These chlorambucil derivatives show that it is possible to achieve tissue-specific, hormone-specific, irreversible inhibitors of peptide hormones. The selective inhibition of the response of guinea pig ileum to angiotensin II makes possible the unequivocal identification of this substance in unknown solutions. The synthesis of **6** containing a radioactive isotope should provide a useful tool for the isolation and characterization of the receptor substance of this tissue.

Experimental Section

The peptides were synthesized by the Merrifield solid phase method,[9] using *N-tert*-butyloxycarbonyl (Boc) derivatives of the L amino acids, in which the reactive side chains were protected with the usual groups, with the exception of histidine, where the imidazole was protected with the *p*-Ts group[10] recently introduced for this purpose into solid phase synthesis.[11] A substantial cleavage of the Ts group was observed during the usual deprotection with 4 *N* HCl in dioxane, though not with 25% (v/v) of trifluoroacetic acid (TFA) in $CHCl_3$. For this reason the latter reagent was used (for 30 min) in all the deprotection steps, with a prewash of the same reagent to avoid excessive dilution by solvent in the resin. Other details of the synthesis were as previously described.[5]

The Boc amino acids were purchased from Schwarz BioResearch, with the exception of Boc-N^{im}-Ts-L-His, obtd from Fox Chemical Co. The purity of all of intermediates was checked by tlc on silica gel in 3 solvent systems: A, $CHCl_3$-MeOH-AcOH (85:10:5); B, $CHCl_3$-AcOH (95:5); C, Me_2CO-AcOH (98:2).

Bioassays were carried out as previously described.[5]

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Synthesis of Di- and Tripeptides and Assay *in Vivo* for Activity in the Thyrotropin Releasing Hormone and the Luteinizing Releasing Hormone Systems†

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Remarkable structure-hormonal activity relationships for the tripeptidic thyrotropin releasing hormone (TRH) of the hypothalamus are emerging. TRH and certain analogs having only one structural change release thyrotropin in the pituitary, but at a dosage differential of 1 to 1000-10,000. Such effective biological activity over such an extreme range of dosage is apparently possible only because of the nanogram potency of TRH and the microgram potency of the analogs. Ten additional tripeptides and two dipeptides, each containing a pGlu moiety and representing two structural changes in the hormone have been synthesized. All these pGlu peptides are inactive even at levels as high as 5000-50,000 times greater than that of an effective dosage of TRH. Since the luteinizing releasing hormone (LRH) of the hypothalamus appears also to have a pGlu moiety, and since it was recently discovered that a synthetic tetrapeptide, pGlu-Tyr-Arg-Trp-NH_2, releases LH like the natural hormone, these same twelve pGlu peptides have been tested for release of LH. Although these pGlu peptide variants did not release LH even at very high dose levels, additional information on the structural specificity of LRH was gained.

Chang, *et al.*,[1] and Bowers, *et al.*,[2] have reported the synthesis and the hormonal activities of structural modifications of the His and Pro moieties of the thyrotropin releasing hormone (TRH), which is pyroglutamylhistidylprolinamide (pGlu-His-Pro-NH_2).[3] Although TRH is relatively specific in releasing TSH, it was found that several tripeptide analogs showed very low but otherwise similar release of TSH. However, some of the active analogs were not inactivated by serum, but the activity of all the active analogs was inhibited by triiodothyronine as is that of TRH.[1,2] These tripeptide analogs have about 0.1-0.3% of the activity of TRH which represents an unusual structure-activity relationship, where the same biological activity is found in an analog at dose levels up to 10,000 times that of the reference compound. Such very great differences in activity can be studied in compounds which are active in a dosage range of nanograms and micrograms, but not when the active dosage range is in milligrams or greater.

To learn more about the structure-activity relationships of TRH, which could indicate that the molecular environment of the functional site of TRH involves some very low incidence of molecular variation, 10 tripeptides and 2 dipeptides containing the pGlu moiety have been synthesized and assayed for TRH activity.

These additional tripeptides have the general structure, pGlu-X-Y-NH_2 where X and Y are 6 different amino acids (Ala, Gly, His, Phe, Trp, Tyr) and, in each case, at least one of the amino acids is His or Tyr. The two dipeptides pGlu-Tyr-NH_2 (**27**) and pGlu-His-NH_2 (**28**) were also synthesized and assayed. These 12 peptides are listed in Table I, for convenience of appraisal.

†Hypothalamic Hormones. 27.

Table I. Peptides Synthesized and Assayed[a]

pGlu-His-Pro-NH_2	TRH	pGlu-Tyr-His-NH_2	**21**
pGlu-Phe-His-NH_2	**4**	pGlu-Tyr-Phe-NH_2	**22**
pGlu-Trp-His-NH_2	**8**	pGlu-Tyr-Trp-NH_2	**24**
pGlu-Trp-Tyr-NH_2	**9**	pGlu-Tyr-Tyr-NH_2	**26**
pGlu-Ala-His-NH_2	**13**	pGlu-Tyr-NH_2	**27**
pGlu-Gly-Tyr-NH_2	**15**	pGlu-His-NH_2	**28**
pGlu-Tyr-Gly-NH_2	**19**		

[a]The peptides were assayed *in vivo* for TRH activity at dose levels up to 50 μg and *in vivo* for LRH activity at dose levels up to 200 μg.

None of these tri- and dipeptides showed TRH activity in the T_3-TRH assay method of Bowers, *et al.*,[4-6] even at dose levels as high as 50 μg, which is about 5000 to 50,000 times greater than an effective dosage range of TRH.

It is now evident that TRH can be modified in one amino acid as in the analogs described[1,2] and still retain hormonal activity. With modifications in the structure of the hormone, as in the peptides described herein, where 2 amino acids have been changed or where the proline moiety has been removed as in the dipeptide, pGlu-His-NH_2 (**28**), the peptide analogs showed no hormonal activity.

Since Currie, *et al.*, found[7] that the bovine luteinizing releasing hormone (LRH) apparently has a pGlu moiety as does the ovine LRH[8] and now the porcine LRH,[9] and that after gel filtration on Bio-Gel P2 bovine LRH has the chromatographic behavior of a relatively small peptide,[7] we also assayed these pGlu peptides for LRH activity. Such assays could give information about the structural specificity of LRH even before the structure of LRH is known particularly when one considers the variety of pGlu peptides[1,2,10] which have TRH activity. The LRH assays were performed as described.[7,11,12]

Although LRH apparently has a pGlu moiety and the presence[7-9] of His and/or Tyr moiety(s) in the molecule has recently been confirmed[9] none of the pGlu peptides, at dose levels as high as 200 μg, showed LRH activity. This inactivity of these tri- and dipeptides is of greater interest for the structure-hormonal activity relationships now that a tetrapeptide, pGlu-Tyr-Arg-Trp-NH_2, has been discovered by Chang, *et al.*,[13] which does have the hormonal activity of LRH. Currently, LRH has been reported as "the nonapeptide isolated from porcine hypothalami" by Schally, *et al.*[9]

The synthesis of the tripeptides **4**, **8**, and **13** have been effected by the following general procedure. *N*-Carbobenzoxypyroglutamic acid (Z-pGlu)[14] was coupled with the appropriate amino acid benzyl ester hydrochloride by the mixed anhydride method (ethyl chloroformate) to give the protected dipeptide, Z-pGlu-X-OBzl (**1**, **5**, **10**) (X = Phe, Trp, or Ala). The two blocking groups were then removed in one step by hydrogenolysis using 5% Pd/C as catalyst to yield the dipeptides, pGlu-X-OH (**2**, **6**, **11**). These dipeptides were then coupled with His Me ester · 2HCl mediated by DCI to give the tripeptides **3**, **7**, **12**. These Me esters were then converted into the corresponding amides **4**, **8**, **13** by treatment with MeOH satd with NH_3.

pGlu-Trp-Tyr-NH_2 (**9**) was obtained by coupling **6** with tyrosine amide using the DCI method.

pGlu-Gly-Tyr-NH_2 (**15**) was prepared by coupling Z-pGlu with Gly-Tyr-NH_2‡ by the mixed anhydride method followed by hydrogenolysis to remove the Z-group.

The pGlu-Tyr-Y-NH_2 peptides, **19**, **21**, **22**, **24**, **26**, were prepared in the following manner. pGlu was coupled with Tyr(Bzl)-OCH_3 by the DCI method to give pGlu-Tyr(Bzl)-OCH_3 (**16**). This latter compound was then hydrolyzed by base to obtain the dipeptide pGlu-Tyr(Bzl)-OH (**17**). This dipeptide was then coupled with Gly-NH_2, His-OCH_3, Phe-NH_2, Trp-NH_2, and Tyr-NH_2 to give the O-Bzl protected tripeptides, **18**, **20**, **30**, **23**, **25**, and **20** was subsequently converted to **29**. The corresponding protected tripeptides were hydrogenated using 5% Pd/C as catalyst, to the unprotected tripeptides, **19**, **21**, **22**, **24**, **26**. The dipeptide pGlu-Tyr-NH_2 (**27**) was obtained by direct coupling of pGlu with Tyr-NH_2 by the DCI method. pGlu-His-NH_2 (**28**) was prepared by treating pGlu-His-OCH_3[15] with MeOH satd with NH_3.

‡Commercially available from Sigma Chemical Co., St. Louis, Mo.

Experimental Section

Melting points were detd on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by the Mikroanalytisches Laboratorium, Bonn, West Germany. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. On silica gel G, R_f^1, R_f^2, R_f^3, and R_f^4 refer to the systems of *n*-BuOH-glacial HOAc-EtOAc-H_2O (1:1:1:1); $CHCl_3$-MeOH-NH_4OH (60:45:20); EtOH-H_2O (7:3), and $CHCl_3$-MeOH-NH_4OH (60:30:5), respectively. The nmr spectra were measured on a Varian A-60 spectrometer with Me_4Si or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standards. Chemical shifts are expressed in τ values. Nmr spectra were recorded for **1**, **2**, **5**, **6**, **7**, **10**, **11**, **12**, **14**, **16**, **17**, **19**, and **20**, and all of the expected signals were obtained. The optical rotations were measured on a Perkin-Elmer Model 141 digital readout polarimeter using a microcell. All of the amino acids used as starting materials were purchased as the L isomers. Hydrolyses of the peptides **3**, **4**, **12**, **13**, **15**, **19**, **22**, and **26** were carried out in a sealed ampoule in 6 *N* HCl at 110° for 24 hr, and the theoretical amino acids were identified.

***N*-Cbz-pGlu-Phe Benzyl Ester (1).** *N*-Carbobenzoxypyroglutamic acid[14] (786 mg) and Et_3N (0.42 ml) were mixed in dry THF (50 ml). After cooling the reaction mixture to 0°, ethyl chloroformate (0.29 ml) was added and the reaction mixture was stirred at 0° for 0.5 hr. Phe benzyl ester · HCl (875 mg) and Et_3N (0.42 ml) in THF (10 ml) were then added and the stirring was continued for 3 hr at room temp. The solvent was evapd *in vacuo* and H_2O (50 ml) was added. A white ppt was obtained which was washed well with H_2O and recrystd from 70% EtOH giving pure **1** (1.29 g, yield 88%): mp 144-146°; $[\alpha]^{22}D$ -0.2 (*c* 1.0 $CHCl_3$); R_f^1 0.84; R_f^2 0.91; R_f^3 0.75; single spot with Cl reagent. *Anal.* ($C_{28}H_{26}N_2O_6 \cdot 2H_2O$) H, N; C, calcd 64.36; found 64.94.

pGlu-Phe (2). Compd **1** (486 ml) in MeOH (50 ml) was hydrogenated at atm pressure and at room temp for 1 hr using 5% Pd/C (0.1 g) as catalyst. After appropriate work-up, the unprotected dipeptide **2** was obtained (253 mg, yield 91%): recrystn from EtOAc-MeOH; mp 223-225° dec; $[\alpha]^{22}D$ +19.2 (*c* 1.0 MeOH); R_f^1 0.64; R_f^3 0.33; single spot with Cl reagent. *Anal.* ($C_{14}H_{16}N_2O_4$) H, N; C, calcd 60.09; found 59.33.

pGlu-Phe-His-OMe (3). pGlu-Phe (**2**) (225 mg), His Me ester · 2HCl (197 mg), Et_3N (0.23 ml), and DCI (168 mg) were mixed in DMF (30 ml) at 0° and the mixture was stirred at room temp for 20 hr. After evapn of the solvent *in vacuo* (40°), the residue was purified by repeated recrystn from MeOH to afford pure **3** (45 mg, yield 15%): mp 235-240°; $[\alpha]^{22}D$ -0.3 (*c* 0.3 DMF); R_f^1 0.62; R_f^2 0.95; R_f^3 0.61; single spot with Pauly and Cl reagents. *Anal.* ($C_{21}H_{25}N_5O_5$) C, H, N.

pGlu-Phe-His-NH_2 (4). Compd **3** (25 mg) was treated with MeOH satd with NH_3. After evapn of the solvent the residue was recrystd from MeOH giving pure **4** (16 mg, yield 73%): mp 220-223° dec; $[\alpha]^{22}D$ -10.9 (*c* 1.0 DMF); R_f^1 0.54; R_f^2 0.56; R_f^3 0.61; single spot with Pauly and Cl reagents. *Anal.* ($C_{20}H_{24}N_6O_4 \cdot 0.5H_2O$) C, H, N.

***N*-Cbz-pGlu-Trp Benzyl Ester (5)** was synthesized by the mixed anhydride method as described for **1**, from *N*-Cbz-pGlu (536 mg) and Trp benzyl ester · HCl, (661 mg). The product was purified by dissolving the reaction mixture in $CHCl_3$-H_2O (50:50 v/v). The org layer was extd with 5% $NaHCO_3$ (2 × 25 ml) and then with H_2O (2 × 25 ml), dried ($MgSO_4$), and evapd. After recrystn from EtOH, pure **5** was obtd (710 mg, yield 66%): mp 149-151°; $[\alpha]^{22}D$ +7.5 (*c* 1.4 $CHCl_3$); R_f^1 0.78; R_f^4 0.95; R_f^3 0.76; single spot with Ehrlich and Cl reagents. *Anal.* ($C_{31}H_{29}N_3O_6$) C, H, N.

pGlu-Trp (6). Compd **5** (211 mg) was hydrogenated as described for **2** and gave **6** (110 mg, yield 90%): R_f^1 0.63; R_f^3 0.69;

R_f^4 0.31; single spot with Ehrlich and Cl reagents; nmr (MeOH-d_4) 2.70 (ArH; 4 H), 6.75 (indole-CH_2, 2 H). This product readily turned colored within 2-3 hr upon storing and was used in the next step without further characterization.

pGlu-Trp-His-OMe (7) was obtd from pGlu-Trp (6) (153 mg) and His Me ester · 2HCl (117 mg) as described for 3. After purification by prep tlc, using $CHCl_3$-MeOH (4:1) as developing solvent, the tripeptide 7 was obtd (51 mg, yield 22%): R_f^1 0.63; R_f^2 0.94; R_f^3 0.61; single spot with Ehrlich, Pauly, and Cl reagents; nmr (MeOH-d_4) 2.40-3.16 (indole and imidazole protons, 7 H), 6.37 (OCH_3, 3 H), 7.80-8.08 (CH_2 protons).

pGlu-Trp-His-NH$_2$ (8). Compd 7 (30 mg) was treated with NH_3 as described for 4. Recrystn from MeOH-Et_2O gave pure 8 (21 mg, yield 73%): no sharp mp; $[\alpha]^{22}D$ -15.5 (*c* 1.0 MeOH); R_f^1 0.55; R_f^3 0.56; R_f^4 0.54; single spot to Ehrlich, Pauly, and Cl reagents. *Anal.* ($C_{22}H_{25}N_7O_4 \cdot 2H_2O$) C, H, N.

pGlu-Trp-Tyr-NH$_2$ (9) was obtd from 6 (315 mg) and Tyr-NH_2 (180 mg) as described for 3. After purification on tlc using MeOH-$CHCl_3$ (1:3) as developing solvent, the product was recrystd from MeOH (48 mg, yield 10%): mp 250-253° dec; $[\alpha]^{22}D$ -25.1 (*c* 0.73 DMF), R_f^1 0.77; R_f^2 0.86; R_f^3 0.68. *Anal.* ($C_{25}H_{27}N_5O_5 \cdot 0.5H_2O$) C, H, N.

***N*-Cbz-pGlu-Ala Benzyl Ester (10)** was synthesized by the mixed anhydride method as described for 1, from *N*-Cbz-pGlu (536 mg) and Ala benzyl ester · HCl (216 mg). After work-up as described for 5, and recrystn from MeOH, pure 10 was obtained (750 mg, yield 88%): mp 128-130°; $[\alpha]^{22}D$ -26.2 (*c* 1.0 $CHCl_3$). *Anal.* ($C_{23}H_{24}N_2O_6$) N.

pGlu-Ala (11). Compd 10 (510 mg) was hydrogenated, as described for 2, and gave 11 (193 mg, yield 80%): mp 203-205° (lit.[16] 205-206°); $[\alpha]^{22}D$ -31.2 (*c* 1.0 MeOH). *Anal.* ($C_8H_{12}N_2O_4$) C, N, H.

pGlu-Ala-His-OMe (12). From pGlu-Ala (160 mg) and His Me ester · 2HCl (134 mg), as described for 3, and after purification on prep tlc using EtOH-H_2O (7:3) as developing solvent, 12 was obtained: recrystn from MeOH (55 mg, yield 20%); mp 217-222° dec; $[\alpha]^{22}D$ -22.6 (*c* 1.0 HOAc); R_f^1 0.34; R_f^2 0.89; R_f^3 0.51; single spot with Pauly and Cl reagents. *Anal.* ($C_{15}H_{21}N_5O_5$) C, H, N.

pGlu-Ala-His-NH$_2$ · HCl (13). Compd 12 (35 mg) was treated with NH_3 as described for 4. The product was converted to the HCl salt (27 mg, yield 73%): no sharp mp; $[\alpha]^{22}D$ -11.0 (*c* 0.67 MeOH); R_f^1 0.22; R_f^2 0.71; R_f^3 0.40; single spot to Pauly and Cl reagents. *Anal.* ($C_{14}H_{20}N_6O_4 \cdot HCl \cdot H_2O$) C, H.

***N*-Cbz-pGlu-Gly-Tyr-NH$_2$ (14)** was synthesized by the mixed anhydride method as described for 1, from *N*-Cbz-pGlu (132 mg) and Gly-Tyr-NH_2‡ (149 mg). The product was purified as described for 5; after recrystn from MeOH-Et_2O (161 mg, yield 67%): mp 132-137° dec; $[\alpha]^{22}D$ -7.2 (*c* 0.7 MeOH); R_f^1 0.74; R_f^2 0.86; R_f^3 0.71; single spot to Pauly and Cl reagents. *Anal.* ($C_{24}H_{26}N_4O_7 \cdot H_2O$) C, N; H, calcd 5.64; found, 6.13.

pGlu-Gly-Tyr-NH$_2$ (15). Compd 14 (100 mg) was hydrogenated as described for 2. After recrystn from MeOH, 15 was obtained (62 mg, yield 84%): mp 227-230° dec; $[\alpha]^{22}D$ +7.9 (*c* 0.9 MeOH); R_f^1 0.62; R_f^2 0.68; R_f^3 0.64. *Anal.* ($C_{16}H_{20}N_4O_5 \cdot 0.25H_2O$) C, H, N.

pGlu-O-Bzl-Tyr-OMe (16). pGlu (2.64 g), O-Bzl-Tyr Me ester · HCl (6.58 g), Et_3N (2.8 ml), and DCI (4.21 g) were combined and the mixture was stirred in MeCN (100 ml) at room temp for 16 hr. After filtration and evaporation *in vacuo* the residue was diluted with H_2O (50 ml) and the soln was extd with EtOAc (3 × 50 ml), dried ($MgSO_4$), and evapd. After recrystn from EtOH-hexane, pure 16 was obtd (7.0 g, yield 86%): mp 124-126° dec; $[\alpha]^{22}D$ +8.1 (*c* 1.76 MeOH); R_f^1 0.87; R_f^3 0.71; single spot to Cl reagent. *Anal.* ($C_{22}H_{24}N_2O_5$) C, H, N.

pGlu-O-Bzl-Tyr (17). A soln of 16 (2.2 g) in 95% EtOH (50 ml) was treated with NaOH (1 g) in H_2O (1 ml). After being stirred at room temp for 1 hr, the reaction mixt was acidified with dil HCl. The solvent was evapd *in vacuo*, and the residue was pptd with H_2O (50 ml). After recrystn from $CHCl_3$-EtOAc, pure 17 was obtd (2.0 g, yield 94%): mp 182-185°; $[\alpha]^{22}D$ +13.1 (*c* 1.53 MeOH); R_f^1 0.82; R_f^2 0.67; single spot to Cl reagent. *Anal.* ($C_{21}H_{22}N_2O_5$) C, H, N.

pGlu-O-Bzl-Tyr-Gly-NH$_2$ (18) was obtd, as described for 3, from 17 (382 mg) and Gly-NH_2 · HCl (111.5 mg). The product was purified by tlc using $CHCl_3$-MeOH (4:1) as developing solvent, and recrystd from MeOH-Et_2O to give pure 18 (115 mg, yield 27%): mp 197-199° dec; $[\alpha]^{22}D$ -7.8 (*c* 0.80 glacial HOAc); R_f^1 0.83; R_f^2 0.69; single spot to Cl reagent. *Anal.* ($C_{23}H_{26}N_4O_5$) C, H, N.

pGlu-Tyr-Gly-NH$_2$ (19). Compd 18 (50 mg) in 95% EtOH (25 ml) was hydrogenated as described for 2. Recrystn from MeOH-Et_2O afforded pure 19 (33 mg, yield 83%): no sharp mp; $[\alpha]^{22}D$ +5.5 (*c* 1.0 MeOH); R_f^1 0.72; R_f^2 0.68; R_f^3 0.68; single spot to Pauly and Cl reagents.

pGlu-O-Bzl-Tyr-His Me Ester (20) was obtd, as described for 3, from 17 (382 mg) and His-OMe · 2HCl (242 mg). After repeated recrystn from MeOH, pure 19 was obtd (328 mg, yield 61%): mp 250° dec; R_f^1 0.57; R_f^2 0.82; R_f^3 0.54; single spot to Pauly and Cl reagents. *Anal.* ($C_{28}H_{31}N_5O_6$) C, H, N.

pGlu-Tyr-His-NH$_2$ · HCl (21). Compd 20 (105 mg) was converted into its corresponding amide 29, as described for 4. The pGlu-O-Bzl-Tyr-His-NH_2 thus obtd (74 mg) was then reduced as described for 2, and converted into its HCl salt. Recrystn from MeOH-Et_2O afforded pure 21 (60 mg, yield 70%): no sharp mp; $[\alpha]^{22}D$ +2.7 (*c* 1.21 DMF); R_f^1 0.42; R_f^2 0.49; R_f^3 0.43; single spot to Pauly and Cl reagents. *Anal.* ($C_{20}H_{24}N_6O_5 \cdot HCl \cdot H_2O$) C, H, N.

pGlu-Tyr-Phe-NH$_2$ (22) was prepared as described for 3 from 17 (300 mg) and Phe-NH_2 · HCl (129 mg). Recrystn from MeOH-$CHCl_3$ afforded pure pGlu-O-Bzl-Tyr-Phe-NH_2 (30, 70 mg) which was reduced as described for 2. Recrystn from EtOH gave pure 22 (48 mg, yield 14%): mp 265° dec; $[\alpha]^{22}D$ -15.1 (*c* 1.11 DMF); R_f^1 0.68; R_f^2 0.69; R_f^3 0.61; single spot with Pauly and Cl reagents. *Anal.* ($C_{23}H_{26}N_4O_5$) H, N. C: calcd, 63.00; found, 63.93.

pGlu-O-Bzl-Tyr-Trp-NH$_2$ (23) was obtd, as described for 3, from 17 (225 mg) and Trp-NH_2 · HCl (141 mg). The product was purified by tlc using $CHCl_3$-MeOH (3:1 v/v) as developing solvent. Recrystn from MeOH yielded pure 23 (50 mg, yield 15%): mp 240-242°; $[\alpha]^{22}D$ -8.4 (*c* 0.78 DMF); R_f^1 0.75; R_f^2 0.78; R_f^3 0.63; single spot to Ehrlich and Cl reagents. *Anal.* ($C_{32}H_{33}N_5O_5 \cdot 0.5H_2O$) C, H, N.

pGlu-Tyr-Trp-NH$_2$ (24). Compd 23 (22 mg) was reduced as described for 2. Recrystn from MeOH-Et_2O afforded pure 24 (15 mg, yield 81%): no sharp mp; R_f^1 0.56; R_f^2 0.77; R_f^3 0.71; single spot to Ehrlich, Pauly, and Cl reagents.

pGlu-O-Bzl-Tyr-Tyr-NH$_2$ (25). As described for 3, this peptide was obtd from 17 (300 mg) and Tyr-NH_2 (142 mg). The product was purified by recrystn from MeOH to afford pure 25 (57 mg, yield 13%): mp 262-266°; $[\alpha]^{22}D$ -13.9 (*c* 1.11 DMF); R_f^1 0.73; R_f^2 0.74; R_f^3 0.64; single spot to Pauly and Cl reagents. *Anal.* ($C_{30}H_{32}N_4O_8$) N.

pGlu-Tyr-Tyr-NH$_2$ (26). Compd 25 (52 mg) was reduced as described for 2. Recrystn from MeOH afforded pure 26 (36 mg, yield 83%): mp 275° dec; $[\alpha]^{22}D$ -16.7 (*c* 1.00 DMF); R_f^1 0.66; R_f^2 0.55; R_f^3 0.62; single spot to Pauly and Cl reagents. *Anal.* ($C_{23}H_{26}N_4O_6$) C, H, N.

pGlu-Tyr-NH$_2$ (27). pGlu (135 mg) and Tyr-NH_2 (180 mg) were treated with DCI (206 mg) as described for 16. The solvent was evapd *in vacuo*, and the residue was purified by repeated recrystn from MeOH giving 27 (121 mg, yield 41%): mp 213-215°; $[\alpha]^{22}D$ +3.1 (*c* 1.0 DMF); R_f^1 0.68; R_f^2 0.76; R_f^3 0.65; single spot to Pauly and Cl reagents. *Anal.* ($C_{14}H_{17}N_3O_4 \cdot 0.5H_2O$) C, H, N.

pGlu-His-NH$_2$ (28) was prepd from pGlu-His-OCH_3[15] (100 mg) by treatment with NH_3 as described for 4. Recrystn from MeOH afforded pure 28 (74 mg, yield 78%): mp 225-227° dec; $[\alpha]^{22}D$ -21.4 (*c* 1.0 HOAc); R_f^1 0.30; R_f^2 0.62; R_f^3 0.39; single spot to Pauly and Cl reagents. *Anal.* ($C_{11}H_{15}N_5O_3$) C, H; N, calcd 26.40; found, 25.53.

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Solid Phase Synthesis of [3,4-Dileucine]-oxytocin and a Study of Some of Its Pharmacological Properties†

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[3,4-Dileucine]-oxytocin has been synthesized by the solid phase method and its pharmacological properties compared with those of [4-leucine]-oxytocin, an analog of the hormone which possesses a marked inhibitory effect on the antidiuretic activity of arginine-vasopressin (anti-ADH). [3,4-Dileucine]-oxytocin was found to possess very weak oxytocic and avian vasodepressor activities and negligible pressor activity. The analog had no antidiuretic activity and no demonstrable anti-ADH activity. However, it had marked natriuretic activity.

[3,4-Dileucine]-oxytocin has been synthesized by the method described in the Experimental Section, and certain pharmacological properties have been studied to explore further the relationship of structure to the marked inhibitory effect exerted by [4-leucine]-oxytocin[1-3] on the antidiuretic activity of arginine-vasopressin, the antidiuretic hormone of the posterior pituitary gland. We have referred to this inhibitory action as anti-ADH. [3,4-Dileucine]-oxytocin (Cys-Tyr-Leu-Leu-Asn-Cys-Pro-Leu-Gly-NH_2) is an analog of the hormone oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2) in which leucine residues replace the Ile and Gln residues of oxytocin in the 3 and 4 positions, respectively.

The effects of [3,4-dileucine]-oxytocin on renal excretion of H_2O and electrolytes were studied in rats by the techniques used by Chan and coworkers.[2,3] Like [4-leucine]-oxytocin, the 3,4-dileucine analog had no antidiuretic activity and had a marked natriuretic activity both in water diuresis and during vasopressin-suppressed water diuresis. Unlike [4-leucine]-oxytocin, the 3,4-dileucine analog had only weak diuretic and no demonstrable anti-ADH activity.

Among the natriuretic oxytocin analogs we have so far studied, [3,4-dileucine]-oxytocin exhibited the highest natriuretic activity. When a single dose (0.7 μg/100 g body weight) was injected iv into a rat under water diuresis, an increase in urinary excretion of Na^+ was noted within 2 min. The peak response was noted between 3 and 6 min *post*injection. For [3,4-dileucine]-oxytocin the average increase in Na^+ excretion at the peak was 300%. For [4-leucine]-oxytocin the increase was 215%,[3] for [2,4-diisoleucine]-oxytocin[4] 75%,[3] and for [2,4-dileucine]-oxytocin[5] 60%.§ It is also of interest to note that Rudinger and coworkers have reported that another 4-leucine analog, [4-leucine, 8-isoleucine]-oxytocin, possesses natriuretic and diuretic properties rather than antidiuretic activity.[6]

[3,4-Dileucine]-oxytocin was also assayed for oxytocic,# avian vasodepressor,** and pressor†† activities against the USP Posterior Pituitary Reference Standard. It was found to possess weak oxytocic (~1.0 unit/mg) and avian vasodepressor (~2 units/mg) potencies and negligible pressor potency (<0.005 unit/mg). No inhibition of the oxytocic or pressor activities of oxytocin was detected. In the case of [3,4-dileucine]-oxytocin, it had been found to have a negligible pressor and no oxytocic activity but to possess a weak antioxytocic activity.

It is interesting that additional leucine substitution of [4-leucine]-oxytocin at the 3 or 2 position produces selective changes in the renal activity of the polypeptide. [4-Leucine]-oxytocin is strongly natriuretic and possesses potent anti-ADH activity. These two activities are responsible for the marked diuretic effect of this analog. [3,4-Dileucine]-oxytocin is more potent than [4-leucine]-oxytocin in its natriuretic activity but it has no anti-ADH effect. The 2,4-dileucine analog has only a weak natriuretic activity and shows no anti-ADH activity. These exptl findings suggest a receptor specificity and that it may be possible to synthesize neurohypophysial analogs with highly selective activity.

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§W. Y. Chan, unpublished data.

#Oxytocic assays were performed according to the method of Holton[7] as modified by Munsick[8] on isolated uteri from rats in natural estrus with the use of Mg-free van Dyke–Hastings solution.

**Avian vasodepressor responses were measured by the method of Coon[9] as modified by Munsick, *et al.*,[10] using conscious prepns.

††Pressor assays were carried out on anesthetized male rats as described in "The Pharmacopeia of the United States of America."[11]