Dopamine Receptor Modulation by Pro-Leu-Gly-NH_2 Analogues Possessing Cyclic Amino Acid Residues at the C-Terminal Position

Rodney L. Johnson,*† G. Rajakumar,‡ and Ram K. Mishra‡

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota, 55455, and Departments of Psychiatry and Neurosciences, McMaster University Health Sciences Center, Hamilton, Ontario L8N 3Z5, Canada. *Received December 6, 1985*

The synthesis of several analogues of L-prolyl-L-leucylglycinamide (PLG) was carried out in which the glycinamide residue was replaced with the following cyclic amino acid residues: L- and D-prolinamide, (+)- and (-)-thiazolidine-2-carboxamide, L- and D-3,4-dehydroprolinamide, L-azetidine-2-carboxamide, L-piperidine-2-carboxamide, and L-thiazolidine-4-carboxamide to give PLG analogues **2**–**10**, respectively. The ability of these analogues to enhance the binding of the dopamine agonist ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) to dopamine receptors was determined by using bovine brain tissue. All of the PLG analogues synthesized in this study enhanced the binding of ADTN to central dopamine receptors. The percent enhancement of ADTN binding produced by analogues **2**, **3**, and **7**–**10** at various concentrations was comparable to the percent enhancement produced by PLG. The PLG analogues Pro-Leu-(+)-thiazolidine-2-carboxamide (**4**), Pro-Leu-(-)-thiazolidine-2-carboxamide (**5**), and Pro-Leu-L-3,4-dehydroprolinamide (**6**), however, produced significantly greater enhancement (2–3-fold) in ADTN binding than did PLG.

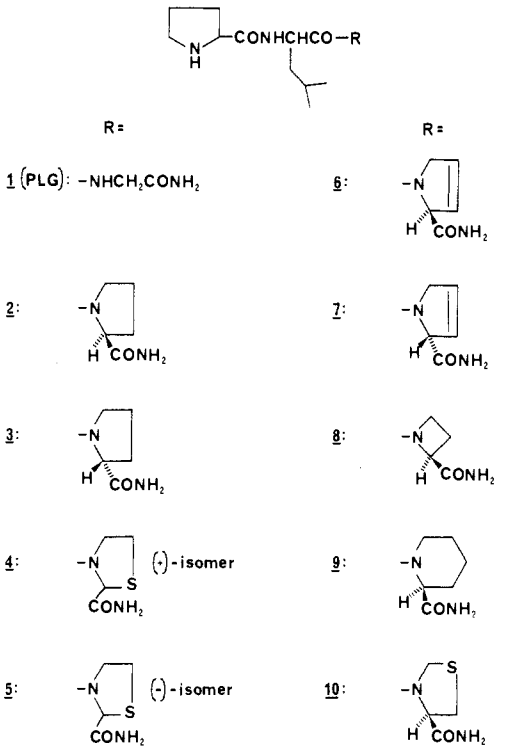
L-Prolyl-L-leucylglycinamide (PLG, **1**) has been shown to possess a pharmacological profile that strongly suggests that this tripeptide is capable of modulating dopamine receptors.[1] For example, PLG has been shown to potentiate the behavioral effects of L-DOPA,[2,3] to antagonize the tremors induced by oxotremorine,[3-5] and to enhance both amphetamine- and apomorphine-dependent rotational behavior in 6-hydroxydopamine-lesioned rats.[6,7] In addition to these activities, PLG has been shown to prevent, as well reverse, the supersensitivity of dopamine receptors produced by neuroleptics such as haloperidol. This effect has been demonstrated through both biochemical[8,9] and behavioral studies.[10,11] Also, previous in vitro studies[12] have demonstrated that PLG preferentially enhances the affinity of the dopamine agonist apomorphine to central dopamine receptors. PLG does not affect, however, the binding of dopamine antagonists such as spiroperidol to dopamine receptors.

Since our earlier studies[13-15] showed that analogues of PLG in which the glycinamide residue had been replaced with either the thiazolidine-2-carboxamide or L-prolinamide residue still possessed activity in the DOPA potentiation and oxotremorine antagonism tests,[13] as well as in a PLG receptor binding assay,[14,15] we undertook the synthesis of additional analogues of PLG in order to explore further the extent to which the glycinamide residue could be replaced with other heterocyclic residues. In the present study, we have synthesized the PLG analogues **2**–**10** in which the glycinamide residue has been replaced with the L- and D-prolinamide, (+)- and (-)-thiazolidine-2-carboxamide (^{2}Thz), L- and D-3,4-dehydroprolinamide ($\Delta^{3,4}$Pro), L-azetidine-2-carboxamide (Aze), L-piperidine-2-carboxamide (Pip), and thiazolidine-4-carboxamide (^{4}Thz) residues, respectively. These analogues have been evaluated for their ability to increase the binding of the dopamine agonist ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) to striatal dopamine receptors.

Results and Discussion

Chemistry. The synthesis of the tripeptides **2**, **4**, **5**, and **10** (see structures in Chart I) was carried out in the same manner as that described by one of us previously.[13] Compound **3** was synthesized by coupling D-prolinamide to Z-Pro-Leu-OH[13] using the mixed anhydride method of Anderson et al.[16,17] Deprotection of the resulting tripeptide by hydrogenolysis yielded the desired analogue Pro-Leu-D-Pro-NH_2 (**3**). L- and D-3,4-dehydroprolinamide

Chart I



† Department of Medicinal Chemistry.
‡ Departmens of Psychiatry and Neurosciences.

(1) Mishra, R. K.; Chiu, S.; Chiu, P.; Mishra, C. P. *Methods Find. Exp. Clin. Pharmacol.* **1983**, *5*, 203.
(2) Plotnikoff, N. P.; Kastin, A. J.; Anderson, M. J.; Schally, A. V. *Life Sci.* **1971**, *10*, 1279.
(3) Huidobro-Toro, J. P.; Scotti de Carolis, A.; Longo, V. G. *Pharmacol. Biochem. Behav.* **1974**, *2*, 105.
(4) Plotnikoff, N. P.; Kastin, A. J.; Anderson, M. S.; Schally, A. V. *Proc. Soc. Exp. Biol. Med.* **1972**, *140*, 811.
(5) Castensson, S.; Sievertsson, H.; Lindeke, B.; Sum, C. Y. *FEBS Lett.* **1974**, *44*, 101.
(6) Kostrzewa, R. M.; Kastin, A. J.; Sobrian, J. K. *Pharmacol. Biochem. Behav.* **1978**, *9*, 375.
(7) Smith, J. R.; Morgan, M. *Gen. Pharmacol.* **1982**, *13*, 203.
(8) Chiu, S.; Paulose, C. S.; Mishra, R. K. *Science (Washington, D.C)* **1981**, *214*, 1261.
(9) Chiu, P.; Rajakumar, G.; Chiu, S.; Johnson, R. L.; Mishra, R. K. *Peptides* **1985**, *6*, 179.
(10) Bhargava, H. N. *Life Sci.* **1981**, *29*, 45.
(11) Bhargava, H. N. *Neuropharmacology* **1984**, *23*, 439.

0022-2623/86/1829-2100$01.50/0

Table I. Enchancement of the Binding of [^{3}H]ADTN[a] to Dopamine Receptors by PLG Analogues Modified at the C-Terminal Position

no.	compd	N^b	% enhancement of [^{3}H]ADTN binding, mean ± SEM, at given concn, nM		
			1	10	100
1	Pro-Leu-Gly-NH_2	4	16.0 ± 3.0	28.0 ± 6.0	46.2 ± 13.4
2	Pro-Leu-Pro-NH_2	3	10.0 ± 2.1	26.0 ± 5.0	37.7 ± 13.9
3	Pro-Leu-D-Pro-NH_2	4	9.0 ± 3.7	21.0 ± 6.5	34.5 ± 13.2
4	Pro-Leu-(+)-^{2}Thz-NH_2	3	18.1 ± 8.9	46.0 ± 7.8	109.0 ± 6.1[c]
5	Pro-Leu-(−)-^{2}Thz-NH_2	3	58.0 ± 20.9	69.5 ± 19.5	138.6 ± 5.9[d,e]
6	Pro-Leu-$\Delta^{3,4}$Pro-NH_2	3	60.0 ± 4.5[f]	85.0 ± 2.5[f]	114.3 ± 2.3[c,g]
7	Pro-Leu-D-$\Delta^{3,4}$-Pro-NH_2	4	11.0 ± 4.6	29.0 ± 8.9	45.7 ± 3.2
8	Pro-Leu-Aze-NH_2	3	18.0 ± 17.5	25.0 ± 16.5	131.7 ± 33.9
9	Pro-Leu-Pip-NH_2	3	19.0 ± 4.6	32.0 ± 9.5	84.0 ± 17.7
10	Pro-Leu-L-^{4}Thz-NH_2	4	16.0 ± 7.5	33.0 ± 9.4	105.7 ± 19.5

[a] ADTN = 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene. [b] Number of independent experiments with triplicate determinations. [c] Significantly different ($P < 0.05$) from PLG (1). [d] Significantly different ($P < 0.01$) from PLG (1). [e] Compound 5 is significantly different ($P < 0.05$) from 4. [f] Significantly different ($P < 0.001$) from PLG (1). [g] Compound 6 is significantly different ($P < 0.01$) from 7.

were synthesized according to the method of Felix et al.[18] These two amides were each coupled to Boc-Pro-Leu-OH using the coupling reagent diphenylphosphorylazide to give Boc-Pro-Leu-L-$\Delta^{3,4}$Pro-NH_2 (**11**) and Boc-Pro-Leu-D-$\Delta^{3,4}$Pro-NH_2 (**12**). The *tert*-butoxycarbonyl protecting group was removed from **11** and **12** with 4 N HCl in dioxane to give the PLG analogues **6** and **7**, respectively.

For the synthesis of analogue **8**, methyl L-azetidine-2-carboxylate was coupled to Z-Pro-Leu-OH using isobutyl chloroformate. The tripeptide ester was converted to the amide Z-Pro-Leu-Aze-NH_2 (**13**) with methanolic ammonia. Hydrogenolysis of **13** yielded analogue **8**. We initially tried to use this same general route in the synthesis of **9**; however, we found that the ester Z-Pro-Leu-Pip-OCH_3 could not be converted to the corresponding amide Z-Pro-Leu-Pip-NH_2 with methanolic ammonia. Even when this mixture was heated in a bomb for 3 h, only a small amount of the desired amide was formed. Thus, an alternate route to **9** was employed wherein L-piperidine-2-carboxamide (**15**) was coupled to Z-Pro-Leu-OH directly. L-Piperidine-2-carboxamide (**15**) was synthesized by reacting Boc-L-Pip-OH[19] with ethyl chloroformate followed by ammonia to give Boc-L-Pip-NH_2 (**14**), which was then deprotected with HCl in doxane to give the desired compound **15**.

Binding Studies. PLG and its C-terminal modified analogues **2–10** were evaluated for their ability to enhance the binding of [^{3}H]ADTN to dopamine receptors isolated from bovine caudate tissue. The results obtained are shown in Table I. The binding assay that was employed for [^{3}H]ADTN was similar to that reported previously by Creese and Snyder.[20] Three concentrations (1, 10, and 100 nM) of peptide were used to test the effect of PLG and its analogues on the binding of [^{3}H]ADTN to dopamine receptors. The dose–response curve for the influence of PLG on the specific binding of [^{3}H]ADTN to bovine caudate tissue is bell-shaped with the peak effect occurring at a concentration of 100 nM. At this dose level PLG increases the binding of [^{3}H]ADTN to dopamine receptors by 46%. This percentage increase is of the same magnitude as that obtained previously by Chiu et al.[12] using another dopamine receptor agonist [^{3}H]apomorphine as the ligand. A bell-shaped dose–response curve was also obtained in this instance. The enhancement of dopamine agonist binding produced by PLG has been shown to be consequential to a decrease in the dissociation constant of the dopamine receptor ligand (i.e., increased affinity) and not to an increase in the number of dopamine receptors.[12]

Like PLG, the analogues **2–10** enhanced the binding of [^{3}H]ADTN to dopamine receptors in a dose-dependent manner. The analogues **2**, **3** and **7–10**, wherein the glycinamide residue had been replaced with the L-prolinamide, D-prolinamide, D-3,4-dehyroprolinamide, L-azetidine-2-carboxamide, L-piperidine-2-carboxamide, and L-thiazolidine-4-carboxamide residues, respectively, produced increases in the binding of [^{3}H]ADTN to dopamine receptors that were comparable to those produced by PLG. On the other hand, analogues **4–6** in which the glycinamide residue had been replaced with the (+)-thiazolidine-2-carboxamide, (−)-thiazolidine-2-carboxamide and L-3,4-dehydroprolinamide residues, respectively, produced increases in [^{3}H]ADTN binding that were significantly different from PLG. At a dose level of 100 nM the percent enhancement of ADTN binding produced by **4–6** was 2–3 times that produced by PLG.

The results obtained in this study suggest that a fair degree of flexibility is allowed in the glycinamide portion of the PLG molecule, at least as it pertains to the replacement of the glycinamide residue with heterocyclic residues. Even the replacement of the glycinamide residue with such divergent residues as the azetidine-2-carboxamide and piperidine-2-carboxamide yielded analogues with the same activity as PLG. Furthermore, differences in activity between the three different pairs of diastereoisomers synthesized in this study were quite variable. No difference in activity was observed between the diastereoisomeric pair Pro-Leu-Pro-NH_2 (**2**) and Pro-Leu-D-Pro-NH_2 (**3**). This was not the case with the diastereoisomeric pairs Pro-Leu-(+)-^{2}Thz-NH_2 (**4**)/Pro-Leu-(−)-^{2}Thz-NH_2 (**5**) and Pro-Leu-$\Delta^{3,4}$Pro-NH_2 (**6**)/Pro-Leu-D-$\Delta^{3,4}$Pro-NH_2 (**7**). Analogue **7**, which possessed the same activity as PLG, was significantly less active than its diastereoisomer **6**. The situation was a little different in the case of the diastereoisomeric pair **4** and **5**. While both of these analogues of PLG were significantly more active than PLG, analogue **5** was also found to be more active than **4**. Like PLG, the two thiazolidine analogues **4** and **5** gave

(12) Chiu, S.; Paulose, C. S.; Mishra, R. K. *Peptides* **1981**, *2*, 105.
(13) Johnson, R. L.; Smissman, E. E.; Plotnikoff, N. P. *J. Med. Chem.* **1978**, *21*, 165.
(14) Chiu, S.; Ferris, J. A.; Johnson, R. L.; Mishra, R. K. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **1982**, *6*, 365.
(15) Chiu, S.; Wang, Y. W.; Ferris, J.; Johnson, R. L.; Mishra, R. K. *Pharmacol. Res. Commun.* **1983**, *15*, 44.
(16) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1966**, *88*, 1338.
(17) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.
(18) Felix, A. M.; Wang, C.-T.; Liebman, A. A.; Delaney, C. M.; Mowles, T.; Burchardt, B. A.; Charnecki, A. M.; Meienhofer, J. *Int. J. Peptide Protein Res.* **1977**, *10*, 299.
(19) Johnson, R. L.; Mishra, R. K.; Rajakumar, G.; Yu, K. L. *J. Med. Chem.* **1986**, *29*, 2104.
(20) Creese, I.; Snyder, S. H. *Eur. J. Pharmacol.* **1978**, *50*, 459.

dose–response curves that were bell-shaped (unpublished observations).

Even though it appears that a variety of heterocyclic residues can be substituted for the glycinamide residue without adversly affecting the ability of the compounds to modulate dopamine receptors, it is also clear that such modifications can give rise to compounds that are significantly more active than PLG. The reason for the increased potency of the PLG analogues **4–6** is not known with certainty at this time. One of the most obvious differences between **4–6** and the other PLG analogues is the electron density at the β position of the C-terminal residue. Analogues **4–6** possess a much higher electron density at this position than the other PLG analogues examined in this study. In the case of **4** and **5**, this higher electron density is the result of the sulfur atom's two sets of lone pair electrons, while in the case of **6** it is due to the π electrons of the olefin. The position of this high electron density appears to be important, since the isomeric analogue of the thiazolidine derivatives **4** and **5**, compound **10** is not more active than PLG. It may be that there is a complementary site on the PLG receptor that recognizes and interacts favorably with the π electrons of the dehydroproline residue or the electron cloud of the sulfur atom in the thiazolidine residue. Another possible explanation for the increase potency seen with analogues **4–6** is that the β-sulfur or β-olefin moiety may affect the donor–acceptor properties of the carboxamide group. Conformational energy calculations[21] as well as X-ray crystallography[22] and nuclear magnetic spectroscopy[23] have indicated a preferred conformation for PLG that consists of a 10-membered β-turn closed by a hydrogen bond between the *trans*-carboxamide hydrogen of the glycinamide residue and the carbonyl moiety of the prolyl residue. Recently, we have shown through the use of conformationally constrained analogues of PLG that this β-turn conformation is also likely to be the biologically active conformation of PLG.[24] Although molecular models indicate that **2–10** are all capable of forming some type of β-turn, it may be that the intramolecular hydrogen bond is enhanced in those analogues possessing a β-sulfur or β-olefin moiety.

Earlier, it had been observed that **5** was effective in the DOPA potentiation test while **2** was effective in the oxotremorine antagonism test.[13] Both of these compounds, however, were less active than PLG. Compounds **4** and **10**, on the other hand, were found to be inactive in both tests. We have also shown[14,15] that **2**, **4**, and **5** are able to displace [^{3}H]PLG from PLG binding sites isolated from either human or rat brain tissue. In this assay system, the IC_{50} values of **2**, **4**, and **5** were 5–9 times higher than the IC_{50} value of PLG (18 nM). It is clear from the above results that there is no correlation between the ability of PLG analogues to enhance the affinity of dopamine agonists to dopamine receptors and their activity in either the DOPA potentiation test or PLG receptor binding assay. This apparent lack of correlation may exist because different species were used in the various assay systems. It also may be that because some of the above assays are in vitro assays while others are in vivo there are differences in metabolism and distribution of the various PLG analogues.

We have demonstrated in another study[19] that replacing the prolyl residue of PLG with the heterocyclic amino acid residues L-piperidine-2-carbonyl, L-azetidine-2-carbonyl, L-pyroglutamyl, and L-3,4-dehydroprolyl residues gives rise to PLG analogues that possess the same level of activity as PLG in enhancing the binding of [^{3}H]ADTN to dopamine receptors. Only when the prolyl residue was replaced with the thiazolidine-4-carbonyl and D-3,4-dehydroprolyl residues were inactive compounds produced. Thus, it appears that a fair degree of flexibility is allowed in the N-terminal residue of PLG. The results of the present study suggest that there is also considerable flexibility in the C-terminal glycinamide residue, since this residue can be replaced with a variety of heterocyclic amino acid residues to give analogues with dopamine receptor modulatory activities that are comparable to that of PLG. In addition, however, this study has shown for the first time that it is possible to obtain PLG analogues that are more potent than PLG through modification of the glycinamide portion of the PLG molecule.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Specific rotations were measured with either a Perkin-Elmer 141 or a Rudolph Autopol III polarimeter. ^{1}H NMR spectra were performed on either a JEOL FX-90-MHz or a Nicolet 300-MHz spectrometer. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Unless otherwise indicated, all analytical results were within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel GF uniplates with the following solvent systems: (A) *n*-propanol/NH_4OH (4:1), (B) *n*-butanol/HOAc/EtOAc/H_2O (1:1:1:1), (C) CH_2Cl_2/MeOH (19:1), (D) EtOAc, (E) *n*-butanol/HOAc/H_2O (4:2:1), (F) CH_2Cl_2/MeOH (1:1). Visualization was done with either I_2 or ninhydrin. Medium-pressure liquid chromatography (20–60 psi) was carried out on silica Woelm (32–63 μm) from ICN Nutritional Biochemicals. 2-[5,8-^{3}H]Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene ([^{3}H]ADTN) was obtained from New England Nuclear, Boston, MA.

L-Prolyl-L-leucyl-D-prolinamide Hydrochloride (3). This analogue was prepared by coupling Z-Pro-Leu[13] (0.46 g, 1.26 mmol) with D-prolinamide[25] (0.19 g, 1.26 mmol) using the same procedures as those described below for **13** to give a 57% yield of Z-Pro-Leu-D-Pro-NH_2 as a foam: $[\alpha]^{24}_D$ −45° (*c* 1.0, MeOH); TLC, R_f (A) = 0.69. This material was deprotected by hydrogenolysis using the same procedure as that described for **8** to give the desired product as a glassy solid: $[\alpha]^{25}_D$ −9° (*c* 0.5, MeOH); TLC, R_f (A) = 0.32, R_f (B) = 0.5; NMR (Me_2SO-d_6) δ 8.86, 8.80 (pair of d as a result of the cis/trans isomerism about the Leu-D-Pro peptide bond, ratio = 1.5:1, 1 H, Leu NH), 7.64, 7.21, 7.06, 6.95 (4 s as a result of the cis/trans isomerism about the Leu-D-Pro peptide bond, ratio = 1:1:1.5:1.5, 2 H, $CONH_2$), 4.55–4.68 (m, 1 H, Pro α-CH) 4.12–4.32 (m, 2 H, Leu and D-Pro α-CH), 3.68–3.8 (m, 1 H, Pro δ-CH) 3.44–3.55 (m, 1 H, Pro δ-CH), 3.12–3.28 (m, 1 H, D-Pro δ-CH_2), 1.7–2.35 (m, 8 H, Pro and D-Pro γ- and β-CH_2), 1.4–1.7 (m, 3 H, Leu β-CH_2 and γ-CH), 0.91 and 0.83 (d and dd, respectively, as a result of the cis/trans isomerism about the Leu-D-Pro peptide bond, 6 H, Leu CH_3). Anal. ($C_{16}H_{29}N_4O_3$-Cl·H_2O) C, H, N.

***N-tert*-Butoxycarbonyl-L-prolyl-L-leucyl-L-3,4-dehydroprolinamide (11).** L-3,4-Dehydroprolinamide hydrochloride[18] (0.4 g, 2.7 mmol) and Boc-Pro-Leu-OH (0.88 g, 2.7 mmol) were dissolved in DMF (10 mL). The mixture was cooled in an ice bath, and diphenylphosphorylazide (0.75 g, 2.8 mmol) was added. This was followed by the addition of NEt_3 (0.54 g, 5.4 mmol). The reaction mixture was stirred at 0 °C overnight and then at room

(21) Ralston, E.; DeCoen, J. L.; Walter, R. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1142.
(22) Reed, L. L.; Johnson, P. L. *J. Am. Chem. Soc.* **1973**, *95*, 7523.
(23) Walter, R.; Bernal, I.; Johnson, L. F. *Chemistry and Biology of Peptides*, Meienhofer, J., Ed.; Ann Arbor Science: Ann Arbor, MI, 1972; p 131.
(24) Johnson, R. L.; Yu, K-L.; Taraporewala, I.; Mishra, R. K.; Rajakumar, G. *Peptides: Structure and Function*, Proceedings of the Ninth American Peptide Symposium; C.M. Deber, C.M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Company: Rockford, IL, 1985; p 671.
(25) Flouret, G.; Morgan, R.; Gendrich, R.; Wilber, J.; Seibel, M. *J. Med. Chem.* **1973**, *16*, 1137.

temperature for 2 h. The precipitate of NEt_3·HCl was removed by filtration, and the DMF was removed from the filtrate in vacuo. The residue was partitioned between EtOAc and 10% citric acid. The EtOAc layer was washed with 1 M $NaHCO_3$ followed by saturated NaCl solution. The EtOAc layer was dried (Na_2SO_4) and then stripped of solvent in vacuo to give 0.51 g of crude product. This material was purified on a silica gel column (1.5 × 50 cm) using CH_2Cl_2/MeOH (18:1) as the eluting solvent. A yield of 330 mg of **11** was obtained as a foam, which was used without further purification: $[\alpha]^{24}_D$ -219.7° (*c* 0.6, CH_2Cl_2); TLC, R_f (C) = 0.2.

L-Prolyl-L-leucyl-L-3,4-dehydroprolinamide Hydrochloride (6). Protected peptide **11** (300 mg, 0.71 mmol) was treated with 10 mL of 4 N HCl in dioxane for 45 min at room temperature. The HCl and dioxane were removed in vacuo and the residue crystallized from a mixture of isopropyl alcohol and Et_2O to give 230 mg (90%) of **6** as a white hygroscopic powder, which sintered at 150 °C: $[\alpha]^{24}_D$ -231.6° (*c* 0.5, MeOH); TLC, R_f (A) = 0.67, R_f (B) = 0.55; NMR (D_2O) δ 6.13 (dd, 1 H, *J* = 1.8 and 4.5 Hz, $\Delta^{3,4}$Pro β-HC=), 5.87 (dd, 1 H, *J* = 2.0 and 4.5 Hz, $\Delta^{3,4}$Pro δ-HC=), 5.13 (dd, 1 H, *J* = 2.11 and 2.42 Hz, $\Delta^{3,4}$Pro α-CH), 4.58–4.68 (m, 2 H, $\Delta^{3,4}$Pro δ-CH and Pro α-CH), 4.46–4.53 (m, 1 H, $\Delta^{3,4}$Pro δ-CH), 4.42 (dd, 1 H, *J* = 5.9 and 2.6 Hz, Leu α-CH), 3.38–3.46 (m, 2 H, Pro δ-CH_2), 2.45–2.48 (m, 1 H, Pro β-CH), 2.0–2.15 (m, 3 H, Pro β-CH and γ-CH_2), 1.70–1.77 (m, 1 H, Leu γ-CH), 1.60–1.67 (m 2 H, Leu β-CH_2), 0.97 (dd, 6 H, Leu CH_3). Anal. ($C_{16}H_{27}N_4O_3Cl·H_2O$) C, H, N.

***N*-*tert*-Butoxycarbonyl-L-prolyl-L-leucyl-D-3,4-dehydroprolinamide (12).** D-3,4-Dehydroprolinamide[18] (0.4 g, 2.7 mmol) was coupled to Boc-Pro-Leu-OH (0.88 g, 2.7 mmol) using the same method as that described for **11**. A yield of 0.6 g of material was obtained after medium-pressure chromatography: $[\alpha]^{24}_D$ +55.7° (*c* 1.0, CH_2Cl_2); TLC, R_f (C) = 0.25. This material was used without further purification.

L-Prolyl-L-leucyl-D-3,4-dehydroprolinamide Hydrochloride (7). The protected peptide **12** (0.6 g, 1.42 mmol) was deprotected in the same manner as that described above for the synthesis of **6**. The hygroscopic product was isolated in a yield of 0.41 g (80.5%): mp 151–155 °C; $[\alpha]^{24}_D$ +116.4° (*c* 0.5, MeOH); TLC, R_f (A) = 0.72, R_f (B) = 0.57; NMR (D_2O) δ 6.13 (br d, 1 H, $\Delta^{3,4}$Pro β-HC=), 5.9 (br m, 1 H, $\Delta^{3,4}$Pro γ-HC=), 5.07–5.10 (m, 1 H, $\Delta^{3,4}$Pro α-CH), 4.62–4.78 (m, 2 H, $\Delta^{3,4}$Pro γ-CH and and Pro α-CH), 4.35–4.54 (m, 2 H, $\Delta^{3,4}$Pro γ-CH and Leu α-CH), 3.34–3.5 (m, 2 H, Pro δ-CH_2), 2.4–2.53 (m, 1 H, Pro β-CH), 1.95–2.16 (m, 3 H, Pro β-CH and γ-CH_2), 1.56–1.78 (m, 3 H, Leu γ-CH_2 and γ-CH), 0.96 (d, 6 H, Leu CH_3). Anal. ($C_{16}H_{27}N_4O_3Cl·H_2O$) C, H, N.

***N*-Benzyloxycarbonyl-L-prolyl-L-leucyl-L-azetidine-2-carboxamide (13).** Z-Pro-Leu-OH[13] (1.55 g, 4.3 mmol) and *N*-methylmorpholine (0.43 g, 4.3 mmol) were dissolved in THF (20 mL). This solution was cooled to -20 °C whereupon a solution of isobutyl chloroformate (0.59 g, 4.3 mmol) in THF (5 mL) was added in a dropwise manner. The reaction mixture was stirred at -20 °C for about 5 min and then treated with a solution of methyl L-azetidine-2-carboxylate hydrochloride (0.65 g, 4.3 mmol) and *N*-methylmorpholine (0.43 g, 4.3 mmol) in CH_2Cl_2 (7 mL). The reaction mixture was allowed to warm up to room temperature where it was kept for 30 min. The mixture was stripped of THF and CH_2Cl_2 in vacuo and the residue partitioned between EtOAc (50 mL) and 10% citric acid (50 mL). The EtOAc layer was washed with 1 M $NaHCO_3$ followed by saturated NaCl solution. After the EtOAc layer was dried (Na_2SO_4), the EtOAc was removed in vacuo to give 1.97 g (100%) of tripeptide ester as an oil: TLC, R_f (D) = 0.43.

The above ester (1.8 g, 3.9 mmol) was dissolved in 60 mL of MeOH saturated with NH_3. The solution was stirred at room temperature for 24 h, after which time the NH_3 and MeOH were removed in vacuo. The residue was placed on a silica gel column (2.5 × 50 cm) and eluted with solvent [CH_2Cl_2/MeOH (19:1)]. The fractions containing the desired amide were combined and evaporated to dryness to give 1.3 g (75%) of **13** as a glassy solid: $[\alpha]^{25}_D$ -147.7° (*c* 1.0, MeOH). Anal. ($C_{23}H_{32}N_4O_5$) C, H, N.

L-Prolyl-L-leucyl-L-azetidine-2-carboxamide Hydrochloride (8). Protected amide **13** (0.7 g, 1.57 mmol) in MeOH (25 mL) was added to a flask containing 5% Pd/C (100 mg) that had been wetted with 10% HCl (0.6 mL, 1.65 mmol). Hydrogen was bubbled into the reaction mixture until TLC analysis showed the reaction to be complete (~1 h). The mixture was filtered through a pad of Celite and the filtrate stripped of solvent in vacuo. The residue was crystallized from a mixture of isopropyl alcohol and Et_2O to give 0.47 g (86%) of **8**: sintered at 125 °C; $[\alpha]^{24}_D$ -122.9° (*c* 1.0, MeOH); TLC, R_f (E) = 0.31, R_f (F) = 0.21; NMR (pyridine-d_5) δ 5.0–5.25 (m, 2 H, Pro α-CH and Aze α-CH), 4.35–4.5 (m, 1 H, Leu α-CH), 4.05–4.3 (m, 2 H, Aze γ-CH_2), 3.3–3.7 (m, 2 H, Pro δ-CH_2), 2.2–2.7 (m, 4 H, Aze β-CH_2 and Pro β-CH_2), 2.6–2.05 (m, 5 H, Pro γ-CH_2, Leu γ-CH, and β-CH_2), 0.8 (dd, 6 H, Leu CH_3). Anal. ($C_{15}H_{27}N_4O_3Cl$) C, H, N.

***N*-*tert*-Butoxycarbonyl-L-piperidine-2-carboxamide (14).** A solution of *N*-butoxycarbonyl-L-piperidine-2-carboxylic acid (0.6 g, 2.6 mmol) and NEt_3 (0.26 g, 2.6 mmol) in THF (10 mL) was cooled to -10 °C and then treated with a solution of ethyl chloroformate in THF (5 mL). The reaction mixture was stirred for a few minutes and then treated with concentrated NH_4OH (0.8 mL). The reaction was allowed to warm up to room temperature where it was kept for 4 h before the solvents were removed in vacuo. The residue was partitioned between EtOAc and 10% citric acid. The EtOAc layer was washed with 1 M $NaHCO_3$ and saturated NaCl solution and then dried ($MgSO_4$). Removal of the EtOAc yielded 470 mg (79%) of **14** as an oil. This material solidified on standing and was collected by filtration with the aid of hexane: mp 91–93 °C; $[\alpha]^{24}_D$ -104.5° (*c* 1.0, CH_2Cl_2). Anal. ($C_{11}H_{20}N_2O_3$) C, H, N.

L-Piperidine-2-carboxamide Hydrochloride (15). Protected amide **14** (0.8 g, 3.5 mmol) was treated with 10 mL of 4 N HCl in dioxane for 45 min at room temperature. The product that precipitated from the reaction mixture was collected with the aid of Et_2O to give 0.58 g (100 %) of pure **15**: mp 253–255 °C dec; $[\alpha]^{24}_D$ -7.6° (*c* 1.0, MeOH); NMR (D_2O) δ 3.95 (m, 1 H, α-CH), 3.48 (dd, 1 H, *J* = 1.35 and 12.7 Hz, $H6_{eq}$), 3.05 (dt, 1 H, *J* = 2.35 and 12.6 Hz, $H6_{ax}$), 2.25 (m, 1 H, $H3_{eq}$), 1.88–2.0 (m, 2 H, $H3_{ax}$ and $H5_{eq}$), 1.56–1.80 (m, 3 H, $H5_{ax}$ and γ-CH_2). Anal. ($C_6H_{13}N_2OCl$) C, H, N.

L-Prolyl-L-leucyl-L-piperidine-2-carboxamide Hydrochloride (9). L-Piperidine-2-carboxamide hydrochloride (**15**; 0.25 g, 1.5 mmol) was coupled to Z-Pro-Leu-OH (0.55 g, 1.5 mmol) using the same mixed anhydride procedure described above for **13** to give a crude yield of 0.63 g (89%) of Z-Pro-Leu-Pip-NH_2. Pure Z-Pro-Leu-Pip-NH_2 (0.44 g of an oil) was obtained by passing the above material through a silica gel column (1.5 × 50 cm) using CH_2Cl_2/MeOH as the eluting solvent. This material was deprotected by hydrogenolysis using the same method as that described above for **8**. The desired hygroscopic product was obtained in a yield of 0.18 g (52%) after recrystallization from a mixture of isopropyl alcohol and Et_2O: mp 142–145 °C; $[\alpha]^{23}_D$ -90.2° (*c* 0.5, MeOH); TLC, R_f (A) = 0.51, R_f (B) = 0.66; NMR (D_2O) δ 5.08 (br, 1 H, Pip α-CH), 4.38–4.48 (m, 1 H, Leu α-CH), 3.91 (br d, 1 H, *J* = 12 Hz, Pip $H6_{eq}$), 3.3–3.5 (m, 3 H, Pip $H6_{ax}$ and Pro δ-CH_2), 2.43–2.57 (m, 1 H, Pro β-CH), 2.0–2.2 (m, 4 H, Pip $H3_{eq}$, Pro β-CH, and γ-CH_2), 1.40–1.88 (m, 8 H, Pip $H3_{ax}$, γ- and δ-CH_2, Leu β-CH_2, and γ-CH), 0.96 (d, 6 H, Leu CH_3). Anal. ($C_{17}H_{31}N_4O_3Cl·H_2O$) C, H, N.

[^{3}H]ADTN Binding Assay. The binding assay for [^{3}H]ADTN was carried out by using a modification of the method of Creese and Snyder.[20] Freshly dissected bovine caudate was initially suspended in 50 vol of 50 mM Tris·HCl buffer (pH 7.7 at 25 °C) and homogenized with a Polytron homogenizer for 20 s. The tissue homogenate was twice centrifuged at 40000 *g* for 10 min in a refrigerated Sorvall centrifuge. The initial pellet was resuspended in fresh Tris buffer. The final pellet was suspended in 50 mM Tris buffer (pH 7.1 at 25 °C) containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2mM $CaCl_2$, and 10 μM pargyline to give an approximate concentration of 20 mg of wet tissue/mL of incubation buffer. The standard assay consisted of 0.4–0.6 mg of protein of the brain homogenate, 8 nM of [^{3}H]ADTN (specific activity equal to 24.4 Ci/mmol), and the buffer with or without 1, 10, or 100 nM of PLG or the PLG analogues in a total incubation volume of 0.6 mL. Incubation was carried out in triplicate in a water shaker bath maintained at 37 °C. The incubation was terminated by the addition of 2.2 mL of ice-cold 50 mM Tris·HCl, and the contents of each incubation tube were rapidly filtered under partial vacuum over Whatman GF/B filters. The filters were washed 4 times with 2.2 mL of ice-cold 50 mM Tris·HCl (pH

7.1). The filters were then placed in liquid scintillation vials and 10 mL of PCS counting cocktail was added (Amersham Corporation, Chicago, IL). The vials were equilibrated for at least 6 h before being counted in a liquid scintillation counter. The specific binding of [^{3}H]ADTN was defined as the difference in binding occurring in the absence and presence of 1 μM (*d*)-butaclamol. The data were analyzed statistically using the Student's *t* test.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (NS 20036) and by a Research Career Development Award (HL 00932) to R.L.J.

Registry No. 1, 2002-44-0; **2**, 65126-64-9; **3**, 103305-45-9; **3** (free base), 103305-58-4; **4**, 65166-58-7; **5**, 65126-62-7; **6**, 103305-46-0; **6** (free base), 103305-57-3; **7**, 103305-47-1; **7** (free base), 103305-56-2; **8**, 103305-48-2; **8** (free base), 103365-86-2; **9**, 103305-49-3; **9** (free base), 103365-87-3; **10**, 65126-63-8; **11**, 103305-50-6; **12**, 103320-93-0; **13**, 103305-51-7; **14**, 78058-41-0; **15**, 78058-42-1; Z-Pro-Leu, 1634-90-8; Z-Pro-Leu-D-Pro-NH_2, 103305-52-8; Boc-Pro-Leu-OH, 68385-28-4; Z-Pro-Leu-Aze-OMe, 103305-54-0; Z-Pro-Leu-Pip-NH_2, 103305-55-1; dopamine, 51-61-6; D-prolinamide, 62937-45-5; L-3,4-dehydroprolinamide hydrochloride, 64869-59-6; D-3,4-dehydroprolinamide, 103305-53-9; methyl L-azetidine-2-carboxylate hydrochloride, 69684-69-1; *N*-butoxycarbonyl-L-piperidine-2-carboxylic acid, 26250-84-0.

Notes

Synthesis of Pro-Leu-Gly-NH_2 Analogues Modified at the Prolyl Residue and Evaluation of Their Effects on the Receptor Binding Activity of the Central Dopamine Receptor Agonist, ADTN

Rodney L. Johnson,*† G. Rajakumar,‡ Kuo-Long Yu,† and Ram K. Mishra‡

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455, and Departments of Psychiatry and Neurosciences, McMaster University Health Sciences Center, Hamilton, Ontario L8N 3Z5, Canada. *Received November 21, 1985*

Several analogues of L-prolyl-L-leucylglycinamide (PLG) were synthesized wherein the prolyl residue was replaced with other heterocyclic amino acid residues. Among the analogues synthesized were D-Pro-Leu-Gly-NH_2 (**2**), <Glu-Leu-Gly-NH_2 (**3**), Thz-Leu-Gly-NH_2 (**4**), Pip-Leu-Gly-NH_2 (**5**), Aze-Leu-Gly-NH_2 (**6**), L-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 (**7**), and D-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 (**8**). These analogues were tested for their ability to enhance the binding of the agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene to central dopamine receptors. Analogues **2**, **3**, and **5–7** showed activity comparable to that of PLG, while the tripeptides **4** and **8** were found to be inactive. The results show that the N-terminal prolyl residue of PLG is not an essential requirement for this tripeptide's ability to modulate dopamine receptors.

The tripeptide L-prolyl-L-leucylglycinamide (**1**, PLG) has been shown to possess activity in a wide variety of in vivo and in vitro neuropharmacological assay systems.[1] In some of the first studies conducted, PLG was found to potentiate the behavioral effects of L-DOPA,[2] to antagonize the central and peripheral effects of oxotremorine,[3,4] and to potentiate the behavioral effects of apomorphine.[5] More recently, PLG has been shown to attenuate both morphine- and haloperidol-induced catalepsy.[6-8] PLG has also been shown to enhance the binding of [^{3}H]apomorphine to dopamine receptors by increasing the affinity of this ligand to these receptors.[7] In contrast to its effects on the binding of dopamine agonists (apomorphine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN)) to dopamine receptors, PLG does not alter the binding of antagonists such as [^{3}H]spiroperidol. PLG does, however, prevent, as well as reverse, the development of dopamine receptor hypersensitivity in the striatum and mesolimbic areas of the brain that are induced by neuroleptic drugs such as haloperidol. This effect has been demonstrated through both biochemical[9-11] and behavioral[10,12] studies. The above pharmacological profile of PLG thus suggests that this tripeptide is capable of modulating dopamine receptors.[1,11]

In an effort to elucidate the structural and conformational requirements of PLG with respect to its ability to modulate dopamine receptor sensitivity, we have undertaken the synthesis of a number of series of analogues of PLG. In the present paper, we wish to report our efforts in modifying the prolyl residue of PLG. We have replaced the prolyl residue of PLG with the D-prolyl, L-pyroglutamyl, L-thiazolidine-4-carbonyl (Thz), L-piperidine-2-carbonyl (Pip), L-azetidine-2-carbonyl (Aze), and L- and D-3,4-dehydroprolyl ($\Delta^{3,4}$-Pro) residues to give the PLG analogues **2–8**, respectively. These particular substitutions have been carried out in order to determine the importance of the pyrrolidine ring system in the ability of PLG to

† Department of Medicinal Chemistry.
‡ Departments of Psychiatry and Neurosciences.

(1) Mishra, R. K.; Chiu, S.; Chiu, P.; Mishra, C. P. *Methods Find. Exp. Clin. Pharmacol.* **1983**, *5*, 203.
(2) Plotnikoff, N. P.; Kastin, A. J.; Anderson, M. J.; Schally, A. V. *Life Sci.* **1971**, *10*, 1279.
(3) Plotnikoff, N. P.; Kastin, A. J.; Anderson, M. S.; Schally, A. V. *Proc. Soc. Exp. Biol. Med.* **1972**, *140*, 811.
(4) Castensson, S.; Sievertsson, H.; Lindeke, B.; Sum, C. Y. *FEBS Lett.* **1974**, *44*, 101.
(5) Plotnikoff, N. P.; Kastin, A. J. *Arch. Int. Pharmacodyn. Ther.* **1974**, *211*, 211.
(6) Chiu, S.; Mishra, R. K. *Eur. J. Pharmacol.* **1979**, *53*, 119.
(7) Chiu, S.; Paulose, C. S.; Mishra, R. K. *Peptides* **1981**, *2*, 105.
(8) Bhargava, H. N. *Gen. Pharmacol.* **1983**, *14*, 609.
(9) Chiu, S.; Paulose, C. S.; Mishra, R. K. *Science (Washington, D.C.)* **1981**, *214*, 1261.
(10) Bhargava, H. N. *Neuropharmacology* **1984**, *23*, 439.
(11) Chiu, P.; Rajakumar, G.; Chiu, S.; Johnson, R. L.; Mishra, R. K. *Peptides* **1985**, *6*, 179.
(12) Bhargava, H. N. *Life Sci.* **1981**, *29*, 45.

0022-2623/86/1829-2104$01.50/0