tected decapeptide 29 (40 mg) in MeOH (10 ml) and glacial HOAc (10 ml) contg 5% Pd–$BaSO_4$ catalyst was hydrogenated at 1 atm. After being stirred at room temp during 24 hr, the reaction mixt was filtered. The filtrate was evapd *in vacuo* to afford the crude peptide. This material was dissolved in H_2O (10 ml), and the soln was added to a CMC column (1 × 15 cm) which was successively eluted with 20 ml each of the following pH 6.9 NH_4OAc buffers: 0.005, 0.01, 0.025, 0.05, 0.075, and 0.1 *M*. Individual fractions (3 ml each) were collected at a flow rate of approximately 3 ml/min. The desired peptide was located in the 0.075-*M* NH_4OAc eluates by the use of Pauly reagent. These fractions were pooled and evapd to a small vol *in vacuo* and the residue was lyophilized to constant wt from small vols of H_2O: 21 mg; yield 67%; $[\alpha]^{22}D$ −27.6° (*c* 0.88, MeOH); R_f^1, 0.65; R_f^3, 0.31; and R_f^5, 0.49; single spot with ninhydrin, Pauly, Ehrlich, and Cl-tolidine reagents. Amino acid ratios in acid hydrolysate: Ser 0.6, Glu 1.0, Pro 1.0, Gly 2.0, Leu 1.0, Tyr 1.0, His 1.0, Lys 1.0, and Trp 0.7. *Anal.* ($C_{55}H_{75}N_{15}O_{13}\cdot 3CH_3COOH$) C, H, N.

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Solid-Phase Synthesis of [6-Arginine]-, [4-Histidine, 6-tyrosine]-, and [8-Isoleucine]angiotensins II†

Mahesh C. Khosla, Satyendra Kumar, Robert R. Smeby, and F. Merlin Bumpus*

Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106. *Received November 18, 1971*

To further evaluate the contribution of positions 6 and 8 for agonistic or antagonistic activity, some analogs of angiotensin II have been synthesized by solid-phase procedure with side-chain variations in both these positions. [Ile^8]angiotensin II has been found to be a very potent and specific competitive antagonist of angiotensin II both *in vitro* on isolated rabbit aortic strips and rat uterus and in the *in vivo* pressor assays in rats and cats, while [Arg^6]- and [His^4, Tyr^6]angiotensins were found to be inactive as pressor or antagonistic agents.

[Ile^5, Ala^8]angiotensin II was synthesized earlier in this laboratory[2] and was found to antagonize the action of angiotensin II on strips of guinea pig ileum.[3] Further testing[4] of this analog on isolated rabbit aortic strips and rat colon revealed that this analog had a specific competitive antagonistic effect against angiotensin II at very low concentrations without having a myotropic effect on these smooth muscle preparations. However, when tested on pentobarbital-anesthetized cats, this compound did not antagonize the pressor and intestinal inhibitory effect of angiotensin II. In view of this, it was thought of interest to synthesize analogs of angiotensin in which position 8 was replaced with other aliphatic amino acids, and as a first step, we have synthesized [Ile^8]angiotensin II.

Position 6, occupied by histidine, appears essential for biological activity of angiotensin[5] and has been studied from different points of view.[6-8] Recently Marshall, *et al.*,[9] showed that [Phe^4,Tyr^8]angiotensin II inhibited the response of the isolated rat uterus to angiotensin II when the pH of the medium was 7.4 or less. It is interesting to note that at this pH the imidazole ring of histidine would be significantly protonated.[10] It therefore appears likely that it is in the protonated form that histidine residue may be interacting with the receptor. In order to study the role of positive charge or an aromatic ring in this residue, we synthesized [Arg^6]-, and [His^4, Tyr^6]angiotensins II.

All the analogs reported in this paper were synthesized by the solid-phase procedure,[11] as previously described by Khosla, *et al.*[12,13]

Biological Results.‡ The pressor activity was determined by pressor assay in vagotomized, ganglion-blocked rat,[15] and the myotropic activity was studied on isolated rabbit aortic strips. Inhibition of angiotensin II myotropic response was studied using isolated rabbit aortic strips *in vitro* and by *in vivo* pressor assay in anesthetized cats and rats. The results obtained indicate that [Ile^8]angiotensin II has a specific competitive antagonistic effect against angiotensin II both *in vitro* on isolated rabbit aorta and rat uterus, and *in vivo* on the rat and cat blood pressure. It has a pA_2 value of 9.21 as compared to 8.3 obtained for [Ala^8]angiotensin II, and a $\log K_2$ value of 8.86 at molar concentrations of $10^{-9.34}$ (Schild's parameters). It antagonizes the pressor effect of angiotensin II at a dose level of 5 ng/g body weight in rat and cat. As compared to other analogs such as [Phe^4, Tyr^8]angiotensin II,[9] it appears to be the most potent competitive inhibitor of angiotensin II so far reported. On the other hand, [Arg^6]-, and [His^4, Tyr^6]angiotensins II were

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‡The antagonistic activities were determined by Yamamoto, *et al.*,[14] and the pressor assays by Dr. S. Sen, Cleveland Clinic, Research Division.

found to be inactive as pressor or myotropic agents and they do not inhibit responses to angiotensin II.

Discussion

Angiotensin II is now well known for its multiplicity of physiological actions but questions such as its "biologically active" conformation, the mechanism of its action, and the identification of its various receptors still remain unanswered. A competitive inhibitor of angiotensin II is an important tool to study some of these questions as well as an important probe to study the etiology of renovascular hypertension, and may provide specific therapy. The results obtained with [Ile^8]angiotensin II represent a major step forward in this direction. It is interesting to note that the replacement of an aromatic residue in position 8 by an aliphatic chain in angiotensin II molecule changes the agonistic activity into antagonistic activity. Since [Ile^8]angiotensin II is a potent antagonist of angiotensin II, one can speculate that it must be strongly bound to the angiotensin II receptor site. It is possible that the branched aliphatic side chain in position 8 may facilitate binding of the molecule to the receptor site because of a more favorable hydrophobic area in the peptide. However, if this side group is involved only in the binding step of the myotropic response due to peptide, it is difficult to explain why there is reduction in agonist properties. This leads to speculation that the aromatic ring may be important for the excitation step of the myotropic response as well as being involved in binding with the receptor site. It has recently been proposed that [Ala^8]angiotensin II has a conformation different from that of parent hormone.§ It is possible that introduction of isoleucine in position 8 may affect the molecule in a similar fashion. This aspect of possible change of conformation on biological activity is being studied.

The biological activities of [Arg^6]-, and [His^4, Tyr^6]angiotensins II are indicative of the fact that neither positive charge alone nor merely aromatic character in position 6 is responsible for the pressor activity of angiotensin II. Transposition of side chains in positions 4 and 6 must place them in a position relative to one another which is not compatible with the receptor since the peptide is neither an agonist nor antagonist. In the parent peptide, therefore, carboxyl of phenylalanine may be associated with the imidazole ring of histidine either by ionic bonding[8] or through chelation with Ca^{2+}, and this interaction is destroyed by transposition.

Experimental Section

Solvents used for ascending paper chromatography (pc) on Whatman No. 1 filter paper and tlc were: (a) *n*-BuOH-AcOH-H_2O (BAW) (4:1:5); (b) *n*-BuOH-AcOH-H_2O-Pyr (BAWP) (30:6:24:20); (c) *n*-PrOH-H_2O (PW) (2:1). E. Merck silica gel chromatogram plates were used for tlc. Ionophoresis was carried out on filter paper strips on S and S 2043A filter paper strips in Beckmann electrophoresis cell (Durrum type) Model R, series D at 450 V, using HCOOH-AcOH buffer prepared by diluting 60 ml of HCO_2H and 240 ml of AcOH to 2 l. with distd H_2O (pH 1.9) and Beckmann barbiturate buffer B-2 (pH 8.6). Glutamic acid was used as a reference compd, and E(Glu) indicates the electrophoretic mobility relative to glutamic acid = 1.00. Detection of the compd on chromatograms was carried out with ninhydrin and/or with diazotized sulfanilic acid. The free peptides were hydrolyzed in 6 *N* HCl at 110° for 36 hr. Amino acid analyses were performed on Jeolco-5AH amino acid analyzer. Melting points were taken on a Leitz Wetzlar hot-stage apparatus and are uncorrected. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values. Angiotensin analogs reported in this paper contain L-isoleucine in position 5.

§P. Fromageot, private communication.

General Procedure for Solid-Phase Peptide Synthesis. For the esterification of C-terminal Boc-amino acid on 2% cross-linked chloromethyl polymer, a soln of Boc-amino acid (10 mmoles) and Et_3N (1.4 ml, 10 mmoles) in EtOH (50 ml) was added to the polymer (10 g) and the mixt stirred at 80° for 24 hr. The esterified polymer was washed with EtOH, H_2O, dil AcOH, H_2O, and MeOH, and dried *in vacuo*. The amount of Boc-amino acid esterified onto the polymer was detd[12] by wt increase, by spectrophotometric estimation of the unreacted aromatic amino acid in the filtrates or by acid hydrolysis of the esterified polymer. The following cycle of reactions was used to introduce each new residue. Unless specified otherwise, all washings were carried out three times for 3 min, and the steps were carried out in the following order: (1) washed with glacial AcOH; (2) Boc group removed with 1 *N* HCl in AcOH for 30 min; (3) washed with AcOH; (4) washed with EtOH; (5) washed with DMF; (6) neutralized the HCl salt with 10% Et_3N in DMF for 10 min; (7) washed with DMF; (8) washed with CH_2Cl_2; (9) introduced 4 equiv of the approp Boc-amino acid in CH_2Cl_2 and the mixt allowed to mix for 10 min; (10) introduced 4 equiv of DCI in CH_2Cl_2 (20 ml) and the mixt shaken for 2 hr with cooling (0-5°) and then shaken overnight at room temp; (11) washed with CH_2Cl_2; (12) washed with EtOH. For Boc-His(Bzl) and Boc-Arg(NO_2) cycles, step 8 was deleted and DMF was substituted for CH_2Cl_2 in steps 9-11. At the end, the protected peptide polymer was washed with AcOH, Boc group removed with 1 *N* HCl in AcOH and the polymer washed with AcOH, CH_2Cl_2, and 1:1 CH_2Cl_2-CF_3COOH. The polymer was now suspended in freshly distd CF_3COOH and a slow stream of HBr (prewashed with 10% resorcinol in AcOH) was passed through the suspension for 35-40 min. The mixt was filtered and the polymer washed with CF_3COOH. The combined filtrates were evapd using a rotary evaporator *in vacuo* and the residue was triturated with Et_2O and filtered, and the ppt washed with Et_2O. The amorphous powder obtained was dissolved in a mixt of MeOH-AcOH-H_2O (10:1:1), treated with charcoal (Darco G-60) at room temp, and filtered, and the mixt hydrogenated at 3.5 kg/cm^2 over 0.5 the peptide wt of Pd black for 48 hr with shaking. The product was purified on a column of Sephadex G-25 (5 × 80 cm) using *n*-BuOH-AcOH-H_2O (4:1:5) (upper phase) as the developing solvent.[16] Yields were in the range of 40-60%.

[Ile^8]angiotensin II had mp 238-240°; $[\alpha]^{27}D$ −96° (*c* 0.5, 1 *N* AcOH); pc R_f 0.43 (BAW), R_f 0.51 (BAWP); tlc R_f 0.15 (BAW), R_f 0.27 (BAWP), R_f 0.24 (PW); E(Glu) 1.13 (pH 1.9), E(Glu) 0.810 (pH 8.4). Amino acid ratios found were: Asp, 1.00; Arg, 1.09; Val, 1.01; Tyr, 0.89; His, 1.04; Ile, 1.91. *Anal.* ($C_{47}H_{73}N_{13}O_{12} \cdot 2H_2O$), N. The compound was very hygroscopic. Loss of H_2O at 100° was 15.16%, but it varied with each sample or with different drying conditions.

[Arg^6]angiotensin II had mp 256-258°; $[\alpha]^{27}D$ −62° (*c* 0.5, 1 *N* AcOH); pc R_f 0.43 (BAW), R_f 0.50 (BAWP); tlc R_f 0.27 (BAW), R_f 0.57 (BAWP); E(Glu) 1.10 (pH 1.9). Amino acid ratios found were: Asp, 0.98; Arg, 2.12; Val, 1.06; Tyr, 0.91; Ile, 1.00; Pro, 1.14; Phe, 1.10. *Anal.* ($C_{50}H_{76}N_{15}O_{11} \cdot CH_3COOH \cdot 4H_2O$) C, H, N.

[His^4,Tyr^6]angiotensin II had mp 235-237°; $[\alpha]^{27}D$ −80° (*c* 0.5, 1 *N* AcOH); pc R_f 0.42 (BAW), R_f 0.58 (BAWP); tlc R_f 0.24 (BAW), R_f 0.51 (BAWP); E(Glu) 1.08 (pH 1.9). Amino acid ratios found were: Asp, 1.00; Arg, 1.15; Val, 1.04; Tyr, 0.92; Ile, 1.10; His, 0.95; Pro, 1.01; Phe, 1.00. *Anal.* ($C_{50}H_{77}N_{13}O_{15} \cdot 3H_2O$) C, H, N.

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Synthesis and Properties of 1-Deamino-8-L-homolysine-vasopressin†

Gunnar Lindeberg, Jaroslav Kynčl, Patricia Dreyfuss, and Miklos Bodanszky*

Biokemiska Institutionen, University of Uppsala, Uppsala, Sweden, The Cleveland Clinic, Cleveland, Ohio 44106, and Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106. *Received December 20, 1971*

An analog of lysine-vasopressin with β-mercaptopropionic acid replacing the cysteine residue in position 1 and with L-homolysine replacing lysine was synthesized. In the rat, the new analog exhibits significantly higher antidiuretic and pressor activities than the parent hormone.

The recent synthesis of 8-L-homolysine-vasopressin[1] (I) and the biological activity exhibited by this hormone analog together with the enhanced and prolonged effects found in the 1-deamino analogs of the pituitary hormones (*e.g.*, ref 2, 3) prompted the synthesis of 1-deamino-8-L-homolysine-vasopressin (II). In this analog the 1-cysteine residue of the parent hormone is replaced by β-mercaptopropionic acid and the 8-lysine moiety by L-homolysine (Figure 1).

Synthesis of the new hormone analog followed the pattern established for deamino-oxytocin[2] and is described in the Experimental Section. Compared with lysine-vasopressin, II proved to be exceptionally active both in the pressor and in the antidiuretic assay. The pharmacological potencies of II are compared with some selected analogs of vasopressin in Table I. These analogs were chosen to illustrate (1) the effect of the length of the side chain of the basic amino acid residue in position 8, (2) the role of the amino group of the cysteine residue in position 1, and (3) the comparison with other very active compounds reported to date.

Table I demonstrates that the pressor potency of II, about 1000 IU/μmole, is significantly higher than that of the other analogs listed. Since, previously, omission of the amino group in position 1 either hardly changed or else slightly suppressed the pressor activity, this high value was unexpected.

The antidiuretic potency of II, about 10,000 IU/μmole, is significantly higher than that reported for any other analog, with the possible exception of 1-deamino-8-D-arginine-vasopressin. In the latter case, however, the activity is extremely dose dependent and cannot be well represented by a single number.[3] In the dose region investigated, the dose-response curve of II is similar to that of lysine-vasopressin.

The reason for the enhanced biological activities of II are not known at the present time. The synthesis of 8-L-homolysine-vasopressin[1] was undertaken in order to explore the role of the length of the side chain of the basic amino acid in position 8. If the length of this side chain plays a significant role in the interaction between the hormone and the antidiuretic receptor site, I should have shown distinctly higher activity than lysine-vasopressin. As shown in Table I, it did not. Nevertheless, it is still possible that increased chain length intrinsically would lead to higher activity, but the required molecular fit with the receptor can only be obtained when the point of attachment through the amino group is absent. The potencies of II are significantly higher than those of 1-deamino-8-lysine-vasopressin. Alternately, it may be that the absence of the amino group extends the lifetime of the compound *in vivo* by making it less susceptible to aminopeptidases (*cf.* ref 4). This would lead to higher response with time. We hope that our further studies can clarify these questions.

Experimental Section‡

Synthesis. β-Benzylmercaptopropionyl-L-Tyr-L-Phe-L-Gln-L-Asn-(Bzl)-L-Cys-N^{ε}-tosyl-L-homolysyl-Gly-NH_2. Z-(Bzl)-L-Tyr-L-Phe-L-Gln-L-Asn-(Bzl)-L-Cys-L-Pro-N^{ε}-tosyl-L-homolysyl-Gly-NH_2[1] (346 mg) was dissolved in AcOH (3.5 ml) with gentle heating. After cooling to room temp, 5 *M* HBr in AcOH (2.5 ml) was added. One hr later, ether (75 ml) was added and the ppt was collected and washed with Et_2O (100 ml). It was dried *in vacuo* over NaOH for 2.5 hr and then dissolved in DMF (2 ml). The soln was filtered and the filter rinsed with DMF (2 × 1 ml). After cooling to 0°, diisopropylethylamine was added dropwise until the vapors gave a slightly alk reaction with a moistened indicator paper. *p*-Nitrophenyl *S*-benzyl-β-mercaptopropionate[5] (93 mg) was added and the soln was stirred at room temp for 1 day when a second portion (47 mg) of the active ester was added. The reaction mixt was kept slightly basic by occasional addns of diisopropylethylamine. After 3 more days, 95% EtOH (45 ml) was added, and the mixt was kept in an ice bath for 5 hr. The pptd solid was filtered and washed with EtOH (2 × 10 ml) and EtOAc (2 × 10 ml). The dried product weighed 239 mg; mp 210–213°, $[\alpha]^{25}D$ −39.5° (*c* 0.52, DMFA). Tlc on silica gel gave single spots in the solvent systems: *n*-PrOH-H_2O (7:3), R_f 0.62, and *n*-BuOH–AcOH–H_2O (3:1:1), R_f 0.60. *Anal.* ($C_{68}H_{86}N_{12}O_{15}S_3$) C, H, N, S.

1-Deamino-8-L-homolysine-vasopressin (II). A portion of the protected nonapeptide (100 mg) was dissolved in boiling liquid NH_3

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*Author to whom correspondence should be addressed at Case Western Reserve University.

‡Capillary melting points were taken and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values. For tlc, precoated plates (E. Merck AG, Darmstadt) were used and peptides were detected by use of the uv, ninhydrin, and chlorination techniques.