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1,8-Disubstituted Analogues of [Ile^5] and [Val^5]Angiotensin II: Difference in Potency and Specificity of Angiotensin II-Antagonistic Activity

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Summary: Depending on the species, position 5 in angiotensin II is occupied by isoleucine or valine. 1,8-disubstituted analogues of [Ile^5]angiotensin II show distinct differences from the corresponding [Val^5]angiotensin II analogues in the potency and specificity of their inhibitory action. The syntheses of new analogues are described.

1,8-Disubstituierte Analoga von [Ile^5]- und [Val^5]Angiotensin II: Unterschiede in Stärke und Spezifität der Angiotensin-II-antagonistischen Wirkung

Zusammenfassung: Position 5 in Angiotensin II ist speziesabhängig von Isoleucin oder Valin besetzt. 1,8-Disubstituierte Analoga von [Ile^5]-Angiotensin II lassen deutliche Unterschiede zu entsprechend substituierten [Val^5]Angiotensin-II-Analoga hinsichtlich Stärke und Spezifität der Angiotensin-II-antagonistischen Wirkung erkennen. Die Synthese neuer Analoga wird beschrieben.

Key words: Renin-angiotensin system, angiotensin II analogues.

From structure-activity relationships of angiotensin II analogues, it is well known that variations in positions 1, 5 and 8 of the parent hormone are of great importance: whereas variations in position 1 may change the duration of action with respect to the influence of aminopeptidases and binding affinity for the receptors[1], in the 5 position a branching or an alicyclic chain is required for high biological activity, indicating the importance of a steric effect of such side chains[2,3]. In position 8, L-phenylalanine is important for stimulation and affinity. The amino acid residue in this position should have the L-configuration, an aromatic ring in the side chain and a free carboxyl group[4,5].

Based on these structure-activity relationships, a number of angiotensin II antagonists have been synthesized as analogues monosubstituted in position 8, or with substitutions in positions 1 and 8.

Abbreviations follow the rules of the IUPAC-IUB Commission on Biochemical Nomenclature (see ref.[7]). Further abbreviations:
Phg, L-*C*-phenylglycine (see footnote to Table 1, p. 826); Sar, sarcosine; NSuc, Succinamoyl (H_2N-CO-CH_2-CH_2-CO-) Z, benzyloxycarbonyl; Boc, tert. butyloxycarbonyl; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; $PhCl_3$, 2,4,5-trichlorophenyl.

These 1,8-disubstituted angiotensin II analogues appear to be most potent antagonists.

Substitution of the *N*-terminal amino acid with sarcosine[6] seems to increase the stability towards hydrolysis by aminopeptidases. Substitution with a succinamoyl group[7,8] in the 1 position has a similar effect. Variations in position 8 showed that replacement of the aromatic ring of L-phenylalanine by an aliphatic chain as in alanine[6], leucine[9] and isoleucine[10], or shortening of the side chain by elimination of one CH_2-group as in L-*C*-phenylglycine (Phg)[7,8], caused a loss of the typical angiotensin II effects and produced potent competitive 1,8-disubstituted angiotensin II antagonists.

However, in accordance with the often confirmed identical biological actions of the natural variants of angiotensin II[11-13] – [Ile^5]angiotensin II in man[14,15], horse[16], pig[17,18], mouse and rat[19,20], [Val^5]angiotensin II in bovine species[21] – it was assumed that the potency and specificity of angiotensin antagonists should not be influenced by Ile or Val in position 5[4].

To check this assumption, pairs of analogues of [Ile^5]angiotensin II and [Val^5]angiotensin II with substitution in position 1 (sarcosine or succinamoyl) and 8 (alanine or *C*-phenylglycine) were synthesized (Table 1) and compared with each other in different specific "in vitro" and "in vivo" systems with special consideration of the renin-angiotensin system.

Table 1. Analogues of angiotensin II:
H-Asp-Arg-Val-Tyr-$\overset{\text{Val}}{\underset{\text{Ile}}{}}$-His-Pro-Phe-OH

Compound No.	Substitution in position		
	1	5	8
I[7]	Sar	Ile	Ala
II[6]	Sar	Val	Ala
III[7]	Sar	Ile	Phg*
IV	Sar	Val	Phg
V[7]	NSuc	Ile	Phg
VI	NSuc	Val	Phg

* We follow the proposal of Wünsch et al.[22] to use the abbreviation Phg for L-*C*-phenylglycine in order to avoid any confusion in the future. In ref.[7] Pgl was used for the same amino acid.

Materials and Methods

Amino acid derivatives were prepared in our laboratory. They met the required purity criteria (thin-layer chromatography, m.p., optical rotation). Solvents were purchased from E. Merck, Darmstadt, and Riedel de Haen, Seelze. The catalyst for hydrogenation was 10% Pd on $BaSO_4$ (Engelhard, Hannover). Reagents p.a. from E. Merck, Darmstadt, were employed for biological experiments. Thin-layer chromatography was performed on SiO_2 plates (Merck).

CHEMICAL METHODS

The melting points are uncorrected. All compounds were examined for purity by thin-layer chromatography (DC-Fertigplatten Merck) in different solvent systems.

For amino acid analysis, peptides were hydrolysed with 6N HCl in sealed tubes for 24 h at 110 °C. The given values are not corrected.

Boc-Sar-PhCl$_3$

21.6 g (0.105 mol) dicyclohexylcarbodiimide was added to a solution of 18.9 g (0.1 mol) Boc-Sar[23] and 19.7 g (0.1 mol) 2,4,5-trichlorophenol in 200 ml of anhydrous tetrahydrofuran with cooling. The reaction mixture was stirred 2 h at 0 °C and 2 h at room temperature. The dicyclohexylurea which formed was filtered and washed with tetrahydrofuran. The filtrate was concentrated in vacuo to yield a yellow oil, which solidified at + 4 °C. The crystallized product was triturated with cold diethylether filtered by suction and washed with a small portion of a 1:1 mixture of diethyl ether and low-boiling light petroleum.

Yield: 29.4 g (80%), m.p. 74 - 76 °C.

$C_{14}H_{16}Cl_3NO_4$ (368.8)

Calcd.	C 45.60	H 4.37	N 3.80
Found	C 46.1	H 4.6	N 3.9

Z-Val-Tyr(Bu^t)OCH$_3$

46 g (0.16 mol) H-Tyr(Bu^t)-OCH$_3$·HCl was dissolved in 180 ml dimethylformamide, the solution was cooled to 0 °C, and 20.2 ml (0.16 mol) *N*-ethylmorpholine was added. A solution of 40 g (0.16 mol) Z-Val and 21.6 g (0.16 mol) 1-hydroxybenzotriazole in 150 ml dimethylformamide was then added. A solution of 36.4 g (0.176 mol) dicyclohexylcarbodiimide in 35 ml dimethylformamide was admixed under stirring at – 5 °C. Stirring was continued for 3 h at 0 °C and then for 3 h at room temperature. After storage overnight at + 4 °C, the dicyclohexylurea which had precipitated was separated by filtration. The filtrate was concentrated at room temperature in vacuo. The residual oil was dissolved in ethyl

acetate, and the ethyl acetate solution was successively washed with 1M $NaHCO_3$, 5% aqueous $KHSO_4$ and water, dried over Na_2SO_4, clarified by means of charcoal and concentrated in vacuo to yield an oil. It was dissolved in 150 ml absolute ether with heating, and after cooling, the product was precipitated by addition of 3 l of low-boiling light petroleum. The precipitate was stored overnight at + 4 °C, filtered and dried in vacuo over P_2O_5.

Yield: 71.5 g (92%), m.p. 91 °C, $[\alpha]_D$: 16.2° (*c* = 1, in methanol).

$C_{27}H_{36}N_2O_6$ (484.6)

Calcd.	C 66.93	H 7.49	N 5.78
Found	C 66.7	H 7.8	N 6.2

Z-Val-Tyr(Bu^t)-OH

68 g (0.145 mol) Z-Val-Tyr(Bu^t)-OCH_3 was dissolved in a mixture of 350 ml dioxane and 150 ml water. 140 ml 1N NaOH was slowly added at pH 12 - 13. After saponification was complete, the solution was neutralized with 1N HCl, concentrated in vacuo to a small volume and acidified to pH 3 by means of hydrochloric acid. The precipitate was filtered, washed with water and dried in vacuo.

Yield: 65.7 g (96%), m.p. 116 °C, $[\alpha]_D$: − 5.2° (*c* = 1, in methanol).

$C_{26}H_{34}N_2O_6$ (470.6)

Calcd.	C 66.36	H 7.28	N 5.95
Found	C 66.5	H 7.3	N 6.2

Z-Val-His-Pro-Phg-OBu^t

1.9 ml (15 mmol) *N*-ethylmorpholine was added at 0 °C to a solution of 5.1 g (15 mmol) H-Pro-Phg-OBu^t·HCl[7] in 35 ml dimethylformamide, and the mixture was combined with a solution of 5.8 g (15 mmol) Z-Val-His-OH[24] and 2.1 g 1-hydroxybenzotriazole (15 mmol) in 50 ml dimethylformamide.

A solution of 3.4 g (16.5 mmol) dicyclohexylcarbodiimide in 5 ml dimethylformamide was admixed under stirring at − 5 °C. Stirring was continued for 1 h at 0 °C and overnight at room temperature.

After filtration the solvent was removed in vacuo. The residue was dissolved in 250 ml ethyl acetate, the solution was washed with 2N Na_2CO_3 and water, dried over $MgSO_4$ and, after clarification with charcoal, concentrated in vacuo to a small volume.

The product was precipitated with dry diethyl ether.

Yield: 8.5 g (84%), m.p. 124 °C, $[\alpha]_D$: − 17° (*c* = 1, in methanol).

$C_{36}H_{46}N_6O_7$ (674.8)

Calcd.	C 64.08	H 6.87	N 12.45
Found	C 63.5	H 6.9	N 11.9

H-Val-His-Pro-Phg-OBu^t·2 HCl

8.3 g (135 mmol) Z-Val-His-Pro-Phg-OBu^t in 200 ml methanol was hydrogenated catalytically. The pH value was maintained at 3.0 by dropwise addition of 1N methanolic HCl.

After filtration, the solution was concentrated in vacuo and precipitated with dry diethyl ether.

Yield: 7.1 g (85.7%), m.p. 170 °C, $[\alpha]_D$: − 17.8° (*c* = 1, in methanol)

$C_{28}H_{40}N_6O_5 \cdot 2HCl$ (613.6)

Calcd.	C 54.81	H 6.90	N 13.70
Found	C 54.2	H 7.2	N 13.3

Z-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t

18.61 g (50 mmol) Z-Tyr(Bu^t)-OH and a solution of 11.3 g (55 mmol) dicyclohexylcarbodiimide in 16 ml dimethylformamide were added at 0 °C to a solution prepared at 0 °C from 30.6 g (50 mmol) H-Val-His-Pro-Phg-OBu^t·2 HCl and 12.6 ml (0.1 mol) *N*-ethylmorpholine in 100 ml dimethylformamide. Stirring was continued for 1 h at 0 °C and for 16 h at room temperature. After filtration the solvent was distilled off, and the residue was dissolved in ethyl acetate. The solution was washed with 1M $NaHCO_3$ and water, clarified with charcoal, dried over Na_2SO_4 and concentrated to dryness in vacuo.

Yield: 44.1 g (98%), $[\alpha]_D$: − 15.5° (*c* = 1, in methanol).

$C_{49}H_{64}N_7O_9$ (895.1)

Calcd.	C 65.75	H 7.21	N 10.95
Found	C 65.4	H 7.3	N 11.1

H-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t·2 HCl

44 g (49 mmol) Z-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t was dissolved in 850 ml CH_3OH, and the solution was hydrogenated as described above. The solvent was removed in vacuo and a colorless, amorphous solid was obtained.

Yield: 38.4 g (94%), $[\alpha]_D$: + 4.8° (*c* = 1, in methanol).

$C_{41}H_{57}N_7O_7 \cdot 2HCl$ (832.9)

Calcd.	C 59.13	H 6.90	N 11.77
Found	C 59.4	H 7.2	N 11.4

Z-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t

38 g (40 mmol) H-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t and 10.12 ml (90 mmol) *N*-ethylmorpholine were dissolved at 0 °C in 280 ml dimethylformamide, then a solution of 10.1 g (40 mmol) Z-Val-OH and 5.4 g (40 mmol) 1-hydroxybenzotriazole in 80 ml dimethylformamide was added. After cooling to − 5 °C, 9.1 g dicyclohexylcarbodiimide in 15 ml dimethylformamide was added. Stirring was continued for 1 h at 0 °C and for 16 h at room temperature. The solution was filtered and concentrated to dryness in vacuo. the residue was dissolved in ethyl acetate, the solution was washed with 2N

Na_2CO_3, dried with Na_2SO_4 and clarified with charcoal. After evaporation of the solvent, the residue was triturated with absolute diethyl ether.

Yield: 31.7 g (80%), $[\alpha]_D$: − 14.8° (*c* = 1, in dimethylformamide)

$C_{54}H_{73}N_8O_{10}$ (994.2)

Calcd.	C 65.20	H 7.40	N 11.27
Found	C 65.3	H 7.3	N 10.7

H-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t · 2 HCl

31.5 g (31.7 mmol) Z-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t in 600 m*l* methanol was hydrogenated and worked up as described above.

Yield: 28.6 g (97%), m.p. 183 °C, $[\alpha]_D$: + 9.6° (*c* = 1, in dimethylformamide)

$C_{46}H_{66}N_8O_8 \cdot 2\,HCl$ (932.0)

Calcd.	C 59.28	H 7.35	N 12.02
Found	C 58.9	H 7.4	N 11.7

Z-Arg(Z)$_2$-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t

6.6 m*l* (52 mmol) of *N*-ethylmorpholine was added at 0 °C to a solution of 27.4 g (28 mmol) H-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t in 300 m*l* dimethylformamide. After addition of 16.5 g (28.6 mmol) Z-Arg(Z)$_2$-OH and 3.8 g (28 mmol) 1-hydroxybenzotriazole the mixture was cooled to − 5 °C.

5.9 g (28.6 mmol) dicyclohexylcarbodiimide in 15 m*l* dimethylformamide was added with stirring. Stirring was continued for 1 h at 0 °C and for 12 h at room temperature. After filtration, the solvent was evaporated in vacuo. The remaining oil solidified on trituration with 2N Na_2CO_3 and water.

Yield: 38.7 g (97%), $[\alpha]_D$: − 6.8°, (*c* = 1, in dimethylformamide).

$C_{76}H_{97}N_{12}O_{15}$ (1418.6)

Calcd.	C 64.35	H 6.89	N 11.85
Found	C 64.0	H 6.5	N 11.8

H-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t · 3 HCl

A solution of 37.5 g (26.5 mmol) Z-Arg(Z)$_2$-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t in 1.3 *l* methanol was hydrogenated and worked up as described above.

Yield: 28.2 g (94%), $[\alpha]_D$: − 7.2° (*c* = 1, in methanol).

$C_{52}H_{78}N_{12}O_9 \cdot 3\,HCl$ (1124.6)

Calcd.	C 55.54	H 7.25	N 14.94
Found	C 55.3	H 7.6	N 14.2

Succinamoyl-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t

16.86 g (15 mmol) H-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t · 3 HCl was dissolved in 200 m*l* dimethylformamide at 0 °C, and 5.70 m*l* (45 mmol) *N*-ethylmorpholine was added. After addition of 6.07 g (45 mmol) 1-hydroxybenzotriazole and 5.71 g (45 mmol) of succinic acid mono-amide in dimethylformamide, the mixture was cooled to − 5 °C under stirring, and 9.27 g (45 mmol) of dicyclohexylcarbodiimide in 15 m*l* dimethylformamide was added. Stirring was continued for 1 h at 0 °C and for 12 h at room temperature. After filtration, the solvent was evaporated in vacuo to yield an oily residue which solidified upon trituration with dry diethyl ether. The product was washed with 2N Na_2CO_3 and water and dried in vacuo.

Yield: 14.5 g (87%), $[\alpha]_D$: 19.2° (*c* = 1, in dimethylformamide).

Succinamoyl-Arg-Val-Tyr-Val-His-Pro-Phg-OH

2 g succinamoyl-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t was dissolved in 20 m*l* 90% trifluoroacetic acid, and the solution was stirred for 60 min. The trifluoroacetic acid was distilled off in vacuo at 25 - 30 °C, the residue was triturated with dry diethyl ether, dissolved in 100 m*l* 90% methanol and passed through the ion-exchange resin Amberlite IR 45 (acetate form) in a column 1 × 50 cm. The resin was washed with 250 m*l* 90% methanol, and the combined solutions were concentrated to dryness in vacuo. The residue was triturated with dry diethyl ether and dried in vacuo.

Yield: 1.4 g. The crude product was purified by partition chromatography on Sephadex LH 20 (column size: 2.5 × 100 cm) in the solvent system n-butanol/acetic acid/water (2:1:10). 350 mg of crude product yielded 160 mg of the chromatographically pure peptide; $[\alpha]_D$: − 38.2° (*c* = 1, in methanol).

Amino acid analysis:

Arg: 0.92	Val: 2.00	Tyr: 0.80	His: 1.04
Pro: 0.95	Phg: 1.03		

Boc-Sar-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t

1.062 g (0.94 mmol) H-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Phg-OBu^t · 3 HCl was dissolved in 10 m*l* dimethylformamide at 0 °C. While stirring 0.380 m*l* (3 mmol) *N*-ethylmorpholine, 0.55 g (1.5 mmol) Boc-Sar-OPhCl$_3$, and 135 mg (1.0 mmol) 1-hydroxybenzotriazole were added. Stirring was continued for 1 h at 0 °C and for 20 h at room temperature when the solvent was evaporated in vacuo. The residue was triturated with dry diethyl ether and dissolved in ethyl acetate. The solution was washed with 2N Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to dryness in vacuo. The product was isolated as a colorless, amorphous powder by triturating the residue with diethyl ether.

Yield: 1.03 g (90%), $[\alpha]_D$: − 15.8° (*c* = 1, in dimethylformamide).

The compound was shown to be nearly pure by thin-layer chromatography (20 µg). R_F = 0.78 (0.14 for the

impurity) in methyl ethyl ketone/pyridine/acetic acid/water (70:15:2:15); R_F = 0.73 (0.15) in methanol/water (8:2).

H-Sar-Arg-Val-Tyr-Val-His-Pro-Phg-OH·CH$_3$COOH

1.66 g (1.45 mmol) of the aforementioned protected sarcosine peptide was dissolved in 10 ml 90% trifluoroacetic acid. The solution was stirred for 50 min at room temperature, then concentrated to dryness in vacuo and the residue was triturated with 100 ml of dry diethyl ether. 1.0 g of a colorless, amorphous powder was obtained, which was dissolved in 20 ml 90% methanol and passed through a column of Amberlite IR 45 (acetate form), 10 ml resin. Another 250 ml of 90% methanol was passed through the column, and the combined solutions were evaporated in vacuo. The remaining residue was precipitated several times from ethanol/ether.

Yield: 748 mg (52%).

Partition chromatography on Sephadex LH 20 in the solvent system butanol/acetic acid/water (2:1:10) yielded 575 mg of the chromatographically pure peptide.

$[\alpha]_D$: − 25.0° (c = 0.5, in dimethylformamide/water 1:1).

Amino acid analysis

Sar: 0.9 Arg: 0.85 Val: 1.99 Tyr: 0.80
His: 0.88 Pro: 0.89 Phg: 1.0

Z-Val-His-Pro-Ala-OBu^t·HCl

9.0 g (31 mmol) H-Pro-Ala-OBu^t·HCl[7] was dissolved in 80 ml dimethylformamide. The solution was cooled to 0 °C and 3.90 ml (31 mmol) *N*-ethylmorpholine was added. The solution of 12.25 g (31 mmol) Z-Val-His[24] and 4.24 g (31.5 mmol) 1-hydroxybenzotriazole in 100 ml dimethylformamide was then poured into the solution of the dipeptide ester with stirring at − 5 °C, and 7.0 g (34 mmol) dicyclohexylcarbodiimide in 10 ml dimethylformamide was admixed. Stirring was continued for 2 h at 0 °C and overnight at room temperature. After filtration the solvent was evaporated in vacuo. The residual oil was dissolved in 350 ml ethyl acetate. The solution was washed with 50 ml 1M $NaHCO_3$ and water, dried over Na_2SO_4, clarified by means of charcoal and concentrated to dryness in vacuo to yield 19.9 g of a white, amorphous solid.

The crude product was purified by dissolving it in 50 ml methylene chloride, cooling to 0 °C, adding 1 equivalent HCl in methylene chloride and precipitating the peptide ester hydrochloride by addition of dry diethyl ether.

Yield: 16.18 g (80%), m.p. 155 - 160 °C, $[\alpha]_D$: − 79.5° (c = 1, in methanol).

$C_{31}H_{44}N_6O_7 \cdot HCl$ (649.2)

Calcd.:	C 57.35	H 6.99	N 12.95
Found	C 57.3	H 7.1	N 12.9

H-Val-His-Pro-Ala-OBu^t·2 HCl

16.2 g (25 mmol) Z-Val-His-Pro-Ala-OBu^t in 300 ml methanol was hydrogenated at pH 3.5 and worked up as described above. 13.2 g (96%) of a white, amorphous solid was obtained.

m.p. 171 °C, $[\alpha]_D$: − 63.8° (c = 1, in methanol).

$C_{23}H_{38}N_6O_5 \cdot 2\,HCl$ (551.5)

Calcd.	C 50.09	H 7.31	N 15.24
Found	C 49.5	H 7.6	N 14.6

Z-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t

13.05 g (23.6 mmol) H-Val-His-Pro-Ala-OBu^t·2 HCl was dissolved in 100 ml dimethylformamide. After cooling to − 10 °C, 6.0 ml *N*-ethylmorpholine and the cooled (− 10 °C) solution of 15.8 g (23.6 mmol) Z-Val-Tyr(Bu^t)-OH and 3.20 g 1-hydroxybenzotriazole in 120 ml dimethylformamide were added. After the addition of 5.35 g (26 mmol) dicyclohexylcarbodiimide in 6 ml dimethylformamide, stirring was continued for 3 h at 0 °C and for 16 h at room temperature. The solution was filtered and the solvent was evaporated in vacuo. The residue was dissolved in ethyl acetate. The solution was washed with 1M $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated in vacuo. The residue was triturated with diethyl ether.

Yield: 21.6 g (98%), m.p. 158 °C, $[\alpha]_D$: − 29.8° (c = 1, in dimethylformamide).

$C_{49}H_{70}N_8O_{10}$ (931.2)

Calcd.	C 63.21	H 7.58	N 12.03
Found	C 63.2	H 7.5	N 11.6

H-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t·2 HCl

21.40 g (23 mmol) Z-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t in 350 ml methanol was hydrogenated and worked up as described above.

Yield: 19.2 g (95%), m.p. 169 °C, $[\alpha]_D$: − 41° (c = 1, in methanol).

$C_{41}H_{64}N_8O_8 \cdot 2\,HCl$ (869.9)

Calcd.	C 56.61	H 7.65	N 12.88
Found	C 56.4	H 7.9	N 12.2

Z-Arg(Z)$_2$-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t

5.56 ml (44 mmol) *N*-ethylmorpholine was added at 0 °C to a solution of 19.1 g (22 mmol) H-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t. 2HCl in 100 ml dimethylformamide. After addition of a solution of 12.7 g (22 mmol) Z-Arg(Z)$_2$-OH and 2.98 g (22 mmol) 1-hydroxybenzotriazole in 80 ml dimethylformamide, the mixture was cooled to − 5 °C. 5.0 g (23.2 mmol) dicyclohexylcarbodiimide in 15 ml dimethylformamide was added with stirring. Stirring was continued for 1 h at 0 °C and for 12 h at room temperature. After filtration, the solvent was evaporated in vacuo. The remaining oily residue solidified on trituration with 2N Na_2CO_3 and water.

Yield: 29.8 g (50%).

For purification this product was precipitated from its solution in dimethylformamide by a 1:1 mixture of diethyl ether and light petroleum (b.p. 40 - 70 °C).

Yield: 17.7 g (30%), $[\alpha]_D$: −15° (*c* = 1, in dimethylformamide).

$C_{71}H_{94}N_{12}O_{15}\cdot H_2O$ (1373.6)

Calcd.	C 62.08	H 7.04	N 12.24
Found	C 61.9	H 7.3	N 12.3

H-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t · 3HCl

22.5 g (16.4 mmol) Z-Arg(Z)$_2$-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t in 1 l methanol was hydrogenated and worked up as described above.

Yield: 17.8 g (99%), $[\alpha]_D$: −21.4° (*c* = 1, in dimethylformamide).

Thin-layer chromatography (20 μg): R_F = 0.70 in n-butanol/acetic acid/water (2:1:1). Trace of impurity with R_F = 0.79.

Boc-Sar-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t

A solution of 8.9 g (8.3 mmol) H-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t · 3HCl in 40 ml dimethylformamide was cooled to −5 °C, then 3.15 ml (25 mmol) *N*-ethylmorpholine, 1.13 g (8.35 mmol) 1-hydroxybenzotriazole, and 4.81 g (12.5 mmol) Boc-Sar-OPhCl$_3$ in 15 ml dimethylformamide were added successively with stirring. Stirring was continued for 1 h at 0 °C and overnight at room temperature. After evaporation of the solvent in vacuo, the residue was triturated with diethyl ether, washed with 2N Na_2CO_3 and water, and dried in vacuo over P_2O_5.

Yield: 8.2 g (87%), $[\alpha]_D$: −38.8° (*c* = 0.5, in dimethylformamide).

Thin-layer chromatography: R_F = 0.78 in n-butanol/acetic acid/water (2:1:1) with a small additional spot, R_F = 0.12.

H-Sar-Arg-Val-Tyr-Val-His-Pro-Ala-OH · CH$_3$COOH

4.0 g (2.81 mmol) Boc-Sar-Arg-Val-Tyr(Bu^t)-Val-His-Pro-Ala-OBu^t was dissolved in 20 ml 90% trifluoroacetic acid. The solution was stirred for 60 min at room temperature, then it was evaporated at 25 °C in vacuo. The residue solidified when treated with absolute diethyl ether. The colorless amorphous solid was dissolved in 50 ml 90% methanol and filtered over Amberlite IR 45 acetate in a column 12 × 200 mm. After washing with 200 ml 90% methanol, the combined solutions were evaporated in vacuo. The residue was triturated with dry ether.

Yield: 2.40 g

Purification of this crude product was achieved by partition chromatography on Sephadex LH 20 in the solvent system n-butanol/acetic acid/water (2:1:10). $[\alpha]_D$: −80.1° (*c* = 0.25, in 1N CH_3COOH) lit.[6] $[\alpha]_D$: −78.0° (*c* = 0.24, in 1N CH_3COOH).

Thin-layer chromatography using Merck silicagel HF 254 plates in the system n-butanol/acetic acid/water (6:2:3) showed one Pauly-positive spot at R_F = 0.1 (lit.[6] 0.1) and a trace of impurity (R_F = 0.28). In n-butanol/acetic acid/water/pyridine (9:2:6:7) the R_F was 0.35 (lit.[6] 0.35).

Amino acid analysis (95% peptide content):

Sar: 0.9 Ala: 1.00 Val: 1.91 Tyr: 0.92 His: 0.97 Arg: 0.86

Proline was not determined quantitatively.

PHARMACOLOGICAL METHODS

Tests in vitro

a) The inhibition of the angiotensin II induced isotonic contraction (4 ng/ml) with angiotensin II antagonists was tested on the isolated *guinea-pig ileum*. The results (expressed as % of the maximum contractile response of the ileum following angiotensin II application) were transformed into probits, a regression analysis performed and the equation of the regression line ($y = A + B \log x$) was calculated; by this means the doses which gave a 50% inhibition of the angiotensin II induced contraction (ID_{50}) could be determined.

b) The inhibition of the angiotensin II induced isometric contraction with angiotensin II analogues was investigated on isolated *rat stomach*[25]. The degree of inhibition with known concentrations can be used as a measure of antagonistic potency of the analogues.

Tests in vivo

a) The angiotensin II analogues were injected intravenously into male anaesthetized normotensive Sprague-Dawley rats with vagotomy and ganglionic blockade at a dose of 16 μg/kg to examine the *intrinsic pressor activity* with high dosage. Arterial blood pressure was measured directly in the carotid artery with a Statham pressure transducer.

b) Angiotensin II in doses of 0.5, 1.0 and 2.0 μg/kg was injected intravenously into male anaesthetized Sprague-Dawley rats with vagotomy and ganglionic blockade during an intravenous infusion of angiotensin II antagonists in different doses [0.1, 1.0, 5.0 and 10.0 μg $kg^{-1} \times (0.01\ ml)^{-1} \times min^{-1}$]. Arterial blood pressure was measured directly in the carotid artery with a Statham pressure transducer. Five rats were used for every analogue and for every dose. The percentage diminution of the angiotensin II pressor effect during infusion of angiotensin II antagonists in comparison to the angiotensin II pressor effect during control infusions was taken as a measure of *antagonistic potency* of the

analogues. The values of the percentage diminution were transformed into probits and a regression analysis was performed for the determination of the ID_{50}.

c) In male anaesthetized normotensive Sprague-Dawley rats with vagotomy and ganglionic blockade, the *duration of the angiotensin II antagonist* (0.1 mg/kg i.v.) *induced blood pressure decrease* during an intravenous angiotensin II infusion [1 $\mu g \times kg^{-1} \times (0.02\ ml)^{-1} \times min^{-1}$] was determined. The duration of the blood pressure decrease was taken as a reference for the duration of the antagonistic action of the analogues. Arterial blood pressure was measured directly in the carotid artery with a Statham pressure transducer.

d) *Acute renal hypertension* in the male anaesthetized Sprague-Dawley rat was produced by unclamping one renal pedicle which had been occluded for 4 h[26-28]. Angiotensin II antagonists were given as intravenous injections in doses of 10 and 100 μg/kg. Arterial blood pressure was measured directly in the carotid artery with a Statham pressure transducer.

e) *In conscious unrestrained rats* with normal blood pressure, spontaneous hypertension and chronic renal hypertension, induced by applying silver clips of 0.2 mm diameter to both renal arteries, *blood pressure* was monitored directly with a chronically inserted catheter in the abdominal aorta[29]. Infusions of angiotensin II antagonists in a dose of 10 $\mu g \times kg^{-1}$ (0.1 $ml)^{-1} \times min^{-1}$ were given through a permanently implanted catheter in the jugular vein for 15 min. For every value six to fifteen animals were used. Rats with a blood pressure > 150 mm Hg were considered to be hypertensive.

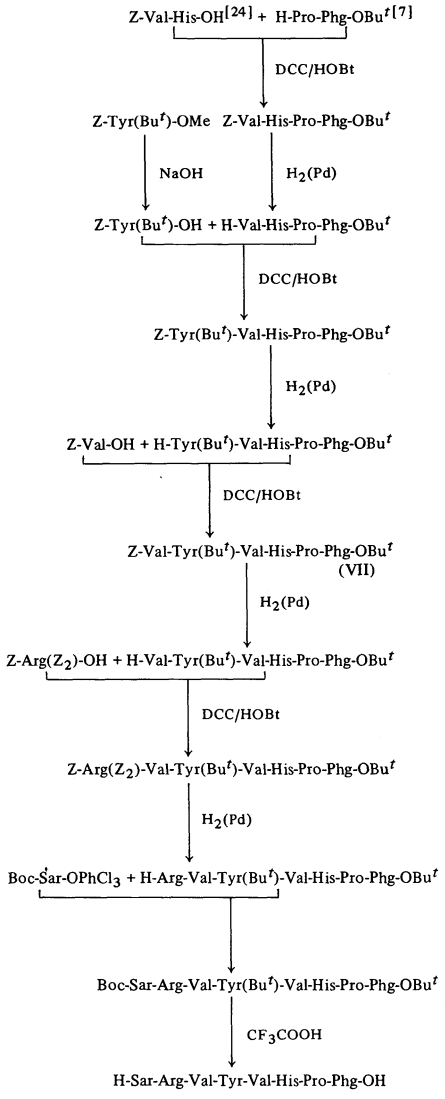
f) In male anaesthetized normotensive Sprague-Dawley rats, angiotensin II analogues in a dose of 50 $\mu g \times kg^{-1} \times (0.02\ ml)^{-1} \times min^{-1}$ were infused intravenously over 15 min. *Plasma renin activity* (ng angiotensin I $ml^{-1}\ h^{-1}$) was determined by radioimmunoassay[30] with a modified commercially available kit (Isotopen-Dienst West, Frankfurt (Main), Germany). Control animals were infused with vehicle alone.

In additional experiments, plasma renin activity was determined in animals with normotension, with bilateral nephrectomy and with acute renal hypertension, induced by unclamping one renal pedicle which had been occluded for 4 h[26-28].

Results

SYNTHESIS OF THE ANALOGUES

The synthesis of [Val^5]angiotensin II analogues with *C*-phenylglycine in position 8 was different from our previous synthesis[7] of the corresponding [Ile^5] compounds.



Scheme 1a. Synthesis of [Sar^1,Val^5,Phg^8]angiotensin II

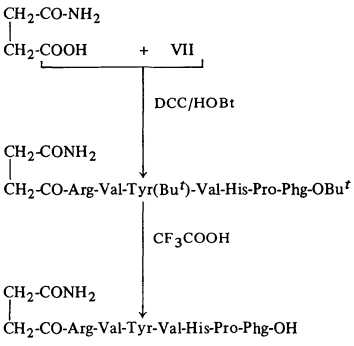
It started with the tetrapeptide Z-Val-His-Pro--Phg-OBu^t, prepared from two dipeptide derivatives as shown in Scheme 1a. The protecting group for the α-nitrogen was always the benzyloxycarbonyl residue. The carboxyl group and the phenolic hydroxyl group of tyrosine were blocked by tertiary butyl radicals, and arginine was introduced as the tris-benzyloxycarbonyl derivative.

The benzyloxycarbonyl groups were eliminated by means of catalytic hydrogenation, the Boc- and *tert*-butyl residues by trifluoroacetic acid. All condensation steps were performed using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole as an additive[31].

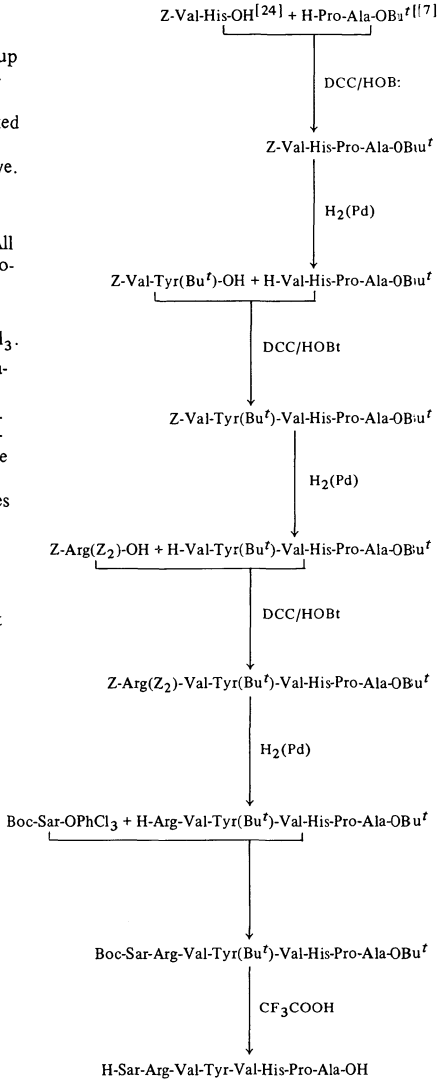
Only sarcosine was introduced as Boc-Sar-OPhCl$_3$.

According to Scheme 1a, the intermediate heptapeptide VII is built up by stepwise introduction of the amino acids tyrosine, valine, and arginine. After deblocking of the α-amino group, Boc-Sar--OPhCl$_3$ is added to form the protected analogue IV. Succinic acid monoamide yields the corresponding analogue VI (Scheme 1b). Both analogues are deblocked and transformed into the acetate by filtration over the weakly basic ion exchange resin Amberlite IR 45 acetate.

Scheme 2 shows the synthesis of the known [Sar^1, Val^5, Ala^8]angiotensin II[6] by a different route.



Scheme 1b.
Synthesis of [NSuc^1,Val^5,Phg^8]angiotensin II



Scheme 2. Synthesis of [Sar^1,Val^5,Ala^8]angiotensin II

The tetrapeptide H-Val-His-Pro-Ala-OBu^t, which is also synthesized from two dipeptides, is elongated by the dipeptide derivative Z-Val-Tyr(Bu^t)--OH. Then the following amino acids are introduced in the same way as already demonstrated in Scheme 1 a.

Amino acid analyses and thin-layer chromatography revealed the purity of each analogue.

PHARMACOLOGICAL EXPERIMENTS

1) Tests in vitro

a) The experiments on the isolated *guinea-pig ileum* showed a dose-dependent inhibition of the angiotensin II induced isotonic contractions by angiotensin II analogues. In Table 2 the antagonistic effect of the angiotensin II analogues is shown in terms of the ID_{50} concerned.

Table 2. ID_{50} value of angiotensin II analogues for the angiotensin II (4 ng/m*l*) induced contractions of the isolated guinea-pig ileum.

Analogue		ID_{50} [ng/m*l*]
[Sar^1,Ile^5, Ala^8]angiotensin II	(I)	2.1
[Sar^1,Val^5,Ala^8]angiotensin II	(II)	8.9
[Sar^1,Ile^5,Phg^8]angiotensin II	(III)	3.9
[Sar^1,Val^5,Phg^8]angiotensin II	(IV)	3.2
[NSuc^1,Ile^5,Phg^8]angiotensin II	(V)	16.7
[NSuc^1,Val^5, Phg^8]angiotensin II	(VI)	17.1

It can be seen that [Sar^1,Ile^5,Ala^8]angiotensin II has the lowest ID_{50}. When Val instead of Ile is brought into the 5-position of [Sar^1,Ala^8]-angiotensin II, a clearly different ID_{50} is determined. The ID_{50} of [Phg^8]analogues with Sar in position 1 lies in a similar range. On the other hand, the substitution of a succinamoyl residue in position 1 of the molecule resulted in higher ID_{50} values.

In this isolated preparation, the difference in the 5-position of [Phg^8]analogues, but not of [Sar^1,Ala^8] analogues, seems to be negligible. Sar in position 1 appears to be of most importance for the potency, expressed as ID_{50}, of the analogues tested in this isolated organ.

b) In the isolated *rat stomach* strip the myotropic effect of angiotensin II (20 ng/m*l*) could be inhibited with the angiotensin II antagonists (6.25 - 100 ng/m*l*) in a close dose-response relation (Table 3).

In both analogue pairs tested with this isolated preparation, the 5-position appears to be of no significance, although it could be demonstrated that the [Phg^8]analogues were more potent and more effective, also in a low dosage, than the [Sar^1,Ala^8]angiotensin II analogues.

2) Tests in vivo

a) In the following table (Table 4), the percentage *blood pressure increase* following intravenous injection of angiotensin II analogues (16 μg/kg) is shown.

Table 3. Inhibition (%) of the myotropic angiotensin II effect (20 ng/m*l*) in the isolated rat stomach strip with angiotensin II analogues.

Analogue	*n*	% Inhibition with analogues at doses [ng/m*l*]				
		6.25	12.5	25	50	100
[Sar^1,Ile^5,Ala^8]angiotensin II	28	0	29	63	64	89
[Sar^1,Val^5,Ala^8]angiotensin II	26	0	19	33	72	93
[NSuc^1,Ile^5,Phg^8]angiotensin II	28	17	49	62	87	98
[NSuc^1,Val^5,Phg^8]angiotensin II	30	26	35	63	81	90

n = number of experiments
0 = no detectable inhibition
100 = complete inhibition of angiotensin II effect

Table 4. Blood pressure increase [% of initial value] following intravenous injection of angiotensin II analogues (16 μg/kg).

Analogue	Increase [%]
[Sar^1,Ile^5,Ala^8]angiotensin II	18
[Sar^1,Val^5,Ala^8]angiotensin II	16
[Sar^1,Ile^5,Phg^8]angiotensin II	17
[Sar^1,Val^5,Phg^8]angiotensin II	18
[NSuc^1,Ile^5, Phg^8]angiotensin II	23
[NSuc^1,Val^5,Phg^8]angiotensin II	22

n = 5 for each analogue.

Table 6. Duration ($M \pm$ SD) of antagonist (0.1 mg/kg i.v.) induced blood pressure decrease during i.v. angiotensin II infusion [1 μg × kg^{-1} × (0.02 ml)$^{-1}$ × min^{-1}].

Analogue	Duration [min]
[Sar^1,Ile^5,Ala^8]angiotensin II	41 ± 11
[Sar^1,Val^5,Ala^8]angiotensin II	66 ± 21
[Sar^1,Ile^5, Phg^8]angiotensin II	78 ± 16
[Sar^1,Val^5, Phg^8]angiotensin II	78 ± 17
[NSuc^1,Ile^5,Phg^8]angiotensin II	51 ± 8
[NSuc^1,Val^5,Phg^8]angiotensin II	89 ± 22

n = 5 for every analogue.

All tested analogues, at a dose of 16 μg/kg i.v., showed a clear pressor activity. No difference concerning the 5-position could be demonstrated.

b) All compounds *inhibited the blood pressure response* of 1.0 μg angiotensin II i.v. per kg body weight. ID_{50} values (μg × kg^{-1} × min^{-1}) based on the percentage of the angiotensin II pressor effect during infusion of antagonists in comparison to the pressor effect during control infusion were calculated (Table 5). In contrast to the [Sar^1,Ala^8]analogues, the [Val^5,Phg^8]analogues have a lower ID_{50} than the [Ile^5, Phg^8]analogues; the latter cause a less marked inhibition of the induced pressor reaction than the [Val^5,Phg^8]-analogues. Substitution in position 1 seems to be unimportant in [Phg^8]analogues in this test model.

Table 5. Inhibition of the angiotensin II induced pressor effect during infusion of analogues.

	ID_{50} [μg × kg^{-1} × min^{-1}]
Dose of angiotensin II [μg/kg i.v.]	1.0
[Sar^1,Ile^5,Ala^8]angiotensin II	2.8
[Sar^1,Val^5,Ala^8]angiotensin II	2.9
[Sar^1,Ile^5,Phg^8]angiotensin II	4.8
[Sar^1,Val^5,Phg^8]angiotensin II	1.0
[NSuc^1,Ile^5,Phg^8]angiotensin II	5.3
[NSuc^1,Val^5,Phg^8]angiotensin II	1.2

c) The *duration* of the antagonist-induced decrease in blood pressure during an intravenous angiotensin II infusion was measured (Table 6).

It appears that the analogues with Val in position 5 depress the blood pressure longer than those with Ile^5.

d) *Unclamping the* left *renal pedicle* after it had been occluded for 4 h results within 10 min in a marked, highly significant increase in blood pressure, which remains elevated for hours. Following angiotensin II analogue injection, blood pressure decreased immediately (Table 7).

With all analogues, a dose-dependent blood pressure decrease could be observed. With respect to the 1 and 5 positions, there was no marked difference between the different analogues.

e) Effect on *blood pressure in conscious rats.* In contrast to normotensive animals, in which a uniform blood pressure increase could be demonstrated, in rats with chronic renal hypertension a significant blood pressure fall occurred after administration of each angiotensin II analogue. In rats with spontaneous hypertension, analogues with valine in the 5-position seemed to be more specific in inducing a blood pressure decrease (Table 8).

f) Intravenous infusion of angiotensin II analogues in normotensive rats resulted almost generally in a marked, significant increase of *plasma renin activity* compared to control infusion (Table 9). This increase in plasma renin activity is also very distinct in comparison to the plasma

Table 7. Blood pressure decrease (%) following injection of angiotensin II analogues (10 and 100 μg/kg i.v.) in acute renal hypertension.

Dose	n	Decrease with 10 μg/kg i.v. [%]	n	Decrease with 100 μg/kg i.v. [%]
[Sar^1,Ile^5,Ala^8]angiotensin II	14	27	10	42
[Sar^1,Val^5,Ala^8]angiotensin II	10	23	11	50
[Sar^1,Ile^5,Phg^8]angiotensin II	14	24	14	43
[Sar^1,Val^5,Phg^8]angiotensin II	11	31	13	44
[NSuc^1,Ile^5,Phg^8]angiotensin II	12	26	14	46
[NSuc^1,Val^5,Phg^8]angiotensin II	11	27	15	44

Table 8. Influence of angiotensin II analogues [10 μg × kg^{-1} × (0.1 ml)$^{-1}$ × min^{-1} i.v.] on blood pressure in conscious rats with normotension, spontaneous hypertension and chronic renal hypertension.
(Change of blood pressure in mm Hg, mean and % of initial value)

Analogue	Normotension		Spontaneous hypertension		Chronic renal hypertension	
	[mm Hg]	[%]	[mm Hg]	[%]	[mm Hg]	[%]
[Sar^1,Ile^5,Ala^8]angiotensin II	+ 8	+ 7	+ 15	+ 8	− 23	− 13
[Sar^1,Val^5,Ala^8]angiotensin II	+ 3	+ 2	− 4	− 2	− 23	− 13
[Sar^1,Ile^5,Phg^8]angiotensin II	+ 8	+ 7	+ 4	+ 2	− 34	− 19
[Sar^1,Val^5,Phg^8]angiotensin II	+ 12	+ 9	+ 4	+ 2	− 28	− 17
[NSuc^1,Ile^5,Phg^8]angiotensin II	+ 24	+ 22	+ 18	+ 11	− 30	− 16
[NSuc^1,Val^5,Phg^8]angiotensin II	+ 7	+ 6	− 5	− 3	− 20	− 12

Table 9. Plasma renin activity M ± SEM following intravenous infusion of angiotensin II analogues (50 μg × kg^{-1} × (0.02 ml)$^{-1}$ × min^{-1})

Analogue	n	Plasma renin activity [ng angiotensin I × ml^{-1} × h^{-1}]	[%]
Control	27	9 ± 1	–
[Sar^1,Ile^5,Ala^8]angiotensin II	12	43 ± 3	+ 378
[Sar^1,Val^5,Ala^8]angiotensin II	11	47 ± 4	+ 422
[NSuc^1,Ile^5,Phg^8]angiotensin II	10	35 ± 4	+ 289
[NSuc^1,Val^5,Phg^8]angiotensin II	11	34 ± 3	+ 278
Normotension	10	12 ± 2	–
Bilateral nephrectomy	22	2 ± 0.5	–
Acute renal hypertension	20	94 ± 11	–

renin activity in animals with normotension, with bilateral nephrectomy and with acute renal hypertension (Table 9).

In contrast to [Phg^8]angiotensin II analogues, the [Sar^1,Ala^8]angiotensin II analogues induce a more pronounced increase of plasma renin activity ($p < 0.01$).

Discussion

It is known that the naturally occurring variants of angiotensin II, [Ile^5]angiotensin II and [Val^5]-angiotensin II, are equiactive even though there are certain inconsistencies[32-35]. The importance of the amino acid in the 5 position is demonstrated by the decreased biological activity of the synthetic analogues, [Leu^5]angiotensin II (25%) [36] and [Ala^5]angiotensin II (5%)[37]. The β-branching side chains of valine or isoleucine in the 5 position may be involved in hydrophobic binding with the receptor protein[37,38], to be consistent with a steric effect, following a specific active conformation for the peptide backbone at the 5 position[2,3].

In agreement with the prevailing equiactivity of the natural variants of angiotensin II, Regoli et al. [4] found that potency and specificity of angiotensin antagonists are not influenced by isoleucine or valine in position 5. However, there are differences in the biological spectra of antagonistic activity of 1,8 disubstituted pairs of analogues of [Ile^5] and [Val^5]angiotensin II. Starting from the evaluation of analogues of [Ile^5]angiotensin II with *C*-phenylglycine instead of phenylalanine in position 8 and additional variations with sarcosine and succinamoyl in position 1, which showed a marked specific competitive antagonism of angiotensin II action[7,8], the corresponding analogues of [Val^5]angiotensin II were synthesized and compared with the [Sar^1,Ala^8] analogues of [Ile^5] and [Val^5]angiotensin II[6] (Table 1).

In the isolated guinea-pig ileum, all analogues showed a dose-dependent inhibition of the angiotensin II induced contraction (Table 2). In this preparation, the [Sar^1,Ile^5,Ala^8]angiotensin II had a stronger antagonistic effect than [Sar^1,Val^5,Ala^8]angiotensin II. This difference concerning the 5 position could not be demonstrated with the [Phg^8] analogues. Sarcosine in position 1, due to the assumed binding and prolonged occupation of receptors[11], seems to increase the potency of the analogues in this isolated preparation. The myotropic effect of angiotensin II in the isolated rat stomach strip was more inhibited by [NSuc^1,Phg^8] analogues than by the [Sar^1,Ala^8] derivatives (Table 3).

With regard to the intrinsic pressor activity (Table 4), no clear-cut difference could be demonstrated concerning the 1, 5 or 8 position of the analogues.

Concerning the angiotensin II antagonistic potency in normotensive animals (Table 5), [Phg^8] analogues with valine in position 5 were more potent than the corresponding analogues with isoleucine in position 5.

The duration of the angiotensin II antagonist induced blood pressure decrease during intravenous angiotensin II infusion was longer with the [Val^5]-analogues (Table 6).

In acute renal hypertension with an extremely high plasma renin activity of 94 ± 11 ng angiotensin I × ml^{-1} × h^{-1} (M ± SEM)[39], in which the antagonism of all tested analogues to endogenous angiotensin II caused a dose-related reduction of blood pressure immediately after intravenous injection, no significant difference was seen concerning the blood pressure decrease (Table 7). Similar results were obtained in the acute, but not in the chronic phase of unilateral renal hypertension, due to constriction of the aorta, in conscious rats with intravenous infusion of [Sar^1,Val^5,Ala^8]angiotensin II[6] and in acute renal hypertension with intravenous infusion of [Cys^8]angiotensin II, [Ile^8]angiotensin II, [*p*-fluoro-Phe^4]angiotensin II and [Phe^4, Tyr^8]-angiotensin II[28].

Experiments with conscious unrestrained rats with normotension, spontaneous hypertension and chronic renal hypertension were performed in an attempt to demonstrate the specific competitive antagonistic properties of the angiotensin II analogues under more physiological conditions (Table 8).

In normotensive animals, infusions of angiotensin II analogues did not produce a hypotensive response, indicating that angiotensin II is not likely to be an important factor in normal blood

pressure regulation[6]. All analogues caused a small transient rise in blood pressure.

In rats with spontaneous hypertension, following infusion of the [Sar^1,Val^5,Ala^8] and [NSuc^1,Val^5,-Phg^8]angiotensin II – in contrast to the same 5-isoleucine substituted analogues – a distinct blood pressure decrease could be seen. An increase in activity of the renin-angiotensin system is observed[40,41] with advancing development of spontaneous hypertension. These results are not in accordance with the earlier observation[6], that infusion of [Sar^1,Val^5,Ala^8]angiotensin II into conscious spontaneously hypertensive rats did not result in significant reduction of blood pressure.

Rats with chronic renal hypertension showed a marked, significant blood pressure decrease following infusion of each angiotensin II analogue. These findings are in accordance with the observation that [Sar^1,Val^5;Ala^8]angiotensin II causes a significant blood pressure drop in chronic renal "two kidney" hypertension, but not in chronic renal hypertension of the "one kidney" type[42]. After central application of 8-*C*-phenylglycine analogues of angiotensin II in conscious rats with chronic renal hypertension, a significant blood pressure decrease could also be observed. In conscious rats with normotension there was no blood pressure fall after central application[39]. This central blood pressure response could be taken as an indication of an intrinsic renin-angiotensin system in the brain[43,44].

All these findings point to the participation of the renin-angiotensin system in these forms of experimental chronic renal hypertension.

Intravenous infusion of angiotensin II analogues resulted in a marked increase of plasma renin activity (Table 9). In comparison to the 8-*C*-phenylglycine angiotensin II analogues, the increase in plasma renin activity was significantly enhanced by [Sar^1,Ala^8]angiotensin II analogues. These findings suggest an inhibition of the negative feedback control of renin release by endogenous angiotensin II by these specific angiotensin II antagonists[45-48].

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