stroys the receptor's ability to respond either to the affinity label or to a subsequent dose of agonist. Inactivation occurs whether the photolyzable functional group is located at the amino terminus of angiotensin (nitroazidobenzoyl-angiotensin) or at the 4 or 8 position.[5]

This long-lasting inactivation is in contrast to the results found with cholecystokinin receptor in the guinea pig exocrine pancreas. In this tissue both nitroazidobenzoylglycine-acylated carboxy-terminal tetra- and octapeptides elicited a long-lasting *agonist* effect after photoaffinity labeling.[2,3] Therefore, there must be a fundamental difference between cholecystokinin receptor in pancreas and angiotensin receptor in aorta, such as the mechanism of hormone recognition by the receptor, the activation of the receptor's initial response to the hormone, or the coupling of this response to a second messenger.

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Potential Thyroliberin Affinity Labels. 1. Chloroacetyl-Substituted Phenylalanylpyrrolidines[1]

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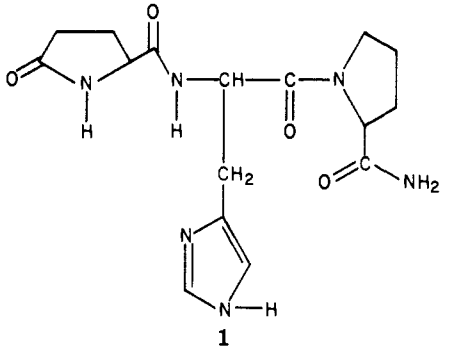
Six analogues of thyroliberin (TRH) that have a chloroacetyl substituent at the amino terminus have been prepared as potential affinity labels for the TRH receptor. These compounds are *N*-(chloroacetyl)-L-alanyl-L-phenylalanylpyrrolidine (ClAc-Ala-Phe-Pyrr; 14), *N*-[*m*-(chloroacetyl)benzoyl]-L-phenylalanylpyrrolidine (*m*-ClAcBz-Phe-Pyrr; 11a), *N*-[*m*-(chloroacetyl)benzoyl]-L-alanyl-L-phenylalanylpyrrolidine (*m*-ClAcBz-Ala-Phe-Pyrr; **15a**), *N*-[*p*-(chloroacetyl)benzoyl]-L-phenylalanylpyrrolidine (*p*-ClAcBz-Phe-Pyrr; 11**b**), and *N*-[*p*-(chloroacetyl)benzoyl]-L-alanyl-L-phenylalanylpyrrolidine (*p*-ClAcBz-Ala-Phe-Pyrr; **15b**). Pyroglutamyl-L-phenylalanylpyrrolidine was also synthesized as a model agonist. Weak agonist activity was observed for 11a, 11**b**, and **15b**. These three analogues do not contain the amide group of the pyroglutamyl moiety that was previously thought to be essential for intrinsic activity. No significant antagonist activity was observed for these compounds at the doses tested.

Thyroliberin (TRH; 1)[2] was the first peptide hormone



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from the hypothalamus to be isolated, structurally characterized, and synthesized.[3,4] The initial knowledge that TRH influenced the secretion of thyrotropin (TSH) from the anterior pituitary[5] was followed by subsequent studies[6-10] with synthetic TRH that showed that TRH concommitantly stimulates the release of prolactin (PRL) both in vitro and in vivo. The development of a radioimmunoassay[11] for TRH led to the identification of TRH and TRH-like immunoreactive substances not only in the hypothalamus but throughout the brain[12-16] and also in the

(1) This work forms part of a thesis submitted by one of us (R. J.G.) to the University of Illinois at the Medical Center, in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the Department of Medicinal Chemistry. This work has been presented in part. See "Abstracts of Papers", 180th National Meeting of the American Chemical Society, Las Vegas, NV, Aug 24-29, 1980, American Chemical Society, Washington, D.C., 1980, Abstr MEDI 47.

(2) Abbreviations used are: TRH, thyrotropin-releasing hormone; Pyrr, pyrrolidine; pGlu, pyroglutamyl; Phe, phenylalanyl; Ala, alanyl; ClAc, chloroacetyl; ClAcBz, chloroacetylbenzoyl; Z, carbobenzyloxy; IBCF, isobutyl chloroformate; NMM, *N*-methylmorpholine; TLC, thin-layer chromatography.

(3) K. Folkers, F. Enzmann, J. Bøler, C. Y. Bowers, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **37**, 123 (1969); J. Bøler, F. Enzmann, K. Folkers, C. Y. Bowers, and A. V. Schally, *ibid.*, **37**, 705 (1969); R. M. G. Nair, J. F. Barrett, C. Y. Bowers, and A. V. Schally, *Biochemistry*, **9**, 1103 (1970).

(4) R. Burgus, T. F. Dunn, D. Desiderio, and R. Guillemin, *C. R. Hebd. Seances Acad. Sci.*, **269**, 1870 (1969); R. Burgus, T. Dunn, D. Desiderio, D. Ward, W. Vale, and R. Guillemin, *Nature (London)*, **226**, 321 (1970).

(5) S. Reichlin, *N. Engl. J. Med.*, **269**, 1182 (1963).

(6) C. Y. Bowers, H. G. Friesen, R. Hwang, H. J. Guyda, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **45**, 1033 (1972); C. Y. Bowers, H. G. Friesen, and K. Folkers, *ibid.*, **51**, 512 (1973); C. Y. Bowers, H. G. Friesen, and K. Folkers, in "Biological Rhythmns in Neuroendocrine Activity", M. Kawakami, Ed., Igaku Shoin, Tokyo, 1974, p 102.

(7) M. L'Hermite, L. Vanhaelst, G. Copinschi, R. Lederig, J. Goldstein, O. D. Bruno, and C. Robyn, *Lancet*, 1, 763 (1972).

(8) A. H. Tashjian, Jr., Y. Yasamura, L. Levine, G. H. Sato, and M. L. Parker, *Endocrinology*, **82**, 342 (1968); P. M. Hinkle, E. E. Woroch, and A. H. Tashjian, Jr., *J. Biol. Chem.*, **249**, 3085 (1974); P. S. Dannies and A. H. Tashjian, Jr., *Nature (London)*, **261**, 707 (1976).

(9) K.-H. Lu, C. J. Shaar, K. G. Kartwright, and J. Meites, *Endocrinology*, **92**, 1540 (1972).

(10) W. Vale, R. Blackwell, G. Grant, and R. Guillemin, *Endocrinology*, **93**, 26 (1973).

(11) R. M. Bassiri and R. D. Utiger, *Endocrinology*, **90**, 722 (1972).

(12) M. J. Brownstein, M. Palkovits, J. M. Saavedra, R. M. Bassiri, and R. D. Utiger, *Science*, **185**, 267 (1974); A. Winokur and R. Utiger, *ibid.*, **185**, 265 (1974).

(13) Y. Grimm-Jorgensen and J. F. McKelvy, *Nature (London)*, **254**, 620 (1975).

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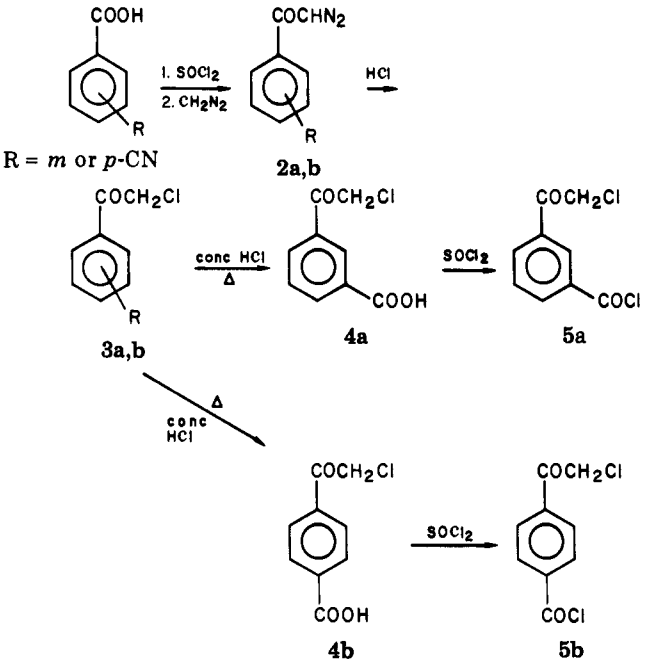
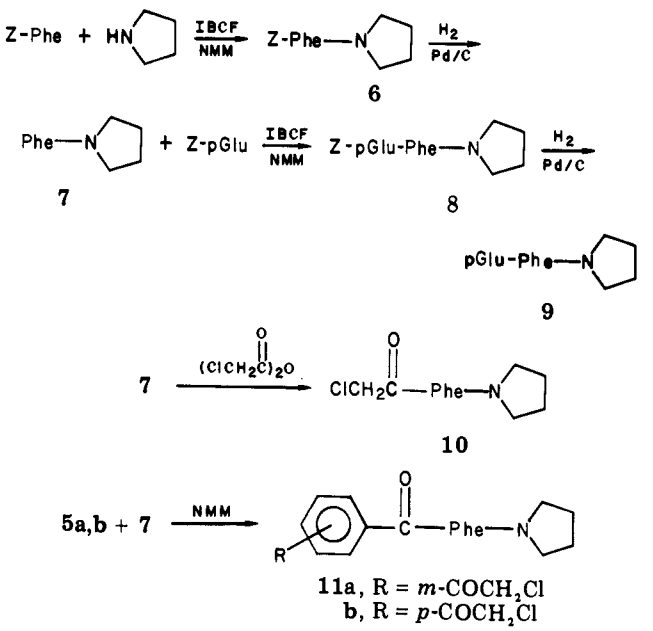
gastrointestinal tract,[17] pancreas,[18] eye,[18] and human placenta.[18,19] These and other observations have led to speculation that TRH functions as a neurotransmitter as well as a neurohormone.[20] Pharmacological activities of TRH associated with the CNS, such as reversal of barbiturate[21] and ethanol[22] hypnosis and other observations,[23-26] support a neurotransmitter-like effect of TRH. The rather unique structure of TRH is resistant to digestive enzymes of the gastrointestinal tract and is thus orally active.[27] These factors have led us to use TRH as a model peptide for our effects to develop peptide analogues that could bind irreversibly to brain or pituitary receptors and thus aid in the characterization of these receptors. Interesting endocrine or CNS properties could also result from this type of analogue.

Previous synthetic analogues of TRH reported by several groups of investigators[28-31] have led to some general conclusions about the structure–activity relationships of TRH, pGlu-His-Pro-NH_2. Intrinsic activity seems to reside primarily in the lactam portion of the pyroglutamyl residue and the C-terminal carboxamide. The imidazole ring and the ring structures of the pyroglutamyl and prolyl residues are important for binding and thus for potency. Almost all of these previous analogues have displayed agonist activity or no activity at all. There are only three reports of TRH analogues that are antagonists. Lybeck et al.[32] and

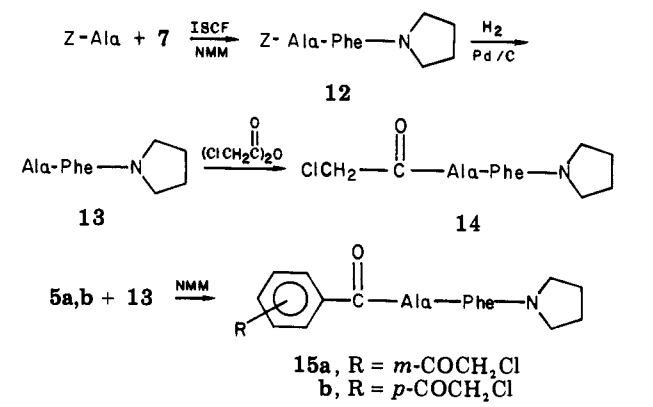
Scheme I



Scheme II



Scheme III



(14) W. F. White, M. T. Hedlund, R. H. Weber, R. H. Rippel, E. S. Johnson, and J. F. Wilber, *Endocrinology*, **94**, 1422 (1974).
(15) I. M. D. Jackson and S. Reichlin, *Endocrinology*, **95**, 854 (1974).
(16) C. Oliver, R. L. Eskay, N. Ben-Jonathan, and J. C. Porter, *Endocrinology*, **95**, 540 (1974).
(17) J. E. Morley, T. J. Garvin, A. E. Pekary, and J. M. Hershman, *Biochem. Biophys. Res. Commun.*, **79**, 314 (1977).
(18) W. W. Youngblood, J. Humm, and J. S. Kizer, *Brain Res.*, **163**, 101 (1979).
(19) G. Shambaugh III, M. Kubek, and J. F. Wilber, *J. Clin. Endocrinol. Metab.*, **48**, 483 (1979).
(20) M. Brownstein, in "Centrally Acting Peptides", J. Hughes, Ed., University Park Press, Baltimore, 1978, pp 37–47.
(21) A. J. Prange, Jr., G. R. Breese, J. M. Cott, B. R. Martin, B. R. Cooper, I. C. Wilson, and N. P. Plotnikoff, *Life Sci.*, **14**, 447 (1974).
(22) A. J. Prange, Jr., G. R. Breese, G. D. Jahnke, B. R. Martin, B. R. Cooper, J. M. Cott, I. C. Wilson, L. B. Alltop, M. A. Lipton, G. Bissette, C. B. Nemeroff, and P. T. Loosen, *Life Sci.*, **16**, 1907 (1975).
(23) N. P. Plotnikoff, A. J. Prange, Jr., G. R. Breese, M. S. Anderson, and I. C. Wilson, in "The Thyroid Axis, Drugs and Behavior", A. J. Prange, Jr., Ed., Raven Press, New York, 1974, pp 103–113.
(24) J. F. Wilber, E. Montoya, N. P. Plotnikoff, W. F. White, R. Gendrick, L. Renaud, and J. B. Martin, *Res. Prog. Hormone Res.*, **32**, 117 (1976).
(25) A. R. Green and D. C. Grahme-Smith, *Nature (London)*, **251**, 524 (1974).
(26) C. N. Jones, L. D. Grant, and A. J. Prange, Jr., *Psychopharmacology*, **59**, 217 (1978).
(27) C. Y. Bowers, A. V. Schally, A. Kastin, A. Arimura, D. S. Schalch, C. Gual, E. Castineda, and K. Folkers, *J. Med. Chem.*, **14**, 477 (1971).
(28) W. F. White, *Annu. Rep. Med. Chem.*, **8**, 204 (1974).
(29) R. Burgus, *Annu. Rep. Med. Chem.*, **7**, 194 (1972); R. Burgus, M. Monahan, J. Rivier, W. Vale, N. Ling, G. Grant, M. Amoss, and R. Guillemin, in "Hypothalamic Hypophysiotropic Hormones: Physiological and Clinical Studies", Proceedings of the Conference, Acapulco, 1972, Excerpta Medica: Amsterdam, 1973, p 12.
(30) C. Y. Bowers, K. Folkers, H. Sievertsson, B. L. Currie, C. Bogentoft, and J. K. Chang, in "Proceedings, IIIrd International Endocrine Symposium, 1971", S. Taylor, Ed., William Heineman Medical Books, Ltd., London, 1972, p 192.
(31) W. Vale, G. Grant, and R. Guillemin, "Frontiers in Neuroendocrinology", W. F. Ganong and L. Martini, Eds., Oxford University Press, London, 1973, p 375.

Table I. Agonist Activity Relative to TRH

peptide	rel act., %
TRH	100[a]
pGlu-Phe-Pyrr (**9**)	0.0017
ClAc-Phe-Pyrr (**10**)	none[b]
m-ClAcBz-Phe-Pyrr (**11a**)	0.0012
p-ClAcBz-Phe-Pyrr (**11b**)	0.0006
ClAc-Ala-Phe-Pyrr (**14**)	none[b]
m-ClAcBz-Ala-Phe-Pyrr (**15a**)	none[b]
p-ClAcBz-Ala-Phe-Pyrr (**15b**)	0.005

[a] TRH dose of 0.6 ng. [b] At a dose up to 30 μg.

Sievertsson et al.[33] developed antagonists which replaced the pyroglutamyl with cyclopentylcarbonyl and the prolinamide with pyrrolidine. This corroborated previous SAR results as previously discussed concerning requirements for intrinsic activity. Some agonist activity could be observed, however, at high dose levels. Bowers et al.[34] have also reported on the TRH antagonist effect of pGlu-Dopa-Pro-NH_2.

Interesting results have previously been reported with biologically active peptides or peptide analogues which contain alkylating functional groups.[35-39] Irreversible inhibition, long-lasting agonists, and metabolizing enzyme inactivation have been observed. A nitrogen mustard analogue of TRH, [chloroambucil[1]]-TRH, was found to be an apparent irreversible inhibitor of TSH, but not PRL, release following initial transient agonist effects.[34]

We now report the development of a series of TRH analogues that were designed as antagonists but have weak agonist activity and also contain a moiety that might react with available nucleophiles.

Chemistry. The required 3- and 4-(chloroacetyl)benzoic acids were synthesized according to the method outlined in Scheme I. This is an adaptation of a method reported previously for the para isomer and also proved suitable for the meta isomer. Several other methods were tried, including direct halogenation,[40] but with only limited success. Peptide portions of the TRH analogues were synthesized according to Schemes II and III. A modification of the mixed anhydride method of Tilak[41] was used for peptide bond formation. Activation times for the formation of the reactive intermediate between the respective amino acids and isobutyl chloroformate were very important. Appropriate times ranged from 30 s or less for Z-Ala to 12 min for Z-pGlu. Carbobenzyloxy (Z) groups were removed by catalytic hydrogenolysis.

Even though the chloroacetyl group is reactive toward nucleophiles, it is sufficiently stable in aqueous media for extraction procedures in reaction workups, including the use of aqueous bicarbonate. The chloroacetyl-substituted benzoic acids can even be recrystallized from hot water. Prolonged standing of this type of compound in methanol, however, has been observed to lead to decomposition.

Biological Assay. The initial evaluation of these compounds as TRH agonists and antagonists was by the method of Bowers et al.[34] Freshly excised whole pituitaries from 20 day old female rats were incubated in Krebs–Ringer bicarbonate for six 1-h periods. The incubation medium was replaced after each hour. Test samples were added during the 3rd through the 6th h (I_3–I_6), and the resultant TSH concentration in the incubation media was measured by radioimmunoassay and compared with the 2nd h (P_2). Agonist activity was measured by the effect of the analogues alone on the pituitaries. Antagonist activity was measured by the net effect of the analogue in combination with synthetic TRH. The bioassay results are summarized in Table I.

Experimental Section

Melting points were determined on a Mel-Temp block and are uncorrected. TLC was performed on 250-μm silica gel G plates that contained fluorescent indicator from E. Merck. The following solvent systems were used: (A) $CHCl_3$; (B) $CHCl_3$–MeOH, 19:1; (C) $CHCl_3$–MeOH–NH_4OH, 38:1:1, lower phase; (D) $CHCl_3$–MeOH–NH_4OH, 18:1:1, lower phase; (E) $CHCl_3$–MeOH–HOAc, 18:1:1; (F) $CHCl_3$–EtOAc, 4:1. Methods of detection are indicated with the data. ^1H NMR spectra were recorded on a Varian T-60A spectrometer equipped with a Nicolet Instrument Corp. TT-7 Fourier transform accessory. The solvents used for ^1H NMR analysis are reported with the data, and spectral values are reported in parts per million (δ) downfield from tetramethylsilane. IR spectra were recorded on a Perkin-Elmer 337 grating infrared spectrophotometer. Optical rotations were determined using a Perkin-Elmer digital readout polarimeter Model 241. All amino acid derivatives used were of the L configuration. Elemental analyses were performed by Micro-Tech Laboratories of Skokie, IL. Where analyses are indicated only by symbols of the elements, analytical results were within ±0.4% of theoretical values.

3-(Chloroacetyl)benzonitrile (3a). 3-Cyanobenzoic acid (2.0 g, 13.6 mmol) was refluxed with $SOCl_2$ (20 mL) for 1 h and concentrated in vacuo to an oil, which was dissolved in ether (50 mL) and added dropwise over a 5-min period to an ice-cold ethereal solution of diazomethane (36.3 mmol) obtained from *N*-(nitrosomethyl)urea (10.0 g) according to the method of Arndt.[43] The resulting mixture was stirred at 0 °C for 1.5 h, and the crude diazo ketone was collected by filtration: yield 1.53 g (66%); NMR ($CDCl_3$) δ 5.92 (s, 1 H), 7.73 (m, 4 H); IR (Nujol) 2250 (CN), 2125 (CHN_2) cm^{-1}.

The diazo ketone **2a** (1.53 g) was resuspended in ether (75 mL), concentrated HCl (50 mL) was added carefully, and the mixture was refluxed for 1 h. Water (50 mL) was added, and the layers were separated. The aqueous layer was extracted with benzene (2 × 50 mL), and the combined organic layers were washed with 5% $NaHCO_3$ (2 × 50 mL) and water (50 mL), then dried ($MgSO_4$), and evaporated to dryness. Crystals were obtained from benzene–petroleum ether: yield 1.0 g (41%); mp 83–86 °C; one spot on TLC (A), UV positive; NMR ($CDCl_3$) δ 4.65 (s, 2 H), 7.89 (m, 4 H); IR (Nujol) 2220 (CN), 1700 (C=O), cm^{-1}. Anal. (C_9H_6ClNO) C, H, Cl, N.

(32) H. Lybeck, J. Leppalnoto, P. Virkkunen, D. Shafter, L. Carlsson, and J. Mulder, *Neuroendocrinology*, **12**, 366 (1973).
(33) H. Sievertsson, S. Castensson, K. Anderson, S. Bjorkman, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **66**, 1401 (1975).
(34) C. Y. Bowers, H. Sievertsson, J. Chang, J. Stewart, S. Castensson, S. Bjorkman, D. Chang, and K. Folkers, Proceedings of the 7th International Thyroid Conference, Boston, June, 1975, Excerpta Medica, Amsterdam, 1976, p 1.
(35) R. Walter, I. L. Schwartz, O. Hechter, T. Dansa, and P. L. Hoffman, *Endocrinology*, **91**, 29 (1972).
(36) D. H. Rich, P. D. Gesellchen, A. Tong, A. Cheung, and C. K. Buckner, *J. Med. Chem.*, **18**, 1004 (1975).
(37) J. M. Stewart and D. W. Woolley, *Nature (London)*, **206**, 619 (1965); F. W. Dunn and J. M. Stewart, *J. Med. Chem.*, 14, 279 (1971); R. J. Freer and J. M. Stewart, *ibid.*, **15**, 1 (1972); T. B. Paiva, A. C. M. Paiva, R. J. Freer, and J. M. Stewart, *ibid.*, **15**, 6 (1972); A. C. M. Paiva, V. L. A. Nouailketas, M. E. Miyamoto, G. B. Medes, and T. B. Paiva, *ibid.*, **16**, 6 (1973).
(38) J. Turk, P. Needleman, and G. R. Marshall, *J. Med. Chem.*, 18, 1135 (1975).
(39) C. Y. Bowers, Y. P. Wan, J. Humphries, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **61**, 598 (1974).
(40) J. Aston, J. Newkirk, P. Jenkins, and J. Dorsky, "Organic Syntheses", Collect. Vol III, E. C. Horning, Ed., Wiley, New York, 1955, p 538; B. Kumar and S. C. Kahra, *J. Indian Chem. Soc.*, **50**, 613 (1973).
(41) M. A. Tilak, *Tetrahedron Lett.*, 849 (1970).
(42) P. Richter, C. Garbe, and G. Wagner, *Pharmazie*, **29**, 256 (1974); G. Wagner, B. Voigt, and K. Steinbruck, *ibid.*, **31**, 254 (1976).
(43) F. Arndt, "Organic Syntheses", Collect Vol. II, A. H. Blatt, Ed., Wiley, New York, 1943, p 165.

3-(Chloroacetyl)benzoic Acid (4a) and Acid Chloride (5a). A solution of **3a** (0.48 g, 2.67 mmol) in dioxane (15 mL) and concentrated HCl (15 mL) was heated for 24 h at 80–85 °C in an oil bath. The resulting mixture was diluted with water (20 mL) and extracted with 5% $NaHCO_3$ (2 × 25 mL). The aqueous layer was acidified to pH 2 with concentrated HCl and, after cooling, the crude product was collected by filtration and recrystallized from benzene: yield 0.35 g (66%); mp 161–164 °C; one spot on TLC (E), UV positive; NMR (acetone-d_6) δ 5.08 (s, 2 H), 8.08 (m, 4 H); IR (Nujol) 1700 (C=O) cm^{-1}. Anal. ($C_9H_7ClO_3$) C, H, Cl.

A solution of **4a** (0.20 g, 1.01 mmol) in $SOCl_2$ (10 mL) was refluxed for 2 h and then concentrated to an oil by azeotroping excess $SOCl_2$ with dry benzene, and the crude **5a** was used without further purification.

4-(Chloroacetyl)benzonitrile (3b). 4-Cyanobenzoic acid (2.0 g, 13.6 mmol) was treated with $SOCl_2$ (20 mL) and diazomethane in a manner similar to the method for **3a**. The crude diazo ketone was collected by filtration: yield 1.87 g (80%); NMR ($CDCl_3$) δ 5.93 (s, 1 H), 7.80 (AA'BB' q, J = 9 and 1 Hz, 4 H); IR (Nujol) 2250 (CN), 2150 (CHN_2) cm^{-1}.

The diazo ketone **2b** (1.87 g) was converted to the chloro methyl ketone as for **3a**. Crystals were obtained from benzene–petroleum ether: yield 1.17 g (48%); mp 95–98 °C (lit.[42] mp 96–98 °C); one spot on TLC (A), UV positive; NMR ($CDCl_3$) δ 4.64 (s, 2 H), 7.69 (AA'BB' q, J = 18 and 9 Hz, 4 H); IR (Nujol) 2220 (CN), 1700 (C=O) cm^{-1}. Anal. (C_9H_6ClNO) C, H, Cl, N.

4-(Chloroacetyl)benzoic Acid (4b) and Acid Chloride (5b). The hydrolysis of **3b** proceeded analogous to **3a**. The product was recrystallized from acetone: yield 0.50 g (56%); mp 235–240 °C (lit.[44] mp 210 °C); one spot on TLC (E), UV positive; NMR (methanol-d_4) δ 4.94 (s, 2 H), 8.10 (s, 4 H); IR (Nujol) 1700, 1590 (C=O) cm^{-1}. Anal. ($C_9H_7ClO_3$) C, H, Cl.

The acid chloride was prepared by the method for **5a** and used without further purification.

L-Phe-Pyrr·HCl (7). Z-Phe (3.34 g, 11.2 mmol) in dry ethyl acetate (75 mL) was stirred and cooled to −35 °C in a dry ice–acetone bath. *N*-Methylmorpholine (1.38 mL, 12.5 mmol) was added, followed immediately with isobutyl chloroformate (1.38 mL, 10.6 mmol). After 7 min, pyrrolidine (0.92 mL, 11.0 mmol) was added. The reaction was warmed to room temperature and stirred overnight. The mixture was washed with 5% $NaHCO_3$ (2 × 25 mL), 1 N HCl (2 × 25 mL), and water (2 × 25 mL). The organic layer was dried ($MgSO_4$) and evaporated to yield 3.90 g of **6** as an oil. The NMR spectrum was consistent with the assigned structure.

Crude **6** was dissolved in methanol (125 mL) and 3 N HCl (8 mL). The mixture was hydrogenolyzed for 1 h at 10 psi and room temperature over 5% Pd/C. Crystalline **7** was obtained from absolute ethanol–ether: yield 1.68 g (62%); mp 208–211 °C; one spot on TLC (C), ninhydrin and Cl–tolidine positive; NMR (D_2O) δ 1.72 [m, 4 H (β-CH_2 of Pyrr)], 3.19 [m, 2 H (β-CH_2 of Phe)], 3.36 [m, 4 H (α-CH_2 of Pyrr)], 4.51 [t, $J \simeq$ 7 Hz, 1 H (α-CH of Phe)], 7.39 [s, 5 H (Ar-H of Phe)]. Anal. ($C_{13}H_{19}ClN_2O$) C, H, Cl, N.

Z-L-pGlu-L-Phe-Pyrr (8). Z-pGlu (0.52 g, 1.98 mmol) in dry ethyl acetate (30 mL) was stirred and cooled to −20 °C. *N*-Methylmorpholine (0.24 mL, 2.18 mmol) was added, followed by isobutyl chloroformate (0.24 mL, 1.85 mmol). After 12 min, **7** (0.24 g, 1.88 mmol) was added, followed by additional *N*-methylmorpholine (0.20 mL, 1.82 mmol). The mixture was warmed to room temperature and stirred overnight. Workup was similar to that for the preparation of **7**. Crystallization from ethyl acetate–ether–cyclohexane gave pure **8**: yield 0.66 g (77%); mp 157–160 °C; one spot on TLC (B), Cl–tolidine and UV positive, ninhydrin negative. The NMR spectrum was consistent with the assigned structure. Anal. ($C_{26}H_{29}N_3O_5$) C, H, N.

L-pGlu-L-Phe-Pyrr (9). Compound **8** (0.20 g, 0.43 mmol) was dissolved in methanol (30 mL) and glacial acetic acid (3 mL). The mixture was hydrogenolyzed for 5 min at 5 psi over 5% Pd/C and filtered through Celite, and the solvent was removed in vacuo. Crystallization from ethyl acetate–cyclohexane–petroleum ether gave pure **9**: yield 0.08 g (56%); mp 146–149 °C; $[\alpha]^{28}_D$ +10.0 (*c* 0.883, $CHCl_3$); one spot on TLC (B), Cl–tolidine and UV positive, ninhydrin negative; NMR ($CDCl_3$) δ 1.72 [m, 4 H (β-CH_2 of Pyrr)], 2.27 [m, 4 H (overlapping CH_2 from pGlu)], 3.04 [m, 2 H (β-CH_2 of Phe)], 3.38 [m, 4 H (α-CH_2 of Pyrr)], 4.18 [m, 1 H (α-CH of pGlu)], 4.96 [m, $J \simeq$ 7 and 7 Hz, 1 H (α-CH of Phe)], 7.25 [s, 5 H (Ar-H of Phe)]. Anal. ($C_{18}H_{23}N_3O_3$) C, H, N.

***N*-(ClAc)-L-Phe-Pyrr (10).** Compound **7** (0.40 g, 1.57 mmol) was dissolved in dry methylene chloride (15 mL). Chloroacetic anhydride (0.26 g, 1.52 mmol) was added, followed by *N*-methylmorpholine (0.18 mL, 1.64 mmol). The mixture was stirred at room temperature for 7 h and then washed with 5% $NaHCO_3$ (15 mL), 1 N HCl (15 mL), and water (15 mL). The organic layer was dried ($MgSO_4$) and evaporated to an oil, which was crystallized from methylene chloride–petroleum ether to yield 0.22 g (49%): mp 60–64 °C; $[\alpha]^{28}_D$ +29.3 (*c* 0.775, $CHCl_3$); one spot on TLC (B), Cl–tolidine and UV positive, ninhydrin negative. The NMR spectrum confirmed the assigned structure. Anal. ($C_{15}H_{19}ClN_2O_2$) C, H, Cl, N.

***N*-[3-(ClAc)Bz]-L-Phe-Pyrr (11a).** To a solution of **7** (0.30 g, 1.18 mmol) in methylene chloride (10 mL) and *N*-methylmorpholine (0.12 mL) was added **5a** (0.21 g), followed by additional *N*-methylmorpholine (0.12 mL). The reaction was stirred for 3 h at room temperature and then worked up as for the preparation of **10**. Pure crystalline **11a** was obtained from methylene chloride–petroleum ether: yield 0.20 g (70%); mp 145–147 °C; $[\alpha]^{28}_D$ +20.2 (*c* 0.983, $CHCl_3$); one spot on TLC (B and F), Cl–tolidine and UV positive, ninhydrin negative. The expected NMR spectrum was observed. Anal. ($C_{22}H_{23}ClN_2O_3$) C, H, Cl, N.

***N*-[4-(ClAc)Bz]-L-Phe-Pyrr (11b).** A solution of **7** (0.30 g, 1.18 mmol) in methylene chloride (10 mL) and *N*-methylmorpholine (0.12 mL) was treated with **5b** (0.20 g), followed by additional *N*-methylmorpholine (0.12 mL) as in the preparation of **11a**. The residue after workup was applied to a chloroform-packed silica gel column [35 g (28 × 2.5 cm)] and eluted with chloroform (600 mL). Crude **11b** was collected in fractions (4 mL) 72 to 130 and crystallized from cyclohexane: yield 0.20 g (50%); mp 65–70 °C dec; $[\alpha]^{28}_D$ +39.0 (*c* 0.800, $CHCl_3$); one spot on TLC (B and F), Cl–tolidine and UV positive, ninhydrin negative. The NMR spectrum was consistent with the assigned structure. Anal. ($C_{22}H_{23}ClN_2O_3$) H, Cl, N; C: calcd, 66.24; found, 65.67.

L-Ala-L-Phe-Pyrr·HCl (13). Z-Ala (1.0 g, 4.48 mmol) was dissolved in dry tetrahydrofuran (50 mL) with stirring and cooled to −40 °C. *N*-Methylmorpholine (0.55 mL, 5.0 mmol) was added, followed by isobutyl chloroformate (0.56 mL, 4.32 mmol). After 15 s, **7** (1.10 g, 4.32 mmol) was added, followed by additional *N*-methylmorpholine (0.48 mL). The mixture was allowed to warm to room temperature and stirred overnight. Workup was similar to that for the preparation of **7**: yield 1.70 g of **12**; one spot on TLC (B), Cl–tolidine and UV positive, ninhydrin negative; NMR ($CDCl_3$) δ 1.34 [d, $J \simeq$ 7 Hz, 3 H (β-CH_3 of Ala)], 1.67 [m, 4 H (β-CH_2 of Pyrr)], 2.99 [m, 2 H (α-CH_2 of Phe)], 3.37 [m, 4 H (α-CH of Ala)], 4.87 [m, $J \simeq$ 7 and 7 Hz, 1 H (α-CH of Phe)], 5.11 [s, 2 H (benzyl CH_2 of Z)], 7.21 [s, 5 H (Ar-H of Z)], 7.33 [s, 5 H (Ar-H of Phe)].

A solution of **12** (1.70 g) in methanol (60 mL) containing gaseous HCl (0.2 g) was hydrogenolyzed for 25 min at 10 psi over 5% Pd/C. Crude **13** (1.07 g) was recrystallized from benzene to yield 0.50 g (36% based on **12**): mp 110–115 °C; one spot on TLC (D), ninhydrin and Cl–tolidine positive; exhibited the expected NMR spectrum.

***N*-(ClAc)-L-Ala-L-Phe-Pyrr (14).** Similar to the preparation of **10**, a solution of **13** (0.19 g, 0.58 mmol) in dry methylene chloride (8 mL) was treated with chloroacetic anhydride (0.10 g, 0.59 mmol), followed by *N*-methylmorpholine (0.08 mL). Crystalline **14** was obtained from methylene chloride–petroleum ether, and exhibited the appropriate NMR spectrum: yield 0.06 g (28%); mp 149–152 °C; $[\alpha]^{28}_D$ +7.36 (*c* 0.883, $CHCl_3$); one spot on TLC (B), UV and Cl–tolidine positive, ninhydrin negative. Anal. ($C_{18}H_{24}ClN_3O_3$) C, H, Cl, N.

***N*-[3-(ClAc)Bz]-L-Ala-L-Phe-Pyrr (15a).** A solution of **13** (0.41 g, 1.24 mmol) in methylene chloride (10 mL) and *N*-methylmorpholine (0.12 mL) was treated with **5a** (0.21 g), followed by additional *N*-methylmorpholine (0.12 mL) and stirred for 3 h at room temperature. The workup was the same as in the

(44) J. Kunskell-Rostock, *Ber. Deutsch. Pharm. Ges.*, 21, 419 (1911).

preparation of 10. Chromatographic purification was required on a chloroform-packed silica gel column [35 g (27.5 × 2.5 cm)], eluted with chloroform (200 mL), 10% ethyl acetate in chloroform (100 mL), 20% ethyl acetate in chloroform (100 mL), 40% ethyl acetate in chloroform (100 mL), 80% ethyl acetate in chloroform (100 mL), and ethyl acetate (700 mL). Crude **15a** was collected in fractions (4 mL) 190–229 and crystallized from ethyl acetate–petroleum ether: yield 0.20 g (42%); mp 135–138 °C; $[\alpha]^{28}_D$ −15.2 (*c* 0.983, $CHCl_3$); one spot on TLC (B), UV and Cl–tolidine positive, ninhydrin negative. The NMR spectrum supported the assigned structure. Anal. ($C_{25}H_{28}ClN_3O_4$) C, H, Cl, N.

***N*-[4-(ClAc)Bz]-L-Ala-L-Phe-Pyrr (15b).** To a solution of **13a** (0.41 g, 1.24 mmol) in methylene chloride (10 mL) and *N*-methylmorpholine (0.12 mL) was added **5b** (0.20 g), followed by additional *N*-methylmorpholine (0.12 mL). The reaction was stirred for 3 h at room temperature and then worked up as described above. Chromatographic purification of **15b** on a silica gel column [40 g (2.5 × 30 cm)] and elution with chloroform (200 mL), 10% ethyl acetate in chloroform (100 mL), 20% ethyl acetate in chloroform (100 mL), 40% ethyl acetate in chloroform (100 mL), 80% ethyl acetate in chloroform (100 mL), and ethyl acetate (700 mL) yielded **15b** in fractions (4 mL) 203 to 238. Crystallization from ethyl acetate gave pure crystalline **15b** with the expected NMR spectrum: yield 0.22 g (46%); mp 194–197 °C; $[\alpha]^{28}_D$ +12.2 (*c* 0.975, $CHCl_3$); one spot on TLC (B), UV and Cl–tolidine positive, ninhydrin negative. Anal. ($C_{25}H_{28}ClN_3O_4$) C, H, Cl, N.

Conclusion

Seven analogues of TRH are reported, six of which have replacements of the pyroglutamic acid moiety. The agonist activity observed for several of these analogues indicated that the pyroglutamyl moiety is not essential for intrinsic activity. It is possible that the carbonyl of the chloroacetyl moiety is fitting the binding site normally occupied by the lactam carbonyl of the pyroglutamic acid. It is interesting to note that in those analogues where the chloroacetyl is part of an amide the agonist activity is not observed. However, when the chloroacetyl group is present as a ketone the agonist activity is apparent. No significant antagonist activity was observed up to a dose of 30 μg.

Synthesis of Peptides by the Solid-Phase Method. 6. Neurotensin, Fragments, and Analogues[1a]

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Neurotensin (NT) and 24 related compounds, including fragments or analogues modified at the C-terminal end of the parent molecule, have been prepared by the solid-phase method. After purification by cation-exchange chromatography, the compounds were characterized by thin-layer chromatography, amino acid analysis, elemental analysis, and high-pressure liquid chromatography. The stimulating effects of the peptides were evaluated in rat stomach strips, in isolated spontaneously beating atria of guinea pigs, and in the coronaries of perfused rat hearts. The differences between the biological activities of these compounds are discussed.

Neurotensin (NT) is a recently discovered peptide hormone[2a] which is largely distributed in the central nervous system[2b,3] and in some regions of the digestive tract[2b,4,5] in various mammals. The peptide exhibits a large spectrum of biological activities, which have been discussed by several authors during the last few years.[6,7] The recent demonstration of the presence of immunoreactive NT in the blood of humans,[8] as well as of the elevated blood concentration of NT following the ingestion of food,[9] more strikingly fat-rich food,[10] seriously raised the possibility that this peptide behaves as a circulating hormone. A likely hypothesis to explain the above results would be that NT is released from endocrine cells located in the wall of the small intestines into the blood and/or the lumen from where it can diffuse into the blood. Recent reports from Swedish[9] and British[11] investigators support these hypotheses. The presence of NT or NT metabolites in the circulating blood raised also the possibility that these peptides influence the function of several organs, including the vasculature and the heart.

At the time we became interested in NT in 1978, this peptide was already known to exert an hypotensive effect in rats[2a] and dogs.[12] Other related actions of NT were an increased vascular permeability in rats[2a] as well as vasodilation in the small intestine and vasoconstriction in subcutaneous adipose tissues in dogs.[12] NT had been reported to be without effect on the heart rate in an-

(1) (a) Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature [*J. Biol. Chem.*, **247**, 977 (1971)]. Other abbreviations used are NT, neurotensin; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; DEA, diisopropylethylamine; DMF, dimethylformamide; 2-BrZ and 2-ClZ, 2-bromo- and 2-chlorocarbobenzoxy; Xan, xanthyl; Me, methyl ether. (b) Chercheur Boursier, Conseil de la Recherche en Santé du Québec. (c) Owner of a Studentship from le Conseil de la Recherche en Santé du Québec. (d) Associate of the Medical Research Council of Canada. (e) Scholar from the Quebec Heart Foundation.

(2) (a) R. Carraway and S. E. Leeman, *J. Biol. Chem.*, **248**, 6854 (1973). (b) R. Carraway and S. E. Leeman, *J. Biol. Chem.*, **251**, 7045 (1976).

(3) G. R. Uhl, M. J. Kuhar, and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 4059 (1977).

(4) L. Orci, O. Baëtens, C. Rufener, M. Brown, W. Vale, and R. Guillemin, *Life Sci.*, **19**, 559 (1976).

(5) J. M. Polack, S. N. Sullivan, S. R. Bloom, A. M. J. Buchan, P. Facer, M. R. Brown, and A. G. E. Pearse, *Nature (London)*, **270**, 183 (1977).

(6) S. E. Leeman, E. A. Mroz, and R. Carraway, in "Peptides in Neurobiology", H. Garner, Ed., Plenum Press, New York, 1977, pp 99–144.

(7) G. Bissette, P. Manberg, C. B. Nemeroff, and A. J. Prange, Jr., *Life Sci.*, **23**, 2173 (1978).

(8) A. M. Blackburn, S. R. Bloom, and J. M. Polack, *J. Endocrinol.*, **79**, 2 (1978).

(9) M. D. Mashford, G. Nilsson, A. Rokaeus, and S. Rosell, *Acta Physiol. Scand.*, **104**, 244 (1978).

(10) S. Rosell and A. Rokaeus, *Acta Physiol. Scand.*, **107**, 263 (1979).

(11) H. S. Besterman, S. R. Bloom, D. L. Sarson, A. M. Blackburn, D. I. Johnston, H. R. Patel, J. S. Stewart, R. Modighani, S. Guerin, and C. N. Mallinson, *Lancet*, 785 (1978).

(12) S. Rosell, E. Burcher, D. Chang, and K. Folkers, *Acta Physiol. Scand.*, **98**, 484 (1976).

0022-2623/81/1824-0370$01.25/0