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Pharmacokinetics and Cardiovascular Effects of YM-21095, a Novel Renin Inhibitor, in Dogs and Monkeys

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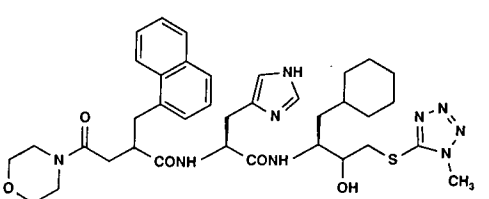
Abstract—The pharmacokinetics and cardiovascular effects of YM-21095 ((2*RS*), (3*S*)-3-[N^{α}-[1,4-dioxo-4-morpholino-2-(1-naphthylmethyl)-butyl]-L-histidylamino]-4-cyclohexyl-1-[(1-methyl-5-tetrazolyl)thio]-2-butanol), a potent renin inhibitor, have been studied in beagle dogs and squirrel monkeys. Plasma levels of YM-21095 after 3 mg kg^{-1} intravenous dosing to dogs declined biphasically and fitted a two-compartment model. Kinetics were as follows: $t_{1/2\alpha}^{1}=4{\cdot}9\pm0{\cdot}2$ min, $t_{1/2\beta}^{1}=2{\cdot}76\pm0{\cdot}79$ h, $Vd_{ss}=3{\cdot}86\pm1{\cdot}04$ L kg^{-1}, plasma clearance $=2{\cdot}22\pm0{\cdot}39$ L kg^{-1}, and AUC $=1445\pm266$ ng h mL^{-1}. After 30 mg kg^{-1} oral dose, maximum plasma concentration, t_{max} and AUC of YM-21095 were $28{\cdot}8\pm9{\cdot}6$ ng mL^{-1}, 0·25 h and $23{\cdot}6\pm7{\cdot}7$ ng h mL^{-1}, respectively. Systemic bioavailability as determined on the basis of the ratio of AUC after intravenous and oral dose was $0{\cdot}16\pm0{\cdot}04\%$. In conscious, sodium-depleted monkeys, YM-21095 at an oral dose of 30 mg kg^{-1} lowered systolic blood pressure and inhibited plasma renin activity without affecting heart rate and plasma aldosterone concentration. Maximum plasma concentration of YM-21095 after 30 mg kg^{-1} oral dose to monkeys was $71{\cdot}8\pm41{\cdot}5$ ng mL^{-1}, which was reached 0·5 h after the dose. At equihypotensive doses, captopril and nicardipine increased plasma renin activity markedly and slightly, respectively. These results suggest that oral absorption of YM-21095 is low in dogs and monkeys, and YM-21095 shows a blood pressure lowering effect by inhibiting plasma renin activity in sodium-depleted monkeys.

The renin-angiotensin system plays an important role in the regulation of blood pressure and in the maintenance of sodium and fluid volume homeostasis. The use of angiotensin-converting enzyme inhibitors, such as captopril, to intervene in this system is well established as an effective approach to controlling hypertension and congestive heart failure (Gavras et al 1978; Cody 1985). This observation has led to considerable interest in compounds that inhibit renin, the enzyme that catalyses the first step in the formation of the biologically active peptide angiotensin II. The pharmacological effects of renin inhibitors have been widely studied in non-primates and in primates, including man (Hiwada et al 1988; Wood et al 1989; Camenzind et al 1991; Gardner et al 1991), but only a few studies of the pharmacokinetics of these agents in animals have been reported in the literature (Greenfield et al 1989; Cumin et al 1990). Moreover, to the best of our knowledge, a therapeutically useful agent as a renin inhibitor has not yet emerged because of the low oral bioavailability and insufficient duration of action. A novel renin inhibitor, YM-21095 ((2*RS*), (3*S*)-3-[N^{α}-[1,4-dioxo-4-morpholino-2-(1-naphthylmethyl)-butyl]-L-histidylamino]-4-cyclohexyl-1-[(1-methyl-5-tetrazolyl)thio]-2-butanol), has been synthesized in our laboratories. It is a highly potent and selective inhibitor of primate renin, and lowers blood pressure after intravenous and oral administration to sodium-depleted squirrel monkeys (Shibasaki et al 1991). In the present study, we examined its pharmacokinetics in dogs and squirrel monkeys, and compared the cardiovascular effects of YM-21095 with those of captopril and nicardipine in squirrel monkeys.



YM-21095

Materials and Methods

Pharmacokinetic study

Three male beagle dogs, 11·0–13·0 kg, were used. YM-21095 was dissolved in 20 mM citric acid. For the intravenous study, food was not restricted. After control blood samples were taken, YM-21095 was injected into a superficial vein in the foreleg in a volume of 0·3 mL kg^{-1}. Venous blood was taken at 5, 10, 15, 30, 60, 120, 180, 240, 360, and 480 min following intravenous administration.

After a 7-day wash-out interval, the oral study was performed. Food was restricted from 16 h before, to 8 h after oral dose, but water was available throughout the study. After control blood was taken, YM-21095 was administered by oral gavage in a volume of 3 mL kg^{-1}. Venous blood was taken at 15, 30, 60, 120, 180, 240, 300, 360, 480, 600, and 1440 min following oral administration.

Blood samples were placed on ice immediately and the plasma was separated within 1 h of sample collection, and stored at −20°C until assay.

Pharmacological study

Six squirrel monkeys of both sexes, 620–1120 g, were used. In

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order to obtain sodium-depletion, animals were fed a diet consisting of a low sodium chow (0·0057 μEq Na^+ g^{-1}) and fruit, and received intramuscular frusemide (2 mg kg^{-1} every other day) for 1 week before the study. Food was restricted for 14 h before the oral dose. On the morning of the study day, animals were seated in primate restraining chairs. Systolic blood pressure and heart rate were measured by a tail-cuff method, using a sphygmomanometer (RS-200, Riken Kaihatsu, Japan). A pneumatic cuff (15–20 mm i.d.) and a piezo-electric pressor sensor were positioned on a shaved portion of the tail. Before recording, animals were placed in a chamber with an ambient temperature of 30–35°C for 10 min. The animals had been previously adapted to this procedure. After systolic blood pressure and heart rate had stabilized, 10 single readings were taken and the mean was calculated. In a separate study, fourteen squirrel monkeys of both sexes, 525–925 g, were used for studying the effect on plasma renin activity and plasma aldosterone concentration. This study was performed under the same conditions as that for the measurement of systolic blood pressure and heart rate described above. Blood samples were taken from the femoral vein and transferred to a tube containing disodium EDTA (final concentration 4 mM), and then stored frozen at −20°C until assayed.

Three female squirrel monkeys, 530–700 g, were studied for the measurement of oral absorption. The animals were fed a regular chow and fruit before the study, and fasted for 14 h before receiving the oral dose. On the morning of the study day, animals were seated in primate restraining chairs. Blood samples were taken from the femoral vein and transferred to a tube containing disodium EDTA (final concentration 4 mM), and then stored frozen at −20°C until assayed.

In all studies, YM-21095 was suspended in a 0·5% methylcellulose solution and administered by gavage in a volume of 1 mL kg^{-1}.

Extraction of plasma and HPLC

Plasma (1 mL for dogs and 0·2 mL for monkeys) was mixed with 100 ng internal standard (KRI-1314, a renin inhibitor) and 1 mL 0·25 M Na_2CO_3-$NaHCO_3$ buffer (pH 9·0), and extracted into 5 mL diethyl ether. After centrifugation at 1000 *g* for 10 min, the organic layer was transferred to a fresh tube containing 0·2 mL of 50 mM phosphoric acid. The sample was mixed and centrifuged, and the organic layer removed by vacuum aspiration. The aqueous layer was used as the extracted sample.

An aliquot of the extracted sample was injected into a Waters Associates HPLC system, consisting of a solvent delivery pump (Hitachi L-600, Japan), an autoinjector (Waters 712 WISP), and a reverse-phase octadecyl silane (Nucleosil C18) column (15 cm × 4 mm i.d.) with a mobile phase of acetonitrile/0·1 M phosphoric acid/0·1 M KH_2PO_4 (35:33:33, v/v/v). The mobile phase had a flow rate of 1·5 mL min^{-1} at 30°C. Column eluent was monitored with a fluorescence detector (Shimadzu RF-535, Japan), with excitation at 283 nm and emission at 337 nm. The sensitivity of these methods permitted quantitation of at least 1 ng mL^{-1} in dog plasma and 5 ng mL^{-1} in monkey plasma.

Pharmacokinetic calculation

Pharmacokinetics after intravenous dosing was assessed by the nonlinear least-square regression program, NONLIN 84 version 2. The program produced estimates of the pharmacokinetic parameters of a two-compartment open model. In the oral study, the total area under the plasma concentration-time curve (AUC) was calculated up to 24 h in dogs and 5 h in monkeys. The maximum drug concentration (C_{max}) and time after the oral dose to peak concentration (t_{max}) were taken from the concentration-time data for each animal. The systemic bioavailability was calculated as:

$$\frac{\text{AUC for oral dose}}{\text{AUC for intravenous dose}} \times \frac{\text{Intravenous dose}}{\text{Oral dose}} \times 100$$

Plasma renin activity and plasma aldosterone concentration

Plasma renin activity was measured by the rate of angiotensin I generation during incubation of endogenous renin and angiotensinogen in plasma at pH 6·0, at 37°C, for 90 min (Shibasaki et al 1991). The generation step utilized 250 μL plasma, 5 μL phenylmethylsulphonyl fluoride, and 25 μL phosphate buffer (pH 6·0). Radioimmunoassay for angiotensin I was carried out in tubes coated with rabbit angiotensin I antibody (Sorin Biomedica RIA kit, Saluggia, Italy). Plasma aldosterone concentration was quantified by radioimmunoassay, using a commercially available radioimmunoassay kit (Aldosterone RIAKIT II, Dainabot, Japan).

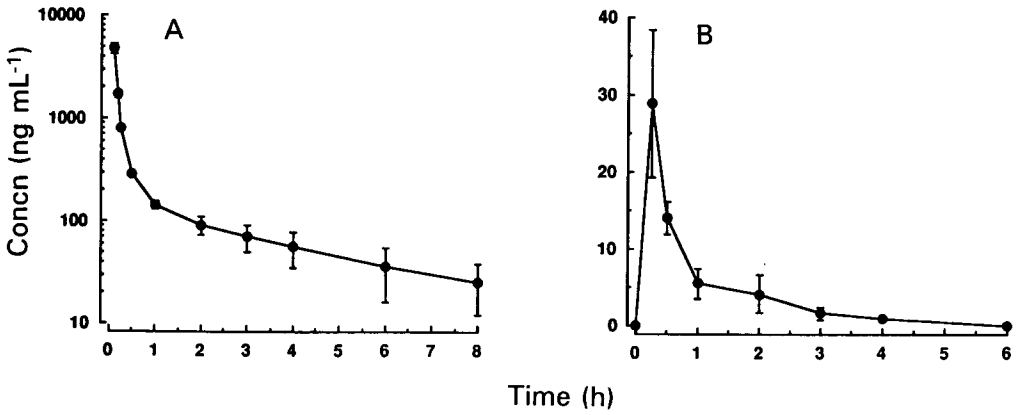

FIG. 1. Plasma concentrations of YM-21095 after a bolus intravenous injection of 3 mg kg^{-1} (A) and after an oral administration of 30 mg kg^{-1} (B) in dogs. Values are the mean ± s.e.m. of three animals.

Table 1. Pharmacokinetic parameters obtained from the analysis of plasma concentrations after a bolus intravenous dose of 3 mg kg^{-1} YM-21095 in dogs.

Dog	$t_{\frac{1}{2}\alpha}$ (min)	$t_{\frac{1}{2}\beta}$ (h)	Vd_{ss} (L kg^{-1})	AUC (ng h mL^{-1})	Clearance (L h^{-1} kg^{-1})
1	4·6	1·84	2·27	1358	2·21
2	4·9	4·33	5·82	1943	1·54
3	5·2	2·11	3·48	1034	2·90
Mean ± s.e.m.	4·9 ± 0·2	2·76 ± 0·79	3·86 ± 1·04	1445 ± 266	2·22 ± 0·39

Table 2. Pharmacokinetic parameters obtained from the analysis of plasma concentrations after an oral dose of 30 mg kg^{-1} YM-21095 in dogs.

Dog	t_{max} (h)	C_{max} (ng mL^{-1})	AUC (ng h mL^{-1})	Bioavailability (%)
1	0·25	25·4	27·9	0·21
2	0·25	46·9	34·2	0·18
3	0·25	14·0	8·7	0·08
Mean ± s.e.m.	0·25 ± 0·00	28·8 ± 9·6	23·6 ± 7·7	0·16 ± 0·04

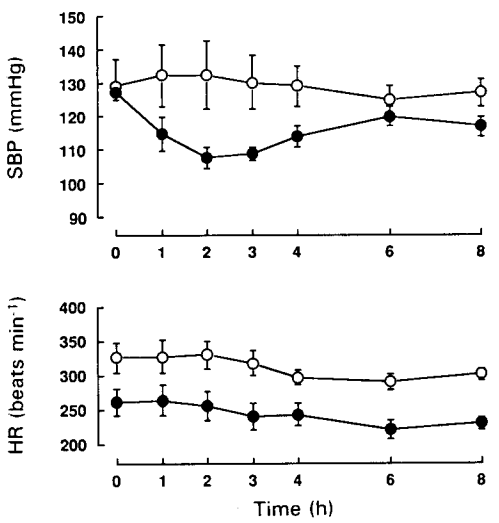

FIG. 2. Effect of YM-21095 at an oral dose of 30 mg kg^{-1} (●) on systolic blood pressure (SBP) and heart rate (HR) in conscious, sodium-depleted squirrel monkeys. Values are the mean ± s.e.m. of three animals. Vehicle (control) ○.

Results

Pharmacokinetic study

After intravenous YM-21095 (3 mg kg^{-1}), the plasma concentration of unchanged YM-21095 declined biphasically and fitted a two-compartment model (Fig. 1). Kinetics obtained from plasma concentration-time data are listed in Table 1.

The plasma concentration-time curve of unchanged YM-21095 after a single oral dose of 30 mg kg^{-1} is shown in Fig. 1. Kinetic parameters after the oral dose of YM-21095 are listed in Table 2. Systemic bioavailability as determined on the basis of the ratio of AUC after intravenous and oral dose was 0·16 ± 0·04%.

Pharmacological study

In conscious, sodium-depleted squirrel monkeys, a single oral administration of YM-21095 of 30 mg kg^{-1} lowered systolic blood pressure without effect on heart rate (Fig. 2). Effects of YM-21095 on plasma renin activity and plasma aldosterone concentrations are shown in Table 3. Maximum plasma concentration of unchanged YM-21095 was 71·8 ± 41·5 ng mL^{-1}, which was reached 0·5 h after oral administration (Fig. 3). The $t_{\frac{1}{2}}$ and AUC values were 53·4 min and 105·7 ± 54·6 ng h mL^{-1}, respectively.

A single oral administration of 3 mg kg^{-1} nicardipine

Table 3. Effects of YM-21095 at an oral dose of 30 mg kg^{-1} on plasma renin activity and plasma aldosterone concentration in conscious, sodium-depleted squirrel monkeys.

Time (h)	Plasma renin activity (ng angiotensin I mL^{-1} h)		Plasma aldosterone concn (ng mL^{-1})	
	Vehicle	YM-21095	Vehicle	YM-21095
Pre	4·0 ± 0·3	2·0 ± 0·6	1·4 ± 0·7	0·6 ± 0·3
0	30·2 ± 3·2	29·3 ± 11·0	16·5 ± 2·7	17·4 ± 4·8
0·5	35·4 ± 7·4	8·3 ± 3·3	18·4 ± 2·4	18·9 ± 3·1
2	33·0 ± 4·5	18·8 ± 4·1	17·9 ± 2·1	21·8 ± 4·5
4	26·4 ± 7·1	24·1 ± 6·9	18·0 ± 2·3	20·3 ± 3·1
6	22·5 ± 3·5	23·7 ± 6·5	16·6 ± 2·3	23·2 ± 4·1
8	24·4 ± 1·4	32·6 ± 8·1	16·9 ± 1·9	21·4 ± 4·1

Each value represents the mean ± s.e.m. of 4–5 animals. Pre; the day before sodium-depletion.

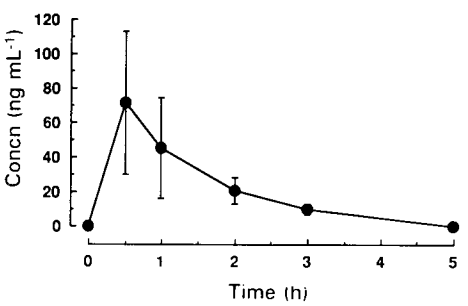

FIG. 3. Plasma concentration of YM-21095 after an oral administration of 30 mg kg^{-1} in squirrel monkeys. Values are the mean ± s.e.m. of three animals.

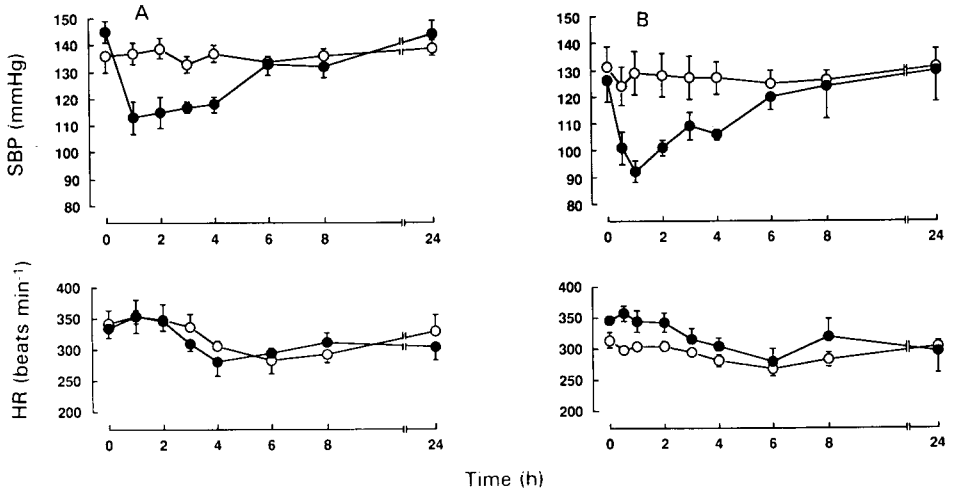

FIG. 4. Effects of captopril (●) at an oral dose of 10 mg kg^{-1} (A) and nicardipine (●) at an oral dose of 3 mg kg^{-1} (B) on systolic blood pressure (SBP) and heart rate (HR) in conscious, sodium-depleted squirrel monkeys. Values are the mean ± s.e.m. of three animals. Vehicle (control) ○.

Table 4. Effects of captopril at an oral dose of 10 mg kg^{-1} and nicardipine at an oral dose of 3 mg kg^{-1} on plasma renin activity and plasma aldosterone concentration in conscious, sodium-depleted squirrel monkeys.

Time (h)	Plasma renin activity (ng angiotensin I mL^{-1} h)			Plasma aldosterone concn (ng mL^{-1})		
	Vehicle	Captopril	Nicardipine	Vehicle	Captopril	Nicardipine
Pre	2·1 ± 0·4	3·5 ± 0·4	3·2 ± 0·8	0·55 ± 0·12	0·88 ± 0·26	1·76 ± 0·67
0	28·7 ± 4·7	25·8 ± 8·7	16·1 ± 4·3	16·3 ± 1·2	17·1 ± 2·1	21·8 ± 4·7
0·5	22·0 ± 5·4	62·0 ± 12·4	19·9 ± 2·3	18·2 ± 1·5	18·3 ± 2·2	23·1 ± 3·7
1	29·6 ± 7·6	74·1 ± 9·1	21·7 ± 10·5	19·9 ± 0·6	17·8 ± 2·2	23·4 ± 4·1
2	31·2 ± 8·4	51·4 ± 13·7	18·8 ± 7·0	19·0 ± 1·8	17·2 ± 2·3	23·8 ± 3·4
4	28·5 ± 10·3	33·0 ± 11·8	16·1 ± 1·9	18·2 ± 1·8	19·1 ± 1·8	22·1 ± 4·4
8	16·1 ± 3·3	61·2 ± 7·9	21·8 ± 5·6	14·6 ± 1·2	12·8 ± 1·4	19·0 ± 3·5
24	31·8 ± 4·4	33·0 ± 9·8	27·6 ± 4·8	20·4 ± 1·8	23·2 ± 1·3	25·4 ± 4·2

Each value represents the mean ± s.e.m. of four animals. Pre; the day before sodium-depletion.

lowered systolic blood pressure without affecting heart rate in conscious, sodium-depleted squirrel monkeys (Fig. 4). Nicardipine induced a slight increase in plasma renin activity, but did not affect plasma aldosterone concentration (Table 4). Captopril at an oral dose of 10 mg kg^{-1} lowered systolic blood pressure without affecting heart rate (Fig. 4). Plasma renin activity was increased 4-fold at 1 h after the oral administration, and was still about three times the pre-treated value at 8 h. Plasma aldosterone concentration was unchanged during the observation period (Table 4).

Discussion

Plasma concentration-time data obtained after intravenous YM-21095 in dogs are consistent with a two-compartment model. The initial rapid fall in plasma concentration ($t_{\frac{1}{2}\alpha}$ = 4·9 min) indicates that the drug was rapidly distributed. The volume of distribution (Vd_{ss} = 3·86 L kg^{-1}) was 6·4-times higher than that of total body water (0·6 L kg^{-1}) (Gibaldi & Perrier 1982), indicating an extensive distribution of the drug.

In the present study with YM-21095, its bioavailability in dogs is low, which is in agreement with that calculated by comparing the hypotensive effect after intravenous dosing with that after oral administration to squirrel monkeys (Shibasaki et al 1991). Greenfield et al (1989) have suggested that U-71038, a novel renin inhibitor peptide, would undergo significant first-pass clearance upon oral administration in the rat, since it was eliminated rapidly from plasma and predominantly excreted in bile. YM-21095 may be poorly absorbed after oral administration or may be subject to an effective first-pass effect. In our experiment, we could not distinguish between the two possibilities.

In squirrel monkeys, the C_{max} of YM-21095 was 71·8 ± 41·5 ng mL^{-1}, much greater than the concentration required to inhibit squirrel monkey plasma renin by 50% (IC50 = 0·9 nM = 0·7 ng mL^{-1}) (Shibasaki et al 1991). At t_{max}, plasma renin activity was inhibited by about 70% and at 2 and 3 h after oral administration, the activity was not inhibited significantly, even though the plasma concentration was still greater than the IC50 value. The pharmacokinetic study was performed using squirrel monkeys fed a normal sodium diet and the IC50 value against squirrel monkey plasma renin was determined using a plasma from squirrel monkey also fed a normal sodium diet. Therefore, it is possible that sodium-depletion may decrease the oral absorption of YM-21095 or that a high plasma renin activity induced by sodium-depletion may require higher concentrations of YM-21095 than the IC50 value obtained using normal plasma renin activity values. In a preliminary study,

we confirmed that sodium in the diet did not affect oral absorption in squirrel monkey. Moreover, the IC50 value obtained by using high plasma renin activity, which was achieved by sodium restriction, was nearly equal to that obtained by using normal values (Shibasaki, unpublished data). Some newly developed renin inhibitors such as Ro 42-5892 and CGP 38560A, have shown a high degree of plasma protein binding (Derkx et al 1991). If YM-21095 also has such high protein binding in plasma, it may not be able to inhibit plasma renin activity efficiently.

The squirrel monkey, a New World primate, has higher plasma aldosterone concentrations than man and Old World primates such as the cynomolgus monkey (Cassorla et al 1982; Chrousos et al 1984). Albertson et al (1988) have reported that elevated plasma aldosterone in squirrel monkey was achieved by an increased 11-hydroxylase activity in the adrenal. In our study, plasma renin activity before sodium-depletion was 1027 ± 384 pg mL^{-1} and higher than those reported in man (68 ± 28 pg mL^{-1}) and in cynomolgus monkey (259 ± 27 pg mL^{-1}) (Chrousos et al 1984; Nussberger et al 1984), confirming the previous observation.

YM-21095, captopril and nicardipine did not affect plasma aldosterone concentration, even though these drugs directly or indirectly affected the renin-angiotensin system. The adrenal renin-angiotensin system may be more important in regulating aldosterone secretion than the circulating renin-angiotensin system, as it is in controlling blood pressure (Thurston et al 1979; Campbell 1989; Higashimori et al 1991). Alternatively, under conditions of sodium restriction, the adrenal response to angiotensin II may already be maximal and other aldosterone secretogogues may have played an increasingly important role in aldosterone secretion.

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