*-prolyl-*L*-tyrosine methylate acts by a receptor mechanism rather than through the inhibition of proteases participating in the neuropeptide processing.

The results of our experiments are indicative of an important role of the Pro^{10} – Tyr^{11} fragment as an active center of neurotensin responsible for the neuroleptic-like activity. This conclusion agrees with the data of [9, 10], according to which the *L*-configuration of the Pro^{10} and Tyr^{11} residues of the neurotensin molecule are necessary for its binding to receptors.

Thus, the action of N-caproylprolyltyrosine methylate (I) is stereoselective and requires that both amino acid residues occur in the *L*-configuration. The optical purity is an important condition for manifestation of the antidopamine activity of this promising atypical neuroleptic.

EXPERIMENTAL CHEMICAL PART

The melting temperatures were determined by the open capillary technique without corrections. The ^1H NMR spectra were measured on a Bruker AC-250 spectrometer (Germany) using DMSO-d_6 as the solvent and TMS as the internal standard. The specific optical rotation was measured on a Perkin-Elmer Model 241 polarimeter. TLC was conducted on Silufol Kavalier plates (Czech Republic) developed by exposure to iodine vapors. The column chromatography was effected in columns filled with Kieselgel 100 (Merck). The solvents were purified and dehydrated by conventional methods. The data of elemental analyses for C, H, N agree with the results of analytical calculations for $C_{21}H_{30}N_2O_5$.

General method for the synthesis of diastereomers of N-caproylprolyltyrosine methylate (I). To a solution 10 ml of the carboxyl component (N-caproylproline) in 50 ml of chloroform are added by dropping from different funnels with stirring and cooling to –15°C (on an ice – NaCl mixture) 10 mmole of N-ethylmorpholine and 10 mmole of isobutylchloroformate, after which the mixture is stirred for 2 – 3 min. To this mixture are slowly added 10 mmole of amino component (tyrosine methylate hydrochloride) in 10 ml of DMF containing 10 mmole of N-ethylmorpholine. The reaction mixture is stirred for 30 min at –15°C and then 1 h at room temperature. The precipitate is separated by filtration and the residual solvent evaporated in vacuum. The precipitate is dissolved in chloroform and washed sequentially with 5% $NaHCO_3$, water, 1 N HCl, and water again un-

TABLE 1. The Activity of Diastereomers of N-Caproylprolyltyrosine Methylate (I) in the Apomorphine-Induced Verticalization Test in Mice

Compound	Dose, mg/kg (i.p.)	Verticalization		Activity, %
		Total rating	%	
$CH_3(CH_2)_4CO$-*L*-Pro-*L*-Tyr-OCH_3	0.4	76 ± 4.6	79*	21*
	0.8	67 ± 18.8	70*	30*
Control		96 ± 16.7	100	
$CH_3(CH_2)_4CO$-*L*-Pro-*D*-Tyr-OCH_3	0.4	90.5 ± 21.1	109	– 9
	0.8	89.5 ± 15.5	108	–8
Control		82.7 ± 30.7	100	
$CH_3(CH_2)_4CO$-*D*-Pro-*L*-Tyr-OCH_3	0.4	90.7 ± 21.4	96	4
	0.8	82.3 ± 30.2	88	12
Control		94 ± 24.8	100	
$CH_3(CH_2)_4CO$-*D*-Pro-*D*-Tyr-OCH_3	0.4	105.2 ± 10.1	149*	– 49*
	0.8	86.1 ± 20.8	122	– 22
Control		70.5 ± 34.1	100	

* Difference from control reliable for $p < 0.05$; each compound tested in a group of six mice.

til reaching pH ~7. The the solution is dehydrated over $MgSO_4$, the solvent evaporated, and the product purified by column chromatography using chloroform as eluent.

N-Caproyl-*L*-prolyl-*L*-tyrosine methylate [$C_3(CH_2)_4C(O)$-*L*-Pro-*L*-Tyr-OMe] was obtained from N-caproyl-*L*-Pro-OH and *L*-Tyr-OMe · HCl with a 54% yield. The product appears as a white crystalline substance; m.p., 115 – 116°C (from ethyl acetate); $[\alpha]_D^{20}$, – 58.4° (*c*, 0.4; chloroform); R_f, 0.50 (chloroform – ethanol, 9 : 1); IR spectrum in KBr disks (ν_{max}, cm^{-1}): 3300 (NH, OH); 1735 (COOMe); 1660, 1638, 1620 (CONH, CON<); ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 0.84, 0.87 (tt, 3H, $CH_3(CH_2)_4$), 1.02 – 2.20 (m, 12H, $C^{\beta}H_2$–$C^{\gamma}H_2$Pro, $CH_3(CH_2)_4$), 2.7 – 3.1 (m, 2H, $C^{\beta}H_2$Tyr), 3.2 – 3.45 (m, 2H, $C^{\beta}H_2$Pro), 3.56, 3.63 (ss, 3H, CH_3O), 4.26, 4.35 (mm, 2H, $C^{\alpha}H$Pro), 4.35, 4.48 (mm, 1H, $C^{\alpha}H$Tyr), 6.65, 6.66, 6.98, 7.00 (mm, 4H, AA′BB′ system, C_6H_4Tyr), 8.04, 8.42 (dd, 1H, NHTyr), 9.23, 9.26 (ss, 1H, OHTyr); $C_{21}H_{30}N_2O_5$.

N-Caproyl-*L*-prolyl-*D*-tyrosine methylate [$CH_3(CH_2)_4C(O)$-*L*-Pro-*D*-Tyr-OMe] was obtained from N-caproyl-*L*-Pro and *D*-Tyr-OMe · HCl with a 47% yield. The product appears as a white crystalline substance; m.p., 143 – 145°C (upon ether treatment); $[\alpha]_D^{25}$, – 104.8° (*c*, 0.4; chloroform); R_f, 0.47 (chloroform – ethanol, 9 : 1); IR spectrum in KBr disks (ν_{max}, cm^{-1}): 3230, 3220, 3070 (NH, OH); 1740 (COOMe); 1665, 1625, 1615 (CONH, CON<); ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 0.85, 0.86 (tt, 3H, $CH_3(CH_2)_4$), 1.10 – 2.27 (m, 12H, $C\delta H_2$–$C^{\gamma}H_2$Pro, $CH_3(CH_2)_4$), 2.7 – 3.03 (m, 4H, $C^{\beta}H_2$Tyr, $C^{\delta}H_2$Tyr), 3.54, 3.61 (ss, 3H, CH_3O), 4.26, 4.29 (mm, 2H, $C^{\alpha}H$Pro), 4.39, 4.43 (mm, 1H, $C^{\alpha}H$Tyr), 6.62, 6.65, 6.96, 6.99 (mm, 4H, AA′BB′ system, C_6H_4Tyr), 8.11, 8.45 (dd, 1H, NHTyr), 9.23, 9.24 (ss, 1H, OHTyr); $C_{21}H_{30}N_2O_5$.

N-Caproyl-*D*-prolyl-*L*-tyrosine methylate [$CH_3(CH_2)_4C(O)$-*D*-Pro-*L*-Tyr-OMe] was obtained from N-caproyl-*D*-Pro and *L*-Tyr-OMe · HCl with a 40% yield. The product appears as a white crystalline substance; m.p., 139 – 142°C (upon ether treatment); $[\alpha]_D^{25}$, + 95.8° (*c*, 0.4; chloroform); R_f, 0.48 (chloroform – ethanol, 9 : 1); IR spectrum in KBr disks (ν_{max}, cm^{-1}): 3240, 3070 (NH, OH); 1740 (COOMe); 1668, 1625, 1618 (CONH, CON<); ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 0.84, 0.87 (tt, 3H, $CH_3(CH_2)_4$), 1.02 – 2.20 (m, 12H, $C^{\beta}H_2$–$C^{\gamma}H_2$Pro, $CH_3(CH_2)_4$), 2.7 – 3.1 (m, 2H, $C^{\beta}H_2$Tyr), 3.2 – 3.45 (m, 2H, $C^{\delta}H_2$Pro), 3.56, 3.63 (ss, 3H, CH_3O), 4.26, 4.35 (mm, 2H, $C^{\alpha}H$Pro), 4.35, 4.48 (mm, 1H, $C^{\alpha}H$Tyr), 6.65, 6.66, 6.98, 7.00 (mm, 4H, AA′BB′ system, C_6H_4Tyr), 8.04, 8.42 (dd, 1H, NHTyr), 9.23, 9.26 (ss, 1H, OHTyr); $C_{21}H_{30}N_2O_5$.

N-Caproyl-*D*-prolyl-*D*-tyrosine methylate [$CH_3(CH_2)_4C(O)$-*D*-Pro-*D*-Tyr-OMe] was obtained from N-caproyl-*D*-Pro and *D*-Tyr-OMe · HCl with a 40% yield. The product appears as a white crystalline substance; m.p., 80 – 84°C (upon ether treatment); $[\alpha]_D^{25}$, +54.4° (*c*, 0.4; chloroform); R_f, 0.48 (chloroform – ethanol, 9 : 1); IR spectrum in KBr disks (ν_{max}, cm^{-1}): 3310, 3230 (NH, OH); 1740 (COOMe); 1660, 1625, 1615 (CONH, CON<); ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 0.84, 0.87 (tt, 3H, $CH_3(CH_2)_4$), 1.0 – 2.3 (m, 12H, $C^{\beta}H_2$–$C^{\gamma}H_2$Pro, $CH_3(CH_2)_4$), 2.81, 2.98 (mm, 2H, C_3H_2Tyr), 3.2 – 3.5 (m, 2H, $C^{\delta}H_2$Pro), 3.56, 3.63 (ss, 3H, CH_3O), 4.25 (m, 2H, $C^{\alpha}H$Pro), 4.33, 4.48 (mm, 1H, $C^{\alpha}H$Tyr), 6.63, 6.65, 6.98, 6.99 (mm, 4H, AA′BB′ system, C_6H_4Tyr), 8.10, 8.42 (dd, 1H, J 7.7 Hz, J 8.4 Hz, NHTyr), 9.21, 9.23 (ss, 1H, OHTyr); $C_{21}H_{30}N_2O_5$.

EXPERIMENTAL PHARMACOLOGICAL PART

Apomorphine-induced verticalization test. The experiments were performed on CC57/BL6 male mice weighing 22 – 25 g. The compounds to be tested were intraperitoneally injected as suspensions in 0.9% NaCl solution containing 2.5% of Tween-80. Apomorphine (5 mg/kg, s.c.) was injected 15 min after test compounds in 0.9% NaCl solution containing 0.1% of ascorbic acid. Animals in the control group in the first stage were injected with physiological solution. The intact (passive control) group received physiological solution in both stages. After apomorphine injection, each animal tested was placed in a wire cylinder with a diameter of 12 cm and a height of 14 cm. The first verticalization characteristics were taken 15 min after apomorphine injections. The observation was continued for 1 h with the characteristics taken every 2 min according to the following scale: all four paws on the floor, 0 (no verticalization); one paw on the wall, 1; two paws on the wall, 2; three paws on the wall, 3; all paws on the wall, 4 (full verticalization). The total rating in the control was taken as 100%. The data were statistically processed in terms of the Wilcoxon – Mann – Whitney criterion.

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