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### **Research Article**

We have assessed the capacity of an analogue of angiotensin II (A II), 1-Sar, 8-Ala A II (P113) in normal man to stimulate and block responses to A II in four systems: blood pressure was monitored directly from an arterial catheter, and renal blood flow was measured with <sup>133</sup>Xe and arterial renin and aldosterone concentrations by radioimmunoassay. The 31 normal subjects were in balance on a daily intake of 200 meq sodium and 100 meq potassium to suppress endogenous renin. P113 administered intravenously induced a dose-related renal blood flow reduction, with a threshold dose of 0.1 mug/kg/min. This dose also induced a small but significant increase in arterial blood pressure and plasma aldosterone as well as a reduction in plasma renin activity. In contrast to its effect on the renal vasculature, no tendency to a progressive response in the latter three parameters was noted as the P113 dose was increased 30-fold, to 3.0 mug/kg/min. P113 also reduced the clearance of para-aminohippurate, creatinine, sodium, and potassium, a pattern similar to that induced by A II. P113 at 0.1 mug/kg/min reduced significantly the blood pressure and renal vascular and aldosterone responses to graded doses of A II. Higher P113 doses totally obliterated all three responses to A II infused at 10 ng/kg/min, a dose that provides arterial A II concentrations in the [...]

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# Blockade and Stimulation of Renal, Adrenal, and Vascular Angiotensin II Receptors with 1-Sar, 8-Ala Angiotensin II in Normal Man

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**ABSTRACT** We have assessed the capacity of an analogue of angiotensin II (A II), 1-Sar, 8-Ala A II (P113) in normal man to stimulate and block responses to A II in four systems: blood pressure was monitored directly from an arterial catheter, and renal blood flow was measured with  $^{133}\text{Xe}$  and arterial renin and aldosterone concentrations by radioimmunoassay. The 31 normal subjects were in balance on a daily intake of 200 meq sodium and 100 meq potassium to suppress endogenous renin.

P113 administered intravenously induced a dose-related renal blood flow reduction, with a threshold dose of 0.1  $\mu\text{g/kg/min}$ . This dose also induced a small but significant increase in arterial blood pressure and plasma aldosterone as well as a reduction in plasma renin activity. In contrast to its effect on the renal vasculature, no tendency to a progressive response in the latter three parameters was noted as the P113 dose was increased 30-fold, to 3.0  $\mu\text{g/kg/min}$ . P113 also reduced the clearance of para-aminohippurate, creatinine, sodium, and potassium, a pattern similar to that induced by A II.

P113 at 0.1  $\mu\text{g/kg/min}$  reduced significantly the blood pressure and renal vascular and aldosterone responses to graded doses of A II. Higher P113 doses totally obliterated all three responses to A II infused at 10 ng/kg/min, a dose that provides arterial A II concentrations in the range found in angiotensin-mediated hypertension.

When A II was infused first, to induce a pressor, renal vascular, and aldosterone response, P113 induced a dose-related reversal of the response in each system.

In conclusion, P113 is a partial agonist in normal man, inducing an angiotensin-like response in settings in which endogenous A II is not playing a tonic role, and

displaying dominant antagonist activity in settings in which A II is active. Moreover, the studies suggest that the receptors mediating the responses to A II are different in the renal vasculature and other systemic vascular beds. The adrenal receptor must also differ. This agent should be useful in dissecting the role of A II in diseases characterized by hypertension or abnormalities of renal and adrenal function.

## INTRODUCTION

A series of analogues that act as antagonists to angiotensin II have recently been identified (1-4). These agents have been useful in exploring angiotensin II's role in normal homeostasis (5, 6) and pathophysiology (4, 5, 7), in characterizing angiotensin II receptors (8-11), and in providing a new potential for diagnosis and therapy (12-14). In this investigation we have characterized the blockade of vascular and adrenal responses to angiotensin II induced by an analogue approved for use in man, 1-Sar, 8-Ala angiotensin II (P113),<sup>1</sup> in normal subjects in whom endogenous renin and angiotensin II levels were suppressed by a high sodium and potassium intake. It is not surprising that agents that arose from modification of a hormone's structure should possess intrinsic activity (10). Because P113 acts as a partial agonist, these studies also provide insight into the angiotensin II receptor mediating the renal, adrenal, and vascular responses to angiotensin II and define P113's potential and its limitations as a diagnostic and therapeutic agent.

<sup>1</sup>Abbreviations used in this paper: A<sub>1</sub>, angiotensin I; P113, 1-Sar, 8-Ala angiotensin II; PAH, para-aminohippurate; PRA, plasma renin activity.

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## METHODS

### Subjects

Studies were carried out in 31 normal potential kidney donors, in 19 at the time of selective renal arterial catheterization for angiography. In an additional 12, clearance studies were performed to assess the renal functional responses. The subjects, ranging in age from 22 to 58 yr, each received a careful evaluation with special emphasis on cardiovascular, renal, and adrenal status, as described earlier (15). They were admitted to a metabolic ward and placed on a diet providing a daily intake of 2,500 ml water, 100 meq potassium, and 200 meq sodium for at least 5 days before study. Balance was assessed by measuring sodium excretion in 24-h urine collections and by a detailed daily dietetic assessment of intake.

### Techniques

Percutaneous selective renal arterial catheterization, the determination of renal blood flow with radioactive xenon and external probe counting, and cardiovascular monitoring during the administration of vasoactive agents have been described in detail (16). The catheter was used for the continuous monitoring of blood pressure and heart rate, for the injecting of xenon, and for drawing arterial blood samples. Blood pressure was measured with a transducer and recorded continuously along with the instantaneous pulse rate and the electrocardiogram. Continuous monitoring insured patient safety and cardiovascular stability.

The study was initiated at least 30 min after aortography. A control blood flow determination was obtained and a control arterial blood sample was drawn for the measurement of renin (PRA), angiotensin II, aldosterone, cortisol, sodium, and potassium concentrations. Then an intravenous infusion of either angiotensin II (Hypertensin, Ciba Pharmaceutical Co., Summit, N. J.) or 1-Sar, 8-Ala angiotensin II (P113; saralasin acetate, Norwich Pharmacal Co., Norwich, N. Y.) was initiated with a motor-driven syringe (Harvard Apparatus Co., Inc., Millis, Mass.). Dilutions were made in 5% glucose and water to allow administration of the agent at 0.3–1.9 ml/min.

### Protocols

*P113 as an agonist.* In 15 subjects the relationship between dose in graded logarithmic increments of P113, from 0.1 to 3.0  $\mu\text{g/kg/min}$  and response was established. Each dose was infused for at least 6 min and renal vascular responses were determined 3 min after the initiation of each dose. Samples were drawn to assess the adrenal and renin response only after a more prolonged infusion, 20 and 40 min at the highest dose used. In seven subjects a single dose ranging from 0.1 to 3.0  $\mu\text{g/kg/min}$  was infused for 40 min to assess the stability of the pressor, renal vascular, adrenal, and renin responses.

*P113 as an angiotensin antagonist.* After assessing the intrinsic responses to P113 alone, a graded infusion of angiotensin II was then superimposed to assess the effect of P113 on the renal, pressor, and adrenal response to angiotensin II in each subject. The angiotensin II doses were 1.0, 3.0, and 10.0  $\text{ng/kg/min}$ , as described previously (15), and P113 was assessed at doses ranging from 0.1 to 3.0  $\mu\text{g/kg/min}$ . As for P113, 6-min infusions were administered at each angiotensin II dose level and the renal blood flow response was assessed at 3 min. The adrenal response was assessed only after a 20-min infusion of angiotensin II at 10  $\mu\text{g/kg/min}$ .

*Responses to P113 in the presence of angiotensin.* In four subjects the sequence of the administration of the agents was reversed. Angiotensin II was administered first at 10  $\text{ng/kg/min}$  for 20 min. After the pressor, renovascular, and adrenal responses to angiotensin II were documented, a graded infusion of P113 was then superimposed, at 0.1, 0.3, and 1.0  $\mu\text{g/kg/min}$ . The first two P113 doses were infused for 6 min to assess the effect on blood pressure and renal blood flow, and the last dose for 20 min to allow assessment of the adrenal response.

*Influence of P113 and angiotensin on renal function.* An additional 12 studies were performed on the metabolic ward to assess the effects of P113 and angiotensin II on renal function. Renal perfusion was assessed by measuring the clearance of *p*-aminohippurate (PAH); glomerular filtration rate by the clearance of creatinine; and sodium and potassium excretion were assessed in each sample. The subjects were studied in their own bed after a 10-h fast. At 7:00 a.m. a water diuresis was initiated with a 20 ml/kg water load taken by mouth and an infusion of PAH was initiated with a motor-driven syringe at a rate calculated to achieve a blood level of about 2  $\text{mg/100 ml}$ . A diuresis of 10–20 ml/min ensued, which allowed serial urine collections by spontaneous voiding at 20–30 min intervals without urethral catheterization. After each collection a volume of water equal to the urine volume of the preceding collection period was ingested, thus maintaining the diuresis. Samples were collected for the measurement of plasma PAH, creatinine, sodium, and potassium concentrations at 20–30 minute intervals during the procedure. The clearance collections began 45–90 min after the water load and initiation of the intravenous administration of PAH. Eight studies were performed during the infusion of graded doses of angiotensin II, from 0.1 to 10.0  $\text{ng/kg/min}$  in log-dose increments. Four separate studies were performed to assess responses to 3.0  $\mu\text{g/kg/min}$  of P113.

### Analytic techniques

All blood samples were collected on ice and immediately spun, and the plasma was separated and frozen until the time for assay. The anticoagulant was EDTA for PRA and angiotensin II samples and heparin for cortisol and aldosterone samples. Diisofluorophosphate was also added to the samples for angiotensin II assay.

Plasma aldosterone and cortisol were measured by displacement analysis techniques after chromatographic separation as described (17). The sensitivity of the cortisol assay system was 0.02  $\text{ng/binding tube}$ , and for aldosterone, 0.002  $\text{ng/binding tube}$ .

Attempts were made to measure angiotensin II by a double-antibody radioimmunoassay method (18). P113, however, showed 0.07% cross-reactivity with the angiotensin antibody, sufficient to interfere with angiotensin II measurements, because P113 was infused in microgram per kilogram quantities. PRA was measured by radioimmunoassay of angiotensin I ( $A_1$ ) generated during a 30-min incubation with endogenous substrate at 37°C. No cross-reactivity was demonstrable between P113 and the angiotensin I antibody.

PAH was measured in the serum and urine by the method of Smith et al. (19). Creatinine concentration in urine and serum was determined by the method of Bonsnes and Taussky (20). Sodium and potassium concentrations in urine and serum were measured by flame photometry, with lithium as an internal standard. Clearances corrected to body surface area were calculated according to standard formulas.

Mean renal blood flow was measured from the initial slope of  $^{133}\text{Xe}$  disappearance from the kidney, determined graphically with a hematocrit-corrected partition coefficient; compartmental analysis was also performed. Curves re-analyzed on a coded basis showed a coefficient of variation of 7% (15, 16).

Group means have been presented with the standard error of the mean as the index of dispersion. The evaluation of statistical probability was carried out, where appropriate, with the Student's *t* test or paired data *t* test. Otherwise, the Wilcoxon rank sum (WRST) or Fisher exact tests for nonparametric data were used. The null hypothesis was rejected when the *P* value was less than 0.05 (21).

The protocol was approved by the Human Experimentation Committees of the Peter Bent Brigham Hospital and Harvard Medical School. Written permission for the procedure was obtained after a careful description of the protocols.

## RESULTS

The relationship between P113 dose infused intravenously and the pressor and renal vascular response is contrasted to the response of these systems to graded doses of angiotensin II in Fig. 1. P113 induced a dose-related reduction in renal blood flow ( $P < 0.01$ ). The lowest P113 dose,  $0.1 \mu\text{g/kg/min}$ , reduced mean renal blood flow by  $-10.3 \pm 2.2 \text{ ml/100 g/min}$ , a reduction of only 3% that did not achieve statistical significance.

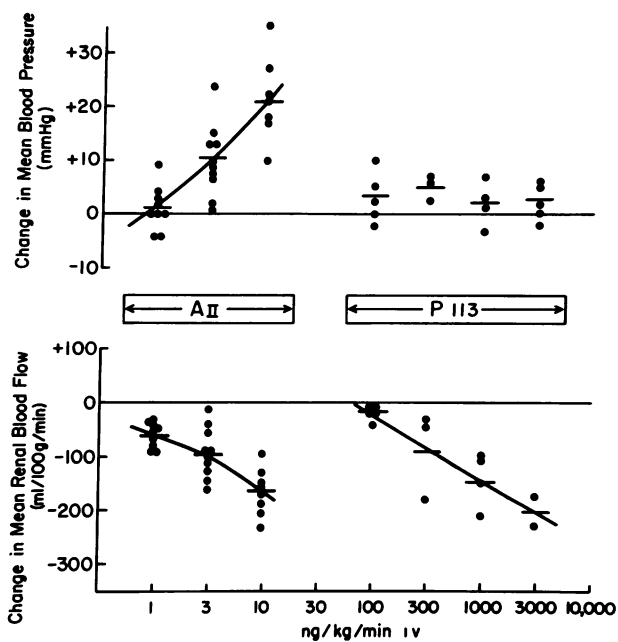


FIGURE 1 Relationship between dose of agent infused (angiotensin II on the left and P113 on the right) and the pressor and renal vascular response in normal subjects. Endogenous renin was suppressed by a 200 meq sodium, 100 meq potassium diet. Note the similarity of the renal vascular responses to the two agents despite angiotensin II's 300-fold greater potency and P113's failure to induce a dose-related pressor response.

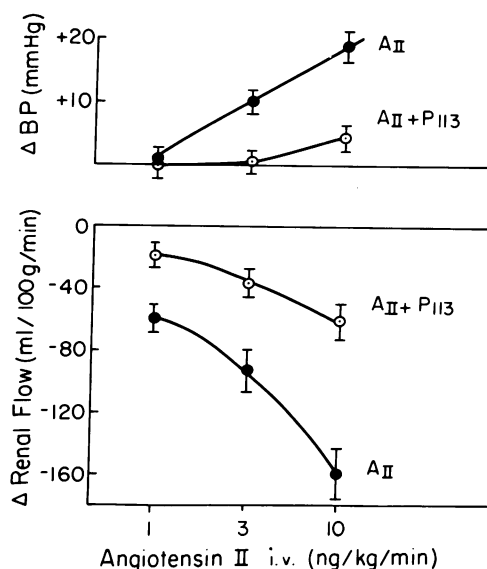


FIGURE 2 Responses to angiotensin II (A II). P113 at its threshold dose for intrinsic activity,  $0.1 \mu\text{g/kg/min}$  administered intravenously, induced a significant reduction in both the pressor (BP) and renal vascular responses to angiotensin II.

An increase in P113 dose to  $1.0 \mu\text{g/kg/min}$  resulted in a 38% reduction in mean blood flow, from  $354 \pm 33$  to  $220 \pm 14 \text{ ml/100 g/min}$  ( $P < 0.005$ ) in association with a parallel 36% fall in the percentage of flow entering the rapid, or cortical flow component ( $80.3 \pm 1.3$  vs.  $51.5 \pm 7.8\%$ ;  $P < 0.02$ ), and the rapid component flow rate ( $431 \pm 25$  vs.  $318 \pm 24 \text{ ml/100 g/min}$ ;  $P < 0.02$ ). The relationship between the log of the P113 dose ( $x$ ) and reduction in mean renal blood flow ( $y$ ) was  $y = 2.047 - 0.583 x$ ;  $r = 0.86$ ;  $F = 37.9$ ;  $P < 0.01$ . This regression line gave a threshold P113 dose of  $111 \text{ ng/kg/min}$ .

A modest blood pressure response, in no case exceeding 10 mm Hg, occurred with the lowest P113 dose used,  $0.1 \mu\text{g/kg/min}$ , but larger doses did not induce a larger blood pressure response. In contrast, the angiotensin dose required to induce an unequivocal pressor response to angiotensin II was about threefold greater than the requirement for a blood flow reduction, and larger angiotensin II doses produced a dose-related blood pressure increase (Fig. 1).

P113 was also an antagonist of pressor and renal vascular responses to angiotensin II at the P113's threshold dose for agonist activity,  $0.1 \mu\text{g/kg/min}$  (Fig. 2). This P113 dose induced a highly significant reduction in both the pressor ( $P < 0.01$ ) and renal vascular ( $P < 0.01$ ) responses to angiotensin II. Larger doses of P113 totally obliterated responses to even the highest dose of angiotensin II used,  $10 \text{ ng/kg/min}$ . However, the interpretation of the influence of larger doses was compli-

TABLE I  
*Effect of P113 (3.0 µg/kg/min) and Angiotensin II (0.1–10.0 ng/kg/min) on Renal Function*

		PAH clearance	Creatinine clearance	FF	Na excretion	K excretion
	ng/kg/min	ml/min/M <sup>2</sup>	ml/min/M <sup>2</sup>		µeq/min	µeq/min
Control	—0—	555±51	167±24	0.30±0.03	160±28	74±25
P113	3,000	444±23 (–20%)*	147±20 (–12%)*	0.33±0.04 (+9%)*	82±16 (–49%)*	49±7 (–34%)*
Control	—0—	622±43	151±7	0.25±0.02	248±67	112±29
Angiotensin II	0.1	532±21 (–15%)*	127±11 (–15%)*	0.25±0.02	229±36 (–8%)*	83±19 (–26%)*
Angiotensin II	0.3	481±18 (–23%)*‡	109±16 (–28%)*	0.23±0.03 (–8%)*	217±41 (–13%)*	70±20 (–38%)*
Angiotensin II	1.0	421±21 (–32%)*§	129±16 (–15%)*	0.30±0.02 (+20%)*‡	113±19 (–46%)*‡	58±13 (–48%)*
Angiotensin II	3.0	361±32 (–42%)*§	121±16 (–20%)*	0.33±0.02 (+32%)*‡	74±9 (–70%)*‡	41±8 (–63%)*
Angiotensin II	10.0	342±17 (–45%)*§	118±9 (–22%)*	0.35±0.03 (+40%)*‡	66±16 (–74%)*‡	45±5 (–60%)*

\*  $P < 0.05$ .

‡  $P < 0.025$ .

§  $P < 0.01$ .

cated by P113's intrinsic activity, especially on the renal vasculature.

The effects of P113 and angiotensin II on renal excretory function are summarized in Table I. Angiotensin induced a dose-related reduction in PAH and creatinine clearance, sodium and potassium excretion. Calculated filtration fraction rose. The pattern of the response to P113 strikingly resembled that to angiotensin II but the P113 dose for an equivalent fall of PAH clearance was four orders of magnitude greater than for angiotensin II. When angiotensin II at 10 ng/kg/min was superimposed on the P113 infusion, no further change in any of the indices of renal function occurred.

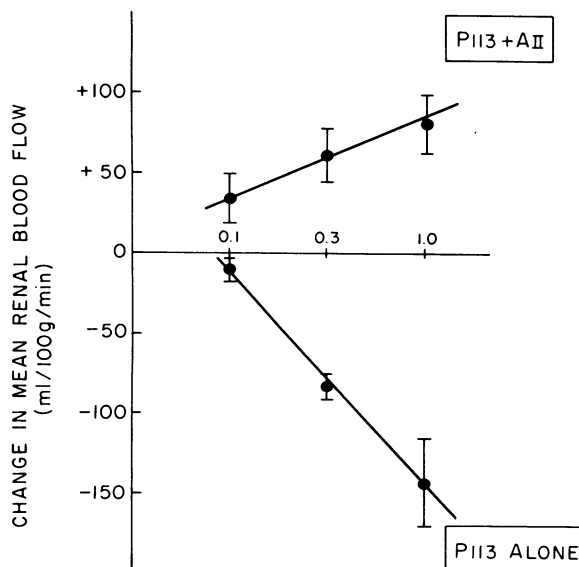


FIGURE 3 The change in renal blood flow in response to P113 is contrasted to the response when P113 was superimposed on an angiotensin (A II) infusion. The flow increase in response to P113 was calculated from the basal blood flow induced by angiotensin II. Similar effects of P113 on angiotensin II-induced pressor and adrenal responses are listed in Table II.

The influence of P113 on blood pressure, renal blood flow, and plasma aldosterone when it was superimposed on a 20-min infusion of 10 ng/kg/min of angiotensin II is shown in Fig. 3 and Table II. The angiotensin II induced the anticipated increase in blood pressure ( $72 \pm 4$ – $96 \pm 7$  mm Hg) and reduction in renal blood flow ( $351 \pm 30$  ml/100 g/min– $178 \pm 11$  ml/100 g/min). The infusion of P113 then induced a dose-related reduction in blood pressure in all, and a return to control at a P113 dose of 1 µg/kg/min in two of the four subjects (Table II). Similarly in this setting, P113 induced a dose-related reversal of the renal blood flow reduction induced by the angiotensin II infusion (Fig. 3). Plasma aldosterone doubled during the 20 min of angiotensin II infusion, rising from 8.5 to 17.2 ng/100 ml, and then returned to control levels during 20 min of P113 infusion at 1.0 µg/kg/min. PRA showed the anticipated fall during angiotensin infusion ( $P < 0.05$ ), but P113 did not reverse this effect of angiotensin II. Prior studies have shown that the renal vascular, pressor, and adrenal responses to an angiotensin II dose of 10 ng/kg/min is well sustained during an interval of 20–40 min of infusion (15).

The response of plasma aldosterone to angiotensin II infusion (10 ng/kg/min) and the influence of P113 at 0.1 µg/kg/min administered simultaneously are shown in Fig. 4. P113 induced a highly significant reduction in the plasma aldosterone response ( $P < 0.01$ ). The influence of a 0.1 µg/kg/min dose of P113 in the absence of exogenous angiotensin II is also shown in Fig. 4. Plasma aldosterone was increased in five of six subjects, but the response was not statistically significant ( $t$  paired data = 1.82;  $P < 0.2$ ).

The relationships between P113 dose and plasma aldosterone and PRA after a 20-min P113 infusion are summarized in Fig. 5. A small but significant increase in plasma aldosterone concentration occurred with the 3.0 µg/kg/min P113 dose, plasma levels rising from  $7.2 \pm 2.8$  to  $15.0 \pm 3.5$  ng/100 ml ( $P < 0.01$ ). In contrast, angiotensin II at 10 ng/kg/min produced a fourfold rise

TABLE II  
The Effect of P113 Superimposed on an Angiotensin II Infusion

Angiotensin II, ng/kg/min P113, µg/kg/min	0 0	10* 0	10 0.1	10 0.3	10 1.0
Blood pressure, mm Hg	72±4	96±7	91±6	84±4	76±4
Renal blood flow, ml/100 g/min	351±30	178±11	212±17	249±18	264±29
Plasma renin activity, ngA <sub>1</sub> /ml/h	2.9±0.4	1.8±0.4	—	—	1.8±0.7*
Plasma aldosterone, ng/100 ml	8.5±5.3	17.2±7.1	—	—	8.5±1.9*

\* Responses to 20 min of infusion.

in plasma aldosterone to  $27.6 \pm 8.2$  ng/100 ml, significantly greater than the aldosterone response to the highest dose of P113 used (Wilcoxon rank sum test;  $P = 0.021$ ). A small but significant reduction in PRA occurred with the 0.1 µg/kg/min P113 dose, from  $4.2 \pm 0.4$  to  $3.4 \pm 0.5$  ngA<sub>1</sub>/ml/h ( $P < 0.01$ ). Larger doses, up to 3.0 µg/kg/min, failed to induce a larger change in the arterial renin activity.

## DISCUSSION

A drug or hormone requires two characteristics for activity. First, it must have affinity for a receptor. Second, it must have intrinsic activity (i.e., once combined with the receptor, it must be capable of initiating the excitation process). According to this concept, a specific competitive antagonist has affinity for the same receptor site as the agent being blocked and thus interferes with the

agent's action by occupying the receptor, without activating it. Stephenson first identified an intermediate series of agents, which he called "partial agonists" (22). These agents are structural analogues of the primary agent or agonist and have affinity for the same receptor, but much less intrinsic activity than the primary agonist. In the absence of the primary agonist, they will stimulate the system, generally less effectively than the primary agonist, but in its presence they will induce competitive blockade. P113, already demonstrated to be a

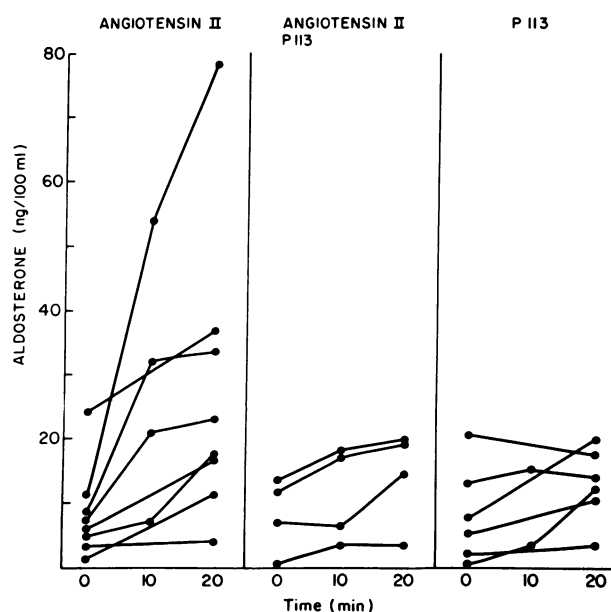


FIGURE 4 The plasma aldosterone responses to A II (10 ng/kg/min: left) and P113 (0.1 µg/kg/min: right). When the two agents were administered simultaneously (center), P113 induced a highly significant reduction in the plasma aldosterone response ( $P < 0.01$ ).

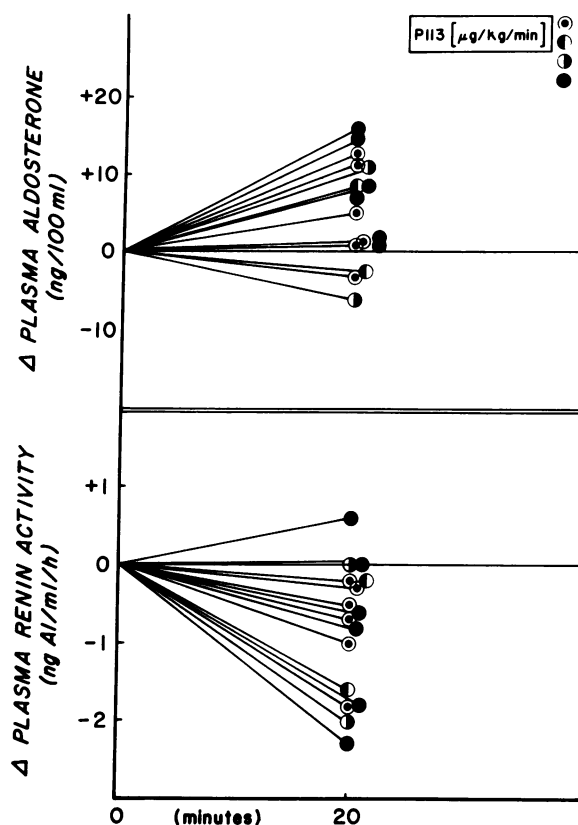


FIGURE 5 The change in plasma aldosterone and plasma renin levels after a 20-min infusion of P113. Both the reduction in plasma renin activity ( $P < 0.05$ ) and the increase in plasma aldosterone ( $P < 0.05$ ) were significant. A dose-response relationship was not evident.

partial agonist in some systems *in vivo* and *in vitro* (10), has now been demonstrated to be a partial agonist in normal man as well, in a setting in which endogenous renin and angiotensin II have been suppressed by a high sodium and potassium intake. The four indices of angiotensin's range of activity assessed in this study, renal blood flow, blood pressure, circulating aldosterone, and circulating renin, all responded to P113 in a direction similar to their response to angiotensin II. The threshold P113 dose for agonist activity also induced striking blockade of responses to angiotensin II and, in the presence of exogenous angiotensin II, P113's dominant action was blockade.

The renal vasculature has a special sensitivity to angiotensin II, particularly in subjects on a high salt intake (15). The intrinsic activity of P113 was also most striking in the kidney, where it exerted a dose-response relationship and a functional influence similar to those of angiotensin II, although the dose of P113 required to induce a response was several hundred times larger. For obvious reasons, maximal renal vascular responses to angiotensin II and P113 were not determined, but over the range that could be examined safely, responses to the two agents were similar. A similar dose-response relationship could not be defined between P113 and blood pressure, which is rather less sensitive to exogenous angiotensin II than the renal vasculature.

We proposed that smooth muscle receptors for angiotensin II could be characterized on the basis of the *in vitro* and *in vivo* responses to angiotensin II and P113 (10). In that study P113 exerted striking intrinsic activity in systems especially sensitive to angiotensin II, including the renal vasculature in the rabbit. The present observations extend this concept to man, and raise the potentially important possibility that the receptors that mediate the response to angiotensin II in the renal vasculature and in other vascular beds differ.

The intrinsic activity displayed by P113 is not unique to this analogue. Virtually all of the competitive antagonists to angiotensin have been developed through a modification in the COOH-terminal amino acid (1-4). Among these analogues, intrinsic activity in sensitive systems has been demonstrated for many (8-11, 23, 24). An alanine substitution, as occurs in P113, appears to lead to considerably less intrinsic activity than, for example, the substitution of isoleucine as the COOH-terminal amino acid (2, 23). The latter results in a more effective antagonist, but it has 30% of angiotensin's intrinsic activity in responsive systems (23). The NH<sub>2</sub>-terminal sarcosine substitution, originally performed to render the agent more resistant to aminopeptidases and thus prolong its biological sojourn, may well contribute to the pronounced intrinsic activity of P113 (25, 26). Another analogue, 1-Sar, 8-Gly angiotensin II, induced

a subcapsular flow reduction evident in renal radioautographs in the dog (24). While 8-cysteine angiotensin II blocked angiotensin II without displaying intrinsic activity in the isolated, perfused rabbit kidney (27), the 8-isoleucine analogue mentioned above was also inactive, and the system was relatively insensitive to angiotensin II, a 150 ng dose was required to reduce blood flow by 50%.

Plasma aldosterone rose in response to P113. Thus, the adrenal glomerulosa cells in the subjects on a high-salt diet are not under a tonic influence of angiotensin II. Plasma renin fell, making it likely that the adrenal responded directly to P113, rather than reflecting a P113-induced activation of the renin-angiotensin system. Steele and Lowenstein also demonstrated that P113 induced an adrenal response in the nephrectomized rabbit, in accord with this interpretation (28). P113 did not induce a response in the rat adrenal *in vitro* (11), and the normal, unanesthetized rabbit's response *in vivo* was considerably greater (28) than that we found in normal man in this study. The observations raise the possibility of important species differences. The modest response of the adrenal in normal man with a high salt intake is also consistent with the hypothesis that responsiveness to P113 reflects the responsiveness of the system to angiotensin II (10). A high salt intake makes the adrenal relatively insensitive to angiotensin II in normal man (15).

There are a number of implications of the present observations relevant to the use of this agent as a diagnostic and therapeutic agent in man. First, the P113 dose required to block pressor responses to large amounts of angiotensin II in normal man was surprisingly low: 0.1  $\mu\text{g/kg/min}$ . This is 3-10% of the dose required to control pressure in patients with renovascular hypertension as reported elsewhere (12-14) and in our experience. The arterial angiotensin II concentration achieved with a 10 ng/kg/min angiotensin II infusion generally ranges from 180 to 340 pg/ml, equivalent to or higher than concentrations in patients with responsive disease. It seems likely, therefore, that when endogenous angiotensin is blocked, its concentration in the biophase—the region of the vascular receptor—is considerably higher than the circulating level.

Second, P113's intrinsic activity has potential utility in the identification of angiotensin-mediated hypertension. With the moment-to-moment variability of blood pressure, it is considerably easier to discriminate between a decrease and increase in blood pressure rather than no change. Moreover, we have seen, in confirmation of a preliminary report with another analogue, 1-Sar, 8-Ile angiotensin II (13), a striking pressor response in patients with low renin hypertension. Thus the agent may be useful in screening patients at both ends of the

renin spectrum. The intrinsic activity also suggests that the agent should be administered in graded dosage, to avoid alarming pressor responses in hypertensive patients.

Finally, the striking intrinsic activity of P113 on the renal vasculature may be an important limitation in its application to the reversal of angiotensin-mediated renal vasoconstriction. That the receptors on the renal vasculature and those of other systemic vascular beds appear to differ raises the hope that analogues with greater specificity, which block the renal vasculature preferentially, may be found. In view of the possibility that angiotensin's action on the kidney plays a role in the pathogenesis of acute renal failure, the hepatorenal syndrome, and the edema states, this avenue merits investigation. Similarly, the adrenal and vascular receptors appear to differ substantially (11). Thus a series of more specific blocking agents should be sought. Certainly this approach has been successful in the development of more specific blocking for adrenergic, cholinergic, and histamine-mediated responses (29).

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