Synthesis and Effect on Gastric Secretion of Several Di- or Tripeptides Related to Proglumide

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Several N-acyl di- or tripeptides related to proglumide (PhCO-DL-Glu-NPr2) were prepared and their effects on gastric secretion were examined by intraperitoneal injection in rats. PhCO-Glu(Phe-NH2)-NPr2, Z-Glu(Phe-NH2)-NPr2, PhCO-Glu(NPr2)-Phe-NH2 and PhCO-Asp-(Phe-NH2)-NPr2 inhibited gastric secretion, while PhCO-Glu(Asp-Phe-NH2)-NPr2 stimulated gastric secretion. Of these peptides, PhCO-Glu(Phe-NH2)-NPr2 showed the most potent inhibitory activity against gastric secretion, and was more potent than proglumide.

Keywords—proglumide; N-acyl-γ-L-glutamyl peptide; γ-glutamylphenylalanine amide; peptide synthesis; anti-gastric secretion

In the previous study, we investigated the effects of N-acyl-γ-D-glutamyl peptides containing a C-terminal small fragment of cholecystokinin (CCK) on gastric secretion. It was found that PhCO-D-Glu(Phe-NH2)—NPr2 and PhCO-D-Glu(Asp-Phe—NH2)—NPr2 inhibited gastric secretion and that Z-D-Glu(Phe—NH2)-NPr2 was the most potent of the peptides investigated by us.

The present investigation on N-acyl-γ-L-glutamyl derivatives showed that the structure-activity relationship of these compounds was not parallel with that of N-acyl-γ-D-glutamyl compounds. Thus, PhCO-Glu(Phe—NH2)—NPr2 (Ia) inhibited gastric secretion more strongly than Z-Glu(Phe—NH2)—NH2 (IIa), while PhCO-Glu(Asp-Phe—NH2)—NPr2 (Ib) stimulated gastric secretion, in contrast to the inhibitory activity of PhCO-D-Glu(Asp-Phe—NH2)—NPr2.

This report describes the syntheses of Ia, b and some analogs of Ia, as well as their effect on gastric secretion.

Compounds Ia, b were synthesized as shown in Fig. 1. Z-Glu—OH reacted with paraformaldehyde to prepare the oxazoline compound according to the procedure of Itoh, followed by reaction with di-n-propylamine (HNPr2) to give Z-Glu—NPr2. Z-Glu—NPr2 was coupled with H—Phe—NH2 or H—Asp—Phe—NH2 by the mixed acid anhydride method to produce the dipeptide derivative (IIa) and tripeptide derivative (IIb). Compounds IIa, b were deprotected by hydrogenolysis over Pd catalyst, then acylated with benzoyl chloride in the presence of NaHCO3 to give Ia, b.

![Fig. 1](image-url)
In order to investigate analogs of Ia, PhCO-Glu(NPr₂)-Phe-NH₂ (III) and PhCO-Asp(Phe-NH₂)—NPr₂ (IV) were prepared. The synthetic scheme for III is shown in Fig. 2. Z-Glu-OMe₆) was condensed with HNPr₂ by the mixed acid anhydride method then saponified to afford Z-Glu(NPr₂)-OH, which was coupled with H-Phe-NH₂ by the mixed acid anhydride method to produce Z-Glu(NPr₂)-Phe-NH₂. This compound was deprotected and acylated to give III in the same way as described for Ia.

The synthetic method for PhCO-Asp(Phe-NH₂)—NPr₂ (IV) is illustrated in Fig. 3. Z-Asp(OBzl)-OH was coupled with HNPr₂ by the mixed acid anhydride method then saponified to give Z-Asp-NPr₂. Z-Asp-NPr₂ was linked with H-Phe-NH₂, then the resulting Z-Asp(Phe-NH₂)—NPr₂ was deprotected and acylated to give IV according to the procedure described for Ia.

The reference compound, proglumide (PhCO–Glu(NPr₂)—Phe—NH₂) (III) and PhCO–Asp(Phe—NH₂)—NPr₂ (IV) were prepared. The synthetic scheme for III is shown in Fig. 2. Z-Glu–OMe₆) was condensed with HNPr₂ by the mixed acid anhydride method then saponified to afford Z-Glu(NPr₂)—OH, which was coupled with H-Phe—NH₂ by the mixed acid anhydride method to produce Z-Glu(NPr₂)—Phe—NH₂. This compound was deprotected and acylated to give III in the same way as described for Ia.

The synthetic method for PhCO–Asp(Phe—NH₂)—NPr₂ (IV) is illustrated in Fig. 3. Z-Asp(OBzl)—OH was coupled with HNPr₂ by the mixed acid anhydride method then saponified to give Z-Asp—NPr₂. Z-Asp—NPr₂ was linked with H-Phe—NH₂, then the resulting Z-Asp(Phe—NH₂)—NPr₂ was deprotected and acylated to give IV according to the procedure described for Ia.

The reference compound, proglumide (PhCO–Glu(NPr₂)—Phe—NH₂) was extracted from a commercial preparation of proglumide (PROMID®). Another reference compound, L-proglumide (l form in proglumide) was prepared from Z-Glu—NPr₂. Thus, Z-Glu—NPr₂ was deprotected by hydrogenolysis, then acylated with benzoyl chloride to give L-proglumide in the same way as described for Ia.

The synthesized peptides were shown to be homogeneous by thin-layer chromatography (TLC) on silica gel and gave the expected elemental analyses. Amino acid analyses of acid hydrolysates of these peptides gave results in good agreement with the theoretically expected values.

The effects of the synthesized peptides (Ia, Ib, IIA, III, IV), proglumide and L-proglumide on gastric secretion in rats were examined in the same manner as described by Watanabe et al. for the evaluation of proglumide.⁸ The test compounds suspended in 1% gum arabic solution were injected intraperitoneally into rats. The control rats were injected with 1% gum arabic solution. The volume of gastric juice secreted during 4 h after injection of the test compound, free acidity and total acidity of the gastric juice were measured and expressed as the ratio (%) with respect to the control value (Table I).

Compound Ia having a Phe—NH₂ residue inhibited gastric secretion more strongly than proglumide or L-proglumide. On the other hand, compound Ib having a Asp—Phe—NH₂ residue stimulated gastric secretion but appeared to induce autoinhibition at a high dose, as pentagastrin and MBOC–Met–Asn–Phe–OH do.⁹ These results may be explained in terms of two types of gastrin receptor, a low-affinity gastrin receptor which leads to inhibition of
secretion and a high-affinity gastrin receptor which activates secretion.9a) Thus, L-proglumide would have specific affinity for the low-affinity gastrin receptor, and the introduction of a Phe–NH$_2$ residue into L-proglumide increased the affinity. On the other hand, the introduction of an Asp-Phe-NH$_2$ residue into L-proglumide resulted in higher affinity for the high-affinity gastrin receptor than the low-affinity gastrin receptor, consequently stimulating gastric secretion. When a high dose of Ib was given, some Ib would bind to the low-affinity gastrin receptor and would induce autoinhibition.

The stimulative activity of Ib forms a contrast with the inhibitory activity of the epimer [PhCO–D-Glu(Asp–Phe–NH$_2$)–NPr$_2$] described in the previous paper.2) The present result for Ib and the previous result for the epimer of Ib show that the configuration of the glutamyl moiety of these tripeptide is very important for binding to the gastrin receptor.

The N-benzyloxycarbonyl compound (IIa) inhibited gastric secretion but appeared to be less potent than the N-benzoyl compound (Ia). In the previous study on D-glutamyl peptides, Z–D-Glu(Phe–NH$_2$)–NPr$_2$ was more potent than PhCO–D-Glu(Phe–NH$_2$)–NPr$_2$.2) Not only the result for Ib but also the result for IIa show that the structure–activity relationship for gastric secretion of N-acyl–γ-L-glutamyl peptides is not parallel with that of N-acyl–γ-D-glutamyl peptides.

In order to investigate further the structural requirements for the glutamyl moiety, the α-glutamyl dipeptide (III) and β-aspartyl dispeptide (IV) were investigated. These compounds inhibited gastric secretion, but their inhibitory activities in terms of the acidity of gastric juice were less than that of Ib. This means that the Glu(Phe–NH$_2$) moiety is preferable to the Glu–Phe–NH$_2$ moiety or Asp(Phe–NH$_2$) moiety.

In conclusion, Ia has a more potent inhibitory activity against gastric secretion than proglumide, and the structure–activity relationship for gastric secretion of N-acyl–γ-L-glutamyl peptides is not parallel with that of N-acyl–γ-D-glutamyl peptides.

**Experimental**

The melting points are uncorrected. Optical rotations were measured with a DIP-181 polarimeter (Japan Spectroscopic Co.). Amino acid analyses of acid hydrolysates were performed according to the procedure of Lee et al.
Elementary analyses were carried out with a Yanagimoto MT-3 CHN Corder. Proton nuclear magnetic resonance (1H-NMR) spectra were recorded on a JEOL JNPS PS-100 high-resolution NMR spectrometer; chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Ascending TLC was performed on silica gel TLC plates (Kieselgel 60 F254, Merck) using the following solvent systems: Rf1, benzene–AcOH (3:1); Rf2, CHCl3–acetone (1:1); Rf3, CHCl3–acetone (3:1); Rf4, n-BuOH–AcOH–H2O (4:1:5).

Z-Glu-NP2r-----Z-Glu–OH3) (80 g) was reacted with paraformaldehyde (14 g) in the presence of p-toluenesulfonic acid hydrate (2.8 g) in benzene (2 l) according to the procedure of Itoh to give benzoyloxycarbonyl-S-oxo-4-oxazolizinopropionic acid as an oil (89.9 g). This oil (89.9 g) and HNPr2 (62 g) were dissolved in THF (180 ml) and the mixture was refluxed for 8 h. After evaporation of the solvent in vacuo, the residue was dissolved in AcEt, then washed with 3% HCl and H2O. The solution was extracted with 1 N NaOH (600 ml). The water layer was acidified with concentrated HCl under cooling with ice and extracted with AcEt (700 ml). The extract was washed with H2O and brine, then dried over anhydrous MgSO4. The solvent was evaporated off in vacuo and the residue was crystallized from ether to give needles. Yield 46 g (44%), mp 82–85 °C, [α] D = −25.8 (c = 5, MeOH). Anal. Calcd for C15H23NO9: C, 62.51; H, 6.94; N, 11.76.

Asp, 1.04; Glu, 0.99; Phe, 0.97 (average recovery, 98%). Anal. Calcd for C31H41N5O7: C, 62.27; H, 6.89; N, 11.76.

PhCO-Glu(N-Phe-NH3) (Ⅰa)-----A solution of Ⅰa (3.2 g) in MeOH (60 ml) containing 5.6 N HCl/dioxane (2 ml) was hydrogenated over a palladium catalyst (10% Pd-C, 0.1 g) with bubbling of hydrogen at room temperature for 2 h. After removal of the catalyst, the solution was evaporated off in vacuo to give H-Glu(Phe-NH2)-HCl, which was dissolved in H2O (50 ml) containing NaHCO3 (1.1 g). To this aqueous solution, benzyl chloride (0.89 g) in ether (20 ml) was added under cooling with ice, and the mixture was stirred for 3 h. The reaction mixture was extracted with AcEt (200 ml), and the extract was washed with H2O and dried over anhydrous MgSO4. AcEt was evaporated off in vacuo and the residue was triturated with ether to afford a crude product, which was recrystallized from AcEt–hexane. Yield 2.2 g (68%), mp 149–151 °C, [α] D = −1.8 (c = 1, DMF), [α] D = +16.1 (c = 1, DMF), Rf1 = 0.42, Rf2 = 0.27. Amino acid ratio in an acid hydrolysate: Glu, 0.96; Phe, 1.04 (average recovery, 94%). Anal. Calcd for C27H36N4O4: C, 67.48; H, 7.55; N, 11.66. Found: C, 67.27; H, 7.64; N, 11.01.

PhCO-Glu(N-Phe-NH2) (Ⅰb)-----Boc–Asp–Phe–NH2111) (3.8 g) was added to TFA (15 ml) under cooling with ice and the mixture was stirred for 1 h. Ether (50 ml) was added to give a precipitate, which was washed with ether to give H–Asp–Phe–NH2–TFA. This material was dissolved in H2O (50 ml) containing NaHCO3 (1.1 g). To this aqueous solution, ethyl chloroformate (0.96 g) and NEt3 (0.37 g) were dissolved in THF (50 ml) at 0–5 °C. After the mixture had been stirred for 15 min, NPr2 (1.6 g) was added and the whole was stirred under cooling with ice. The resulting precipitate was collected by filtration, washed with ether and H2O, then recrystallized from iso-PrOH. Yield 3.9 g (70%), mp 196.0–197.5 °C, [α] D = −37.8 (c = 1, MeOH), Rf1 = 0.25, Rf2 = 0.69. Anal. Calcd for C, 60.95; H, 7.03; N, 11.18.

PhCO-Glu(N-Phe-NH2) (Ⅰb)-----Compound Ⅰb (1.6 g) was deprotected and acylated with benzyloxycarbonyl chloride (0.37 g) in the same way as described for Ⅰa. The reaction mixture was washed with ether to give H–Asp–Phe–NH2–TFA. This material was dissolved in H2O (50 ml) containing NaHCO3 (1.1 g). To this aqueous solution, benzoyl chloride (0.89 g) in ether (20 ml) was added under cooling with ice, and the mixture was stirred for 3 h. The reaction mixture was extracted with AcEt (200 ml), and the extract was washed with H2O and dried over anhydrous MgSO4. AcEt was evaporated off in vacuo and the residue was triturated with ether to afford a crude product, which was recrystallized from AcEt–hexane. Yield 2.2 g (68%), mp 149–151 °C, [α] D = −1.8 (c = 1, DMF), [α] D = +16.1 (c = 1, DMF), Rf1 = 0.42, Rf2 = 0.27. Amino acid ratio in an acid hydrolysate: Glu, 0.96; Phe, 1.04 (average recovery, 94%). Anal. Calcd for C27H36N4O4: C, 67.48; H, 7.55; N, 11.66. Found: C, 67.27; H, 7.64; N, 11.01.

PhCO-Glu(N-Phe-NH2) (Ⅱb)-----Compound Ⅱb (1.6 g) was deprotected and acylated with benzyloxycarbonyl chloride (0.37 g) in the same way as described for Ⅰa. The reaction mixture was washed with ether to give H–Asp–Phe–NH2–TFA. This material was dissolved in H2O (50 ml) containing NaHCO3 (1.1 g). To this aqueous solution, ethyl chloroformate (0.96 g) and NEt3 (0.37 g) were dissolved in THF (50 ml) at 0–5 °C. After the mixture had been stirred for 15 min, NPr2 (1.6 g) was added and the whole was stirred under cooling with ice. The resulting precipitate was collected by filtration, washed with ether and H2O, then recrystallized from iso-PrOH. Yield 1.1 g (71%), mp 206–208 °C, [α] D = −48.4 (c = 1, DMF), Rf1 = 0.24, Rf2 = 0.69. Amino acid ratio in an acid hydrolysate: Asp, 1.04; Glu, 0.99; Phe, 0.97 (average recovery, 98%). Anal. Calcd for C31H34N4O4: C, 62.51; H, 6.94; N, 11.76. Found: C, 62.27; H, 6.89; N, 11.76.

Z-Glu(NP2r)–OH-----A mixture of 15 g-Z-Glu–OMe–DCHA6) (17 g) and 5% KHSO4 aqueous solution (150 ml) was stirred vigorously at room temperature for 1 h and the resulting oil product was extracted with AcEt (150 ml). The extract was dried over anhydrous MgSO4 and the solvent was evaporated off in vacuo to give Z-Glu–OMe as an oil, which was dissolved in AcEt (200 ml). The solution was washed successively with 3% HCl, 3% NaHCO3 and H2O, then dried over anhydrous MgSO4. The solvent was evaporated off in vacuo and the residue was crystallized from ether to give needles. Yield 46 g (44%), mp 82–85 °C, [α] D = −25.8 (c = 5, MeOH). Anal. Calcd for C15H23NO9: C, 62.51; H, 6.94; N, 11.76.
acid (meq/1) and the acidity of total acid (meq/1), respectively. The stomachs were removed according to Watanabe et al.8) The content in the stomach was centrifuged to obtain a supernatant. An aliquot of the supernatant was titrated with 0.02 N NaOH by using phenolphthalein and methyl yellow as indicators to determine the acidity of free supernatant. The gastric juice volume was measured by measuring the supernatant.

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**References and Notes**

1) The customary l. indication for amino acid residues is omitted. Standard abbreviations for amino acids and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [Biochemistry, 5, 2485 (1966); 6, 362 (1967); 11, 1726 (1972)]. Other abbreviations used are: Boc, tert-
butyloxycarbonyl; Z, benzyloxycarbonyl; PhCO, benzyol; OMe, methyl ester; OBzl, benzyl ester; NPr$_2$, di-n-propylamino; DCHA, dicyclohexylamine; DMF, N,N-dimethylformamide; THF, tetrahydrofuran; TFA, trifluoroacetic acid; AcOEt, ethyl acetate; AcOH, acetic acid; iso-PrOH, 2-propanol; n-BuOH, 1-butanol.