Notes

Dopamine Receptor Modulation by a Highly Rigid Spiro Bicyclic Peptidomimetic of Pro-Leu-Gly-NH_2

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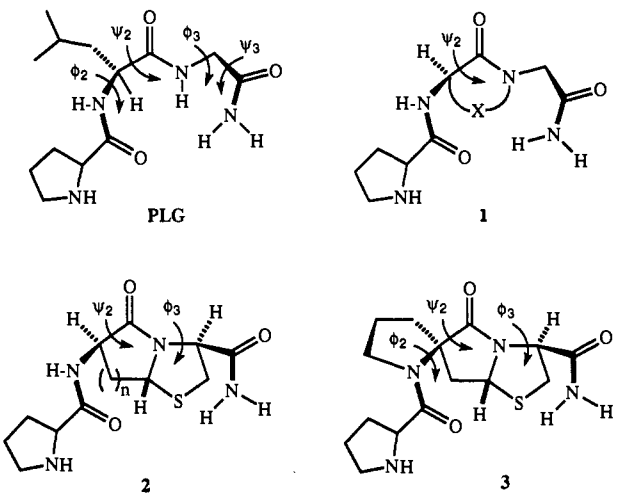
Received June 15, 1993•

A peptidomimetic analogue of Pro-Leu-Gly-NH_2 (PLG), compound **3**, has been synthesized that contains a highly constrained spiro bicyclic type-II β-turn mimic. Peptidomimetic **3** enhanced the binding of the dopamine receptor agonist ADTN to dopamine receptors by 40% at 10^{-6} M. At this same concentration PLG enhanced the binding of ADTN by 26%. Like PLG, **3** exhibited a bell-shaped dose–response curve with the maximum effect occurring at a concentration of 10^{-6} M. Because of the highly rigid nature of the spiro bicyclic type-II β-turn constraint found in **3**, these results lend strong support for the hypothesis that the biologically active conformation of PLG is a type-II β-turn.

The discovery of numerous biologically active peptides has stimulated much work in the areas of peptide–receptor interactions and the relationship between peptide conformation and biological activity. The tripeptide Pro-Leu-Gly-NH_2 (PLG), which is one such biologically active peptide, has been shown to have modulatory effects on dopamine receptors in the central nervous system. For example, PLG increases the affinity of dopamine receptor agonists to dopamine receptors[1] as well as increasing the number of high-affinity agonist binding sites.[2] In addition, PLG has been shown to down-regulate neuroleptic drug-induced supersensitized dopamine receptors.[3,4]

We have previously synthesized a number of conformationally restricted analogues of PLG in order to elucidate its biologically active conformation. These include a series of lactam analogues illustrated by structure **1** in which the ψ_2 torsion angle is restricted[5,6] and a series of bicyclic thiazolidine lactam analogues illustrated by structure **2** in which the ψ_2 and ϕ_3 torsion angles are restricted.[7] The dopamine receptor modulating activity of those analogues in these two series whose backbone torsion angles were restricted to values similar to those found in type-II β-turn led us to hypothesize that this is the bioactive conformation of PLG. However, the lactams (**1**) and bicyclic thiazolidine lactams (**2**) still retain a high degree of conformational freedom, since only one and two, respectively, of the four peptide backbone torsion angles that describe a turn are restricted.

In the present study we report the synthesis and biological activity of peptidomimetic **3** which incorporates the highly rigid spiro bicyclic system designed previously by us as a type-II β-turn mimic.[8] This peptidomimetic is the most highly constrained analogue of PLG made to date.



Synthesis

The synthesis of peptidomimetic **3** is depicted in Scheme I. The key starting material for this synthesis was the *N-tert*-butoxycarbonyl spiro bicyclic methyl ester **4**, the synthesis of which has been described previously by us.[8] Compound **4** was converted to spiro lactam amide **5** by a sequence of three reactions in which the intermediates were not purified, but rather were immediately carried on to the next step. In this sequence of reactions, the *tert*-butoxycarbonyl group was removed from **4** and the product which was obtained was coupled to Boc-Pro-OH using standard peptide coupling conditions. Finally, ammonolysis of the prolyl spiro bicyclic ester provided spiro bicyclic amide **5**. Removal of the *tert*-butoxycarbonyl group from **5** with HCl/dioxane afforded the desired PLG peptidomimetic **3** as its hydrochloride salt.

Pharmacology

The highly constrained PLG analogue **3** was tested for its ability to enhance the binding of the dopamine receptor agonist [^{3}H]-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene ([^{3}H]ADTN) to striatal dopamine receptors *in vitro*. This assay, which has been described previously by us,[5,6] is used as a measure of the ability of PLG and its analogues to modulate dopamine receptors. In this assay, PLG has a bell-shaped dose–response curve with a

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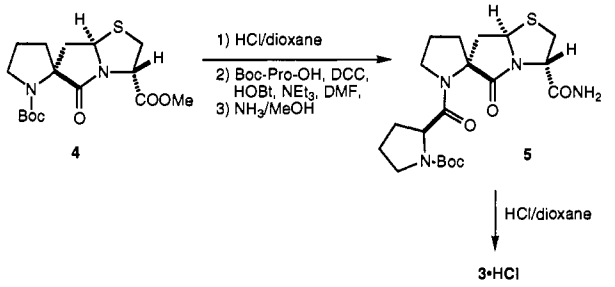
• Abstract published in *Advance ACS Abstracts*, October 1, 1993.

0022-2623/93/1836-3481$04.00/0

Scheme I



maximum effect of 25-40% typically being observed at a concentration of 10^{-6} M.

Spiro bicyclic peptidomimetic 3 was also found to increase the binding of [^{3}H]ADTN to dopamine receptors. Like PLG, it exhibited a bell-shaped dose–response curve with a maximum effect at 10^{-6} M (Figure 1). However, in the present assay, the 40% increase in [^{3}H]ADTN binding produced by 3 was slightly greater than the 26% increase produced by PLG.

Discussion

The highly constrained peptidomimetic 3 was designed in order to investigate the biologically active conformation of PLG. We previously proposed that the bioactive conformation of PLG is a type-II β-turn on the basis of the activity of the lactam (1) and bicyclic thiazolidine lactam (2) analogues of PLG,[5-7] since these two types of constraints restrict the ψ_2 and ψ_2, Φ_3 torsion angles, respectively to values close to those of the classical type-II β-turn.

In the present study, we have incorporated into the PLG molecule a highly rigid spiro bicyclic system, which has previously been shown by us to be a very good mimic of a type-II β-turn.[8] This system restricts three of the four torsion angles that define a β-turn. Modeling studies have shown that this highly constrained system restricts the Φ_2, ψ_2, and Φ_3 torsion angles to values of -40°, 108°, and 78°, respectively.[8] These values are in close agreement with those found in a classical type-II β-turn (Φ_2 = -60°, ψ_2 = +120°, Φ_3 = +80°, ψ_3 = 0°).[9]

Peptidomimetic 3 was slightly more effective than PLG in enhancing the binding of [^{3}H]ADTN to the striatal dopamine receptor. Several other conformationally constrained analogues of PLG have also been shown to possess greater effectiveness than PLG. These include several analogues that possess cyclic amino acids in place of the glycinamide residue,[10] several compounds in the lactam (1) series,[6] and the bicyclic thiazolidine lactams (2).[7] We speculate that the basis of the greater enhancement in ADTN binding to dopamine receptors by these compounds is related to their ability to prevent GTP-induced conversion of the high-affinity state of the dopamine receptor to the low affinity state.[2,11]

In summary, the highly rigid nature of the spiro bicyclic system contained in 3 dictates that this analogue exist in a bent conformation. Thus, the high activity of peptidomimetic 3 provides the strongest evidence to date in support of our hypothesis that the biologically active conformation of PLG is in fact a type-II β-turn.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt 6406K apparatus and are uncorrected. Specific rotations were measured with a Rudolph Autopol II polarimeter at 589 nm.

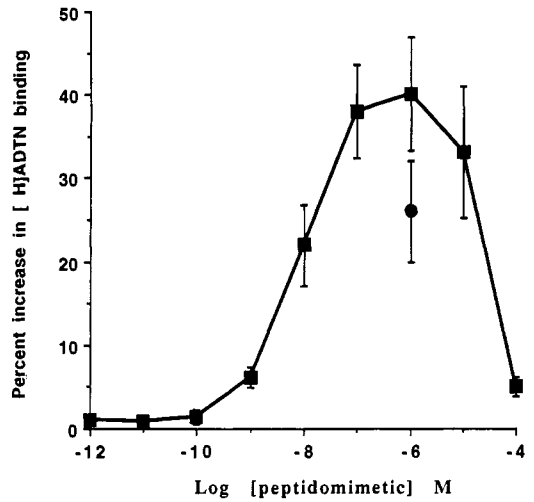


Figure 1. Percent enhancement over the control value of specific [^{3}H]ADTN binding to striatal membranes incubated with the indicated concentrations of peptidomimetic 3 (■) and PLG (●). In these experiments PLG was used for comparison purposes and thus was tested only at the concentration at which it produces its maximum effect. A complete dose–response curve for PLG can be found in ref 5. Results are the means ± SEM of three of four experiments each carried out in triplicate.

FAB mass spectra were recorded on a Kratos MS25 spectrometer. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^{1}H and ^{13}C NMR spectra were measured on a General Electric GN-Ω-300 spectrometer in either $CDCl_3$ or DMSO-d_6 at 300 and 75.5 MHz, respectively. Tetramethylsilane (δ 0.0) served as the internal reference for ^{1}H, and either $CDCl_3$ (δ 77.0) or DMSO-d_6 (δ 39.5) served as the internal standard for ^{13}C. Flash column chromatography was performed with silica gel, Merck, grade 60 (240–400 mesh, 60 Å) from Aldrich Chemical Co., Inc. Amino acids were obtained from the Sigma Chemical Co., and 4 N HCl in dioxane was obtained from the Pierce Co. All other reagents were obtained from the Aldrich Chemical Co.

[3′*S*-[3′α,6′α*R,7′aα]]-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]tetrahydro-5′-oxospiro[pyrrolidine-2,6′-pyrrolo[2,1-*b*]thiazolidine]-3′-carboxamide (5).** Spiro bicyclic ester 4[8] (150 mg, 0.42 mmol) was deprotected in 4 N HCl/dioxane (5 mL) for 30 min at room temperature under N_2. Solvent and excess HCl were removed in vacuo, and the residue obtained was dissolved in CH_2Cl_2. This solution was stripped of solvent under reduced pressure. The residue which was obtained was then dried in vacuo overnight. This material was dissolved in dry DMF (5 mL) along with Boc-Pro-OH (200 mg, 1.0 mmol), HOBt (140 mg, 1.0 mmol), and NEt_3 (0.06 mL, 0.42 mmol). To this solution was added a solution of DCC (210 mg, 1.0 mmol) in dry DMF (5 mL). The reaction mixture was stirred at room temperature under N_2 for 3 days. The mixture was poured into H_2O and extracted with EtOAc (3 × 50 mL). The EtOAc extracts were combined, washed with H_2O and saturated NaCl solution, and dried ($MgSO_4$). Solvent was evaporated in vacuo, and the residue obtained was chromatographed on a 2.5- × 40-cm silica gel flash column with EtOAc/hexane (3:1) as the eluting solvent. The N-protected prolyl spiro bicyclic ester which was formed was isolated as a colorless oil in a yield of 130 mg (68%). This material was immediately treated overnight with a saturated methanolic ammonia solution (10 mL). The excess NH_3 and MeOH were removed in vacuo, and the residue that was obtained was chromatographed on a 1- × 40-cm silica gel flash column with CH_2Cl_2/MeOH (20:1) as the eluting solvent. The product was isolated as a colorless oil which solidified in Et_2O to yield 100 mg (55%) of 5 as a fine white powder: mp 87–89 °C; $[\alpha]_D$ +40.2° (*c* 1.23 MeOH). The presence of rotamers about the carbamate bond were observed in both ^{1}H and ^{13}C NMR spectra: ^{1}H NMR ($CDCl_3$) δ 1.41 and 1.43 (s, 9 H, Boc CH_3), 1.78–2.24 (m, 8 H, Pro γ-CH_2, Pro β-CH_2, pyrrolidine β-CH_2, and pyrrolidine γ-CH_2), 2.29–2.41 (m, 1 H, lactam β-CH_2), 2.72–2.87 (m, 1 H, lactam β-CH_2), 3.33–3.60 (m, 5 H, SCH_2, pyrrolidine δ-CH_2, and Pro δ-CH_2), 3.74–3.80 and 3.93–4.00 (m, 1 H, Pro δ-CH_2), 4.36 and

4.42 (dd, J = 8.5 and 3.6 Hz, 1 H, Pro α-CH), 4.79–4.85 (m, 1 H, thiazolidine α-CH), 5.16-5.21 (m, 1 H, SCHN), 5.51 and 5.55 (br s, 1 H, *cis* $CONH_2$), 7.17 and 7.24 (br s, 1 H, *trans* $CONH_2$); ^{13}C NMR ($CDCl_3$), δ 23.51 and 24.17 (Pro γ-C), 24.34 and 24.45 (pyrrolidine γ-C), 28.36 and 28.39 (Boc CH_3), 29.26 and 30.15 (Pro β-C), 36.10 (SCH_2), 37.65 and 37.74 (pyrrolidine β-C), 38.73 (lactam β-C), 46.68, 46.94, 47.64, and 47.66 (Pro δ-C, and pyrrolidine δ-C), 57.37, 57.40, 57.68, and 57.78 (Pro α-C, and thiazolidine α-C), 62.62 and 62.91 (SCN), 69.83 and 69.99 (lactam α-C), 79.55 and 79.67 (Boc C-O), 154.51 and 154.54 (Boc C=O), 171.65 and 171.91 ($CONH_2$), 172.15 and 172.36 (lactam C=O), 172.91 and 173.20 (Pro C=O); FAB-MS m/z 439 $[MH]^+$. Anal. ($C_{20}H_{30}N_4O_5S$) C, H, N, S.

[3′*S*-[3′α,6′αR^*,7′aα]]-1-[2-(*S*)-Pyrrolidinylcarbonyl]tetrahydro-5′-oxospiro[pyrrolidine-2,6′-pyrrolo[2,1-*b*]thiazolidine]-3′-carboxamide Hydrochloride (3·HCl). Spiro bicyclic amide 5 (24 mg, 0.05 mmol) was deprotected in cold 4 N HCl/dioxane (5 mL) for 30 min under N_2. The reaction was diluted with Et_2O, and the precipitated product was filtered, washed with copious amounts of Et_2O, and dried in vacuo. This material was dissolved in H_2O (1 mL) and lyophilized to give the pure product as a white solid in a yield of 15 mg (80%): mp 98–101 °C; ^1H NMR (DMSO-d_6) δ 1.63-1.74 (m, 1 H, γ-CH_2), 1.83–2.05 (m, 6 H, Pro γ-CH_2, Pro β-CH_2, pyrrolidine β-CH_2 and pyrrolidine γ-CH_2), 2.27 (d, J = 14.7 Hz, 1 H, lactam β-CH_2), 2.35–2.46 (m, 1 H, pyrrolidine β-CH_2), 2.69 (dd, J = 14.1 and 8.1 Hz, 1 H, lactam β-CH_2), 3.14-3.21 (m, 3 H, SCH_2 and pyrrolidine δ-CH_2), 3.30–3.49 (m, 2 H, pyrrolidine δ-CH_2 and Pro δ-CH_2), 3.71–3.76 (m, 1 H, Pro δ-CH_2), 4.42–4.48 (m, 1 H, Pro α-CH), 4.65 (dd, J = 8.0 and 5.6 Hz, 1 H, thiazolidine α-CH), 5.21 (d, J = 7.2 Hz, 1 H, SCHN), 7.27 (br s, 1 H, *cis* $CONH_2$), 7.40 (br s, 1 H, *trans* $CONH_2$), 8.66 (br s, 1 H, $^+NH_2$), 9.51 (br s, 1 H, $^+NH_2$); ^{13}C NMR (75.5 MHz, DMSO-d_6), δ 23.60 and 23.70 (Pro γ-C and pyrrolidine γ-C), 27.77 (Pro β-C), 31.46 (SCH_2), 33.26 (β-C), 36.25 (lactam β-C), 45.66 (pyrrolidine δ-C), 47.11 (Pro δ-C), 58.35 and 58.50 (Pro α-C and thiazolidine α-C), 63.59 (SCN), 68.47 (pyrrolidine α-C), 166.54 ($CONH_2$), 171.19 (lactam C=O), 175.14 (Pro C=O); FAB-MS m/z 339 $[MH]^+$. Anal. ($C_{15}H_{23}N_4O_3SCl$) C, H, N, S.

[^{3}H]ADTN Binding Assay. The detailed protocol for measuring the enhancement of binding of ADTN to dopamine receptors has been described earlier by us.[5] In short, striatal synaptosomal membranes from bovine caudate along with [^{3}H]-ADTN are incubated with or without (control) different concentrations (10^{-12} to 10^{-4} M) of the peptidomimetics. Incubation is carried out in triplicate at 37 °C for 10 min. The mixtures are filtered and the filters counted on a liquid scintillation counter. In the present experiment the control total binding was 4850 dpm's and nonspecific binding was equal to 1885 dpm's. The nonspecific binding is defined as the amount of [^{3}H]ADTN remaining bound in the presence of 10 μM (+)-butaclamol. The statistical significance of the data at various doses was determined using the student t-test. The unpaired t-test was performed on the actual dpms obtained at each dose level before converting them to precent enhancement. Values of p less than 0.05 are considered significantly different from the control.

Acknowledgment. This work was supported in part from a grant (NS 20036) from the National Institutes of Health to R.L.J. M.J.G. acknowledges the support of an NIH predoctoral traineeship (GM 07994). The authors thank Thomas Krick for the FAB mass spectra.

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