Notes

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New Angiotensin Analogues: 8-(L-α-Methyl-3,4-dihydroxyphenylalanine)-Angiotensin II

Kikuo Arakawa,[1a] Mitsuteru Hirata, Koichiro Watanabe, Hiroshi Kuga, and Eizou Hattori[1b]

2nd. Department of Internal Medicine, Fukuoka University School of Medicine[1a] *and Tokyo Research Laboratories, Kowa Co., Ltd.*[1b]

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Two analogues of [Ile^5]-angiotensin II with the substitution of C-terminal phenylalanine by L-α-methyl-3,4-dihydroxyphenylalanine have been synthesized. The agonistic activity in the rat blood pressure was largely diminished, and the antagonist activity to parent angiotensin II was also much weaker than that of [Sar,^{1} Ile^8]-angiotensin II. Both two analogues did not act as antagonists to norepinephrine.

Keywords——angiotensin-antagonist; angiotensin analogue; new peptides; α-methylamino acid; α-methyldopa; liquid- an solid-phase peptide synthesis; angiotensin II; blood pressure; norepinephrine

C-Terminal amino acid residue of angiotensin II molecule plays a key role in the biological activity.[2] Substitution of the C-terminal phenylalanine with aliphatic amino acids largely diminishes the agonistic activity of angiotensin II and discloses antagonistic activity to parent angiotensin II.[3]

Recently, new types of angiotensin II inhibitors have been reported, in which C-terminal phenylalanine is replaced by aromatic amino acids, such as N-methylphenylalanine[4] and phenylglycine.[5] Moreover, [α-methylphenylalanine^8]-antiotensin II proved to be quite resistant to the action of carboxypeptidase A.[6]

Biologically angiotensin shares many activities, such as pressor and oxytocic activities, with catecholamines, and, they tend to act cooperatively. Chemically, they have phenyl group in common. In conjunction with this point, it occurred to us that substitution of phenylalanine with an antihypertensive quaternary amino acid, L-α-methyl-3,4-dihydroxyphenylalanine (α-methyldopa), or an inhibitor of catecholamine synthesis and activity, might invoke antagonistic action to both angiotensin II and norepinephrine.

[α-methyldopa^8]- and [Sar^1, α-methyldopa^8]-angiotensin II were synthesized by the solid-phase method in combination with the liquid method. They were investigated for the agonistic and antagonistic actions to angiotensin II and norepinephrine pressor activity in rat blood pressure.

Experimental

The synthesis of angiotensin II analogues reported here was started first of all from the synthesis of C-terminal dipeptide *tert*-butyloxycarbonyl (Boc)-Pro-α-methyl-3,4-dibenzyloxyphenylalanine. The sequence

1) Location: *a*) *34, Nanakuma, Nishi-ku, Fukuoka*; *b*) *2-17-43, Noguchi-cho, Higashimurayama, Tokyo.*
2) F.M. Bumpus, P.A. Khairallah, K. Arakawa, I.H. Page and R.R. Smeby, *Biochim. Biophys. Acta*, **46**, 38 (1961); K. Arakawa, R.R. Smeby and F.M. Bumpus, *J. Am. Chem. Soc.*, **84**, 1424 (1962); R.R. Smeby, K. Arakawa, F.M. Bumpus and M.M. Marsh, *Biochim. Biophys. Acta*, **58**, 550 (1962).
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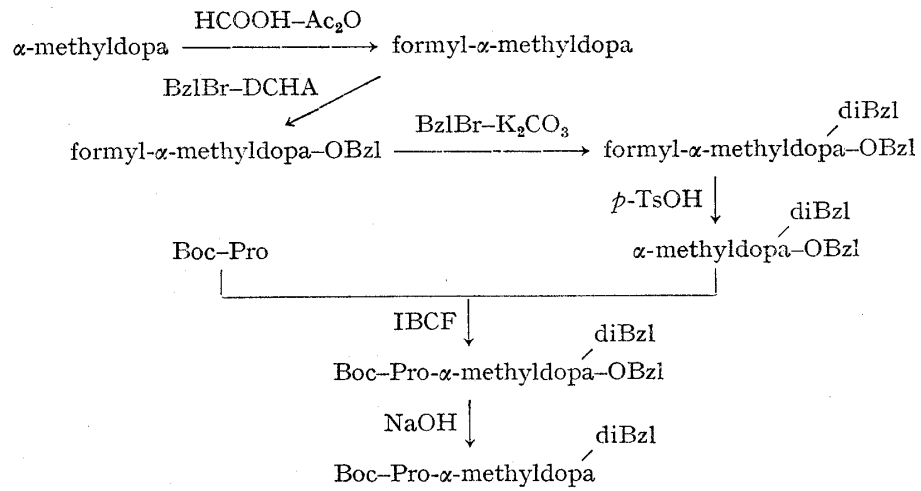

Ac_2O=acetic anhydride; DCHA=dicyclohexylamine; Bzl=benzyl; *p*-TsOH=*p*-toluenesulfonic acid; IBCF=isobutylchloroformate

Chart 1

of reaction employed for the synthesis of this dipeptide is shown in Chart 1. In the second place, Boc-Pro-α-methyl-3,4-dibenzyloxyphenylalanine was esterified to 2% cross-linked chloromethyl polymer as a starting material in the solid-phase method.[7] All coupling reactions were repeated with 3 eq. of Boc- or tert-amyloxycarbonyl (Aoc)-amino acid and dicyclohexylcarbodiimide, and completeness of coupling at each stage was checked by the ninhydrin color test.[8] Also, the swell-shrink-swell wash was used between all coupling and deprotection steps.[9] Functional side group of Boc-Asp was protected by benzyl, Boc-Tyr by 3-bromobenzyloxycarbonyl, and, Aoc-Arg and Boc-His by tosyl groups. At the end of the synthesis, the peptide was cleaved from the polymer and deblocked by treatment with anhydrous HF in the presence of anisole. After removal of HF and anisole, the desired peptides were purified by Bio-Gel P-2 and CM-cellulose column chromatographies. The purity was confirmed by thin-layer chromatography, paper electrophoresis and amino acid analysis.

Blood pressure was measured in female Wistar rats (Shizuoka Agricultural Cooperative Association for Laboratory Animals, 140—180 g) anesthetized with sodium amobarbital (150 mg/kg, i.p.) and ganglion-blocked with pentolinium tartrate (5 mg/kg, *s.c.*).[10] The carotid artery pressure was measured by using an electromanometer (Nihon Kohden, MPU-0.5). Angiotensin analogues (0.5 ml/kg) were injected into the cannulated femoral vein to assay the pressor activity. To evaluate the antagonistic action of test materials to angiotensin II or norepinephrine, both femoral veins were cannulated: one vein was used for infusions of the substances to be assayed at a constant infusion rate of 0.01—0.05 ml/min by using an infusion pump (Harvard Apparatus, Model 901) and the other for injections of a bolus (0.5 ml/kg) of angiotensin II or norepinephrine.

Table I. Physical Properties of the Angiotensin II Analogues

Compound	$[\alpha]_D$	*Rf* on TLC[e]		
		A	B	C
[Des-Asp[1], α-methyldopa[8]]-AT II[a]	−40.4[b]	0.16	0.38	0.65
[α-methyldopa[8]]-AT II	−67.4[c]	0.13	0.27	0.58
[Sar[1], α-methyldopa[8]]-AT II	−67.9[d]	0.12	0.30	0.52

a) AT II=angiotensin II.
b) *c*=0.76, 1 N AcOH.
c) *c*=0.88, 1 N AcOH.
d) *c*=1.36, 1 N AcOH.
e) Solvent systems, A=*n*-BuOH–AcOH–H_2O (4:1:5, upper layer); B=*n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2); C=*n*-BuOH–AcOH–H_2O–AcOEt (1:1:1:1).

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Results

$[\alpha]_D$, and *Rf* values of the synthesized analogues on a Silica Gel (Merck, 0.25 mm) are shown in Table I. As shown in Fig. 1, agonistic activity in rat blood pressure of [α-methyldopa^8]- and [Sar^1, α-methyldopa^8]-angiotensin II were about 1/200 and 1/30 of parent angiotensin II, respectively. During the infusion of the angiotensin II analogues at a rate of 1 μg/kg/min, neither [α-methyldopa^8]- nor [Sar^1, α-methyldopa^8]-angiotensin II inhibited angiotensin II pressor activity, while [Sar^1, Ile^8]-angiotensin II did inhibit. At high infusion levels (5 μg/kg/min), as shown in Fig. 2, the response to angiotensin II was suppressed completely by [Sar^1, Ile^8]-angiotensin II, but faintly by either [α-methyldopa^8]- or [Sar^1, α-methyldopa^8]-angiotensin II. The pressor response to norepinephrine (0.1, 0.3 and 1.0 μg/kg, *i.v.*) was inhibited neither by [α-methyldopa^8]-angiotensin II infused at a rate of 1 or 5 μg/min, nor by [Sar^1, α-methyldopa^8]-angiotensin II infused at a rate of 1 or 2 μg/kg/min (Fig. 3).

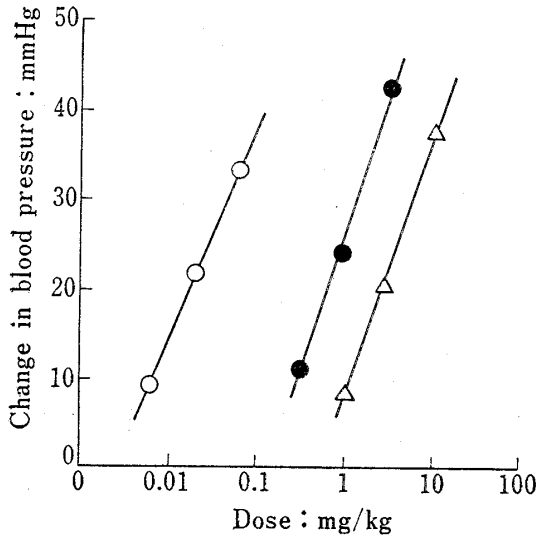

Fig. 1. The Agonistic Activity of Angiotensin II Analogues in Rat Blood Pressure

—○—, AT II.
—●—, [Sar^1, α-methyldopa^8]-AT II.
—△—, [α-methyldopa^8]-AT II.

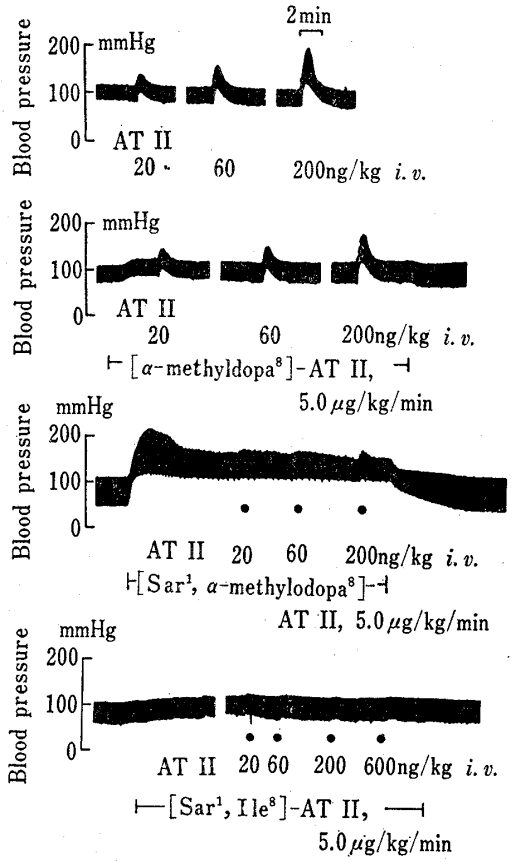

Fig. 2. Effect of Angiotensin II Analogues Infusions on the Rat Blood Pressure Responses to Angiotensin II injected *i.v.*

Discussion

The presence of a methyl group on the position 8-α-carbon did not result in a significant decrease in agonistic potency.[6] And, substitution by hydroxy group in *p*-position of the phenyl ring reduced the pressor activity to 10%,[11] or to 83%.[12] Therefore, a significant

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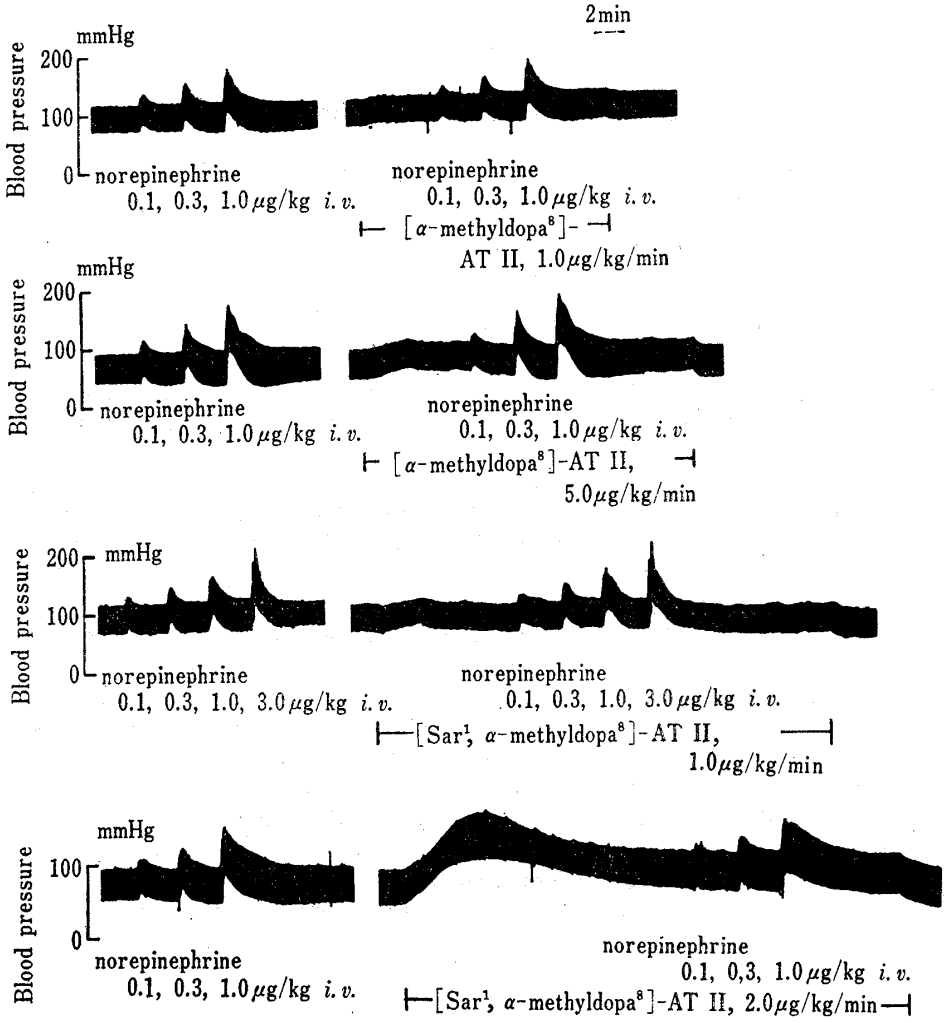

Fig. 3. Effect of Angiotensin II Analogues Infusions on the Rat Blood Pressure Responses to Norepinephrine injected *i.v.*

reduction in pressor activity following the replacement of C-terminal phenylalanine with α-methyldopa may not be due to the presence of the 8-α-methyl group, but to the introduction of hydroxy group into *m*- and *p*-position of the phenyl ring.

It has been reported that the aromatic ring in the side chain of C-terminus was essential for the pressor activity but not necessary for the inhibition of norepinephrine uptake.[13] We thought the similarity in the biological action of catecholamines and angiotensin might be attributed to the chemical structure in common shared by the both compounds, *i.e.*, phenyl group. On the other hand, α-methyldopa, a modification of catecholamines, has been a well known inhibitor of catecholamines. It occurred to us that [α-methyldopa⁸]-angiotensin II might also antagonize, in similar fashion, at the site of angiotensin receptor, as well as acting as a catecholamine inhibitor, simultaneously. The results were negative to our expectation. Since the hypotensive action of α-methyldopa is also considered to be, in part, mediated by an effect on the central nervous system,[14] the effects of [α-methyldopa⁸]-angiotensin II analogues on norepinephrine uptake or on the central nervous system must be investigated otherwise.

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