*Br. J. clin. Pharmac* (1976), 3, 483-487

# AGONIST AND ANTAGONIST EFFECTS OF Sar$^1$–ala$^8$–ANGIOTENSIN II IN SALT-LOADED AND SALT-DEPLETED NORMAL MAN

G.A. MACGREGOR

Department of Medicine, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF

P.M. DAWES

Department of Pharmacology, ICI, Macclesfield, Cheshire

1 Three normal subjects were infused with Sar$^1$-ala$^8$-angiotensin II (Saralasin, P113) whilst on a high sodium (200 mEq + normal diet) and a low sodium (10 mEq diet) intake.

2 On the high sodium intake when angiotensin II and plasma renin activity (PRA) were suppressed, P113 infusion (5-10 $\mu g\ kg^{-1}\ min^{-1}$) caused a slight rise in BP and a marked drop in urine flow and sodium excretion, with a fall in glomerular filtration rate, and effective renal plasma flow.

3 On the low sodium intake, when angiotensin II and PRA were increased, P113 infusion (5-10 $\mu g\ kg^{-1}\ min^{-1}$) caused no change in blood pressure, urine flow or sodium excretion. However, when P113 was infused at an incremental rate starting at 0.25 $\mu g\ kg^{-1}\ min^{-1}$ there was a fall in standing BP, which was maximal at an infusion rate of 1 $\mu g\ kg^{-1}\ min^{-1}$, and this fall in standing BP was largely abolished as the rate of infusion was increased to 10 $\mu g\ kg^{-1}\ min^{-1}$.

4 These results show firstly that angiotensin II is involved in maintaining standing blood pressure during dietary sodium depletion in normal man and secondly that P113 does have agonist as well as antagonist activity in normal man, the effect depending on the level of angiotensin II and sodium intake. When looking for angiotensin II mediated hypertension it may be important to use an incremental rate of infusion of P113 as the agonist activity of larger doses may mask its hypotensive action.

## Introduction

Sar$^1$-ala$^8$-angiotensin II (Saralasin, P113), a competitive inhibitor of angiotensin II has been shown to lower blood pressure in hypertensive patients in whom angiotensin II has been thought to be responsible for the increased blood pressure (Brunner, Gavras, Laragh & Keenan, 1973; Streeten, Anderson, Freiberg & Dalakos, 1975). However, P113 has been noted to cause a rise in blood pressure in some patients (Streeten *et al.*, 1975), and these presumed agonist (Angiotensin II) effects of P113 have been observed in animal isolated tissues and in sodium loaded rabbits (Mimran, Hinrichs & Hollenberg, 1974). The agonist activity seeming to depend on the tissue studied, the endogenous level of angiotensin II and the sodium intake. We report here studies on the blood pressure and kidney function of three normal volunteers infused with P113 whilst on a high sodium intake when angiotensin II was suppressed, and a low sodium intake when angiotensin II was increased.

## Methods

Three normal males volunteered to take part in the study, the purpose of which was fully explained to them. They were given 200 mEq/day of slow sodium (Ciba) in addition to their normal diet for 5 days. On the final day of this added sodium intake, the blood pressure weight, 24 h urinary sodium excretion, upright angiotensin II and plasma renin activity (PRA) were measured. A water diuresis was then induced with 5% dextrose (500 ml/h intravenously) which was continued throughout the infusion. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured using ($^{51}$Cr) EDTA and ($^{125}$I) Hippuran. After equilibration for 1 h they were instructed to pass urine every 30 min, venous blood being taken at the mid-point of each collection period. Two 0.5 h control periods were followed by infusion of P113 at a rate of 5 $\mu g\ kg^{-1}\ min^{-1}$ for 30 min and then at 10 $\mu g\ kg^{-1}\ min^{-1}$ for 1 hour. P113 was then stopped and two further 30 min urine collections

were obtained. Blood pressure was measured every 1-2 min with an arteriosonde (Roche) 1217. Standing blood pressure was measured one minute after standing upright, and repeated three or four times at one minute intervals.

The subjects were then put on a 10 mEq sodium diet. On the fifth day of this diet the same measurements and infusion were carried out except that P113 was given at rates of 0.25, 0.5, 1.0, 2.5, 10 $\mu g\ kg^{-1}\ min^{-1}$ each rate being given for 15 min. Two of the same subjects were also studied a second occasion after another five days of 10 mEq sodium diet with a dose of P113 of 5 $\mu g kg^{-1}\ min^{-1}$ for 30 min and then 10 $\mu g\ kg^{-1}\ min^{-1}$ for 1 hour.

Plasma angiotensin II concentration was measured by the method of Düsterdieck & McElwee (1971). PRA was measured by radioimmunoassay of angiotensin I generated during plasma incubation for 90 min at 37° C and pH 6.0 in the presence of EDTA, 8-hydroxyquinoline and phenylmethylsulphonylfluoride.

## Results

On the high sodium intake, mean angiotensin II and PRA were 15.5 $\rho g\ ml^{-1}$ and 2.2 $ng\ ml^{-1}\ h^{-1}$ respectively (Table 1). P113 infusion at a rate of 5 and 10 $\mu g\ kg^{-1}\ min^{-1}$ caused a slight rise in blood pressure, especially standing diastolic pressure (Figure 1). The change in lying blood pressure was particularly seen immediately after starting the infusion and on increasing the rate of infusion. There was a pronounced fall in urinary sodium excretion and urine flow associated with a fall in GFR and ERPF during infusion of P113 (Table 2). Supine PRA showed a slight decrease during infusion of P113 (Table 2).

On the low sodium intake, mean angiotensin II and PRA were 44.3 $pg\ ml^{-1}$ and 8.30 $ng\ ml^{-1}\ h^{-1}$ respectively (Table 1). P113 infusion at doses of 5 and 10 $\mu g\ kg^{-1}\ min^{-1}$, caused no change in lying or standing blood pressure and no change in urine flow or urinary sodium excretion. However, when infused at an incremental rate starting at

**Table 1** Mean values of 24 h urinary sodium, blood pressure (BP), angiotensin II and PRA on the fifth day of high and low sodium diet (± 1 s.d.)

| | *High sodium diet* | *Low sodium diet* |
|---|---|---|
| Urinary sodium (mEq/24 h) | 346 (77) | 9.6 (2.9) |
| Supine BP (mm Hg) | 119/79 | 108/75 |
| Upright A II (pg/ml) | 15.5 (6.1) | 44.3 (4.9) |
| Upright PRA ($ng\ ml^{-1}\ h^{-1}$) | 2.20 (0.45) | 8.30 (3.9) |
| Weight change (kg) | +0.43 | −2.2 |

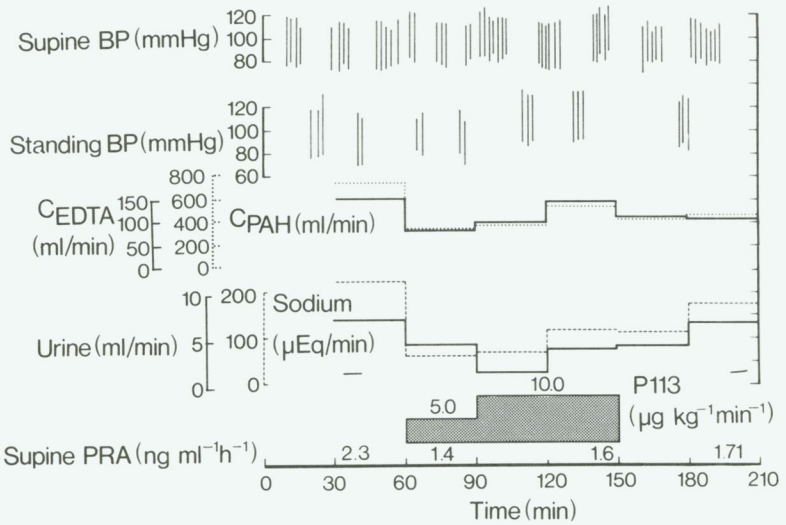


**Figure 1** Effect of P113 infusion on a sodium-loaded subject.

**Table 2** Mean effect of P113 on kidney function in three subjects after high sodium intake (± 1 s.d.)

| | | *P113* ($\mu g\ kg^{-1}\ min^{-1}$) | | |
|---|---|---|---|---|
| | *Control* | *5* | *10* | *Control* |
| Urinary sodium excretion ($\mu$Eq/min) | 393 (190) | 71.7 (49.2) | 126 (31.6) | 173 (97.3) |
| Urine flow (ml/min) | 11.4 (3.7) | 4.28 (3.4) | 7.70 (5.1) | 7.27 (1.8) |
| GFR (ml/min) | 161 (30.0) | 98.8 (12.25) | 120 (3.8) | 120 (6.2) |
| ERPF (ml/min) | 672 (30.4) | 401 (72.8) | 454 (2.1) | 475 (36.8) |
| Supine PRA ($ng\ ml^{-1}\ h^{-1}$) | 1.6 (0.76) | 1.26 (0.23) | 1.40 (0.20) | 1.23 (0.50) |

0.25 $\mu g\ kg^{-1}\ min^{-1}$ there was a fall in standing blood pressure, with no change in lying blood pressure. This fall in standing blood pressure was greatest at an infusion rate of 1 $\mu g\ kg^{-1}\ min^{-1}$, (Figure 2, Table 3), and at this dose one subject fainted when standing. As the dose of P113 was progressively increased the fall in standing blood pressure was largely abolished (Table 3). The effects of an incremental rate of infusion of P113 on urine flow and sodium excretion were more variable. Overall, there was a fall in urine flow and sodium excretion with a reduction in GFR and ERPF (Figure 2). Supine PRA increased during infusion of P113 (Table 3).

There was no consistent change in plasma sodium or potassium before, during and after infusion with P113.

## Discussion

P113 infusion caused a reduction in standing blood pressure in three salt depleted normals, but had no effect on their lying blood pressure. These results indicate that angiotensin II is concerned in maintaining standing but not lying blood pressure during dietary sodium depletion in normal man and confirm results using a tilt table and an enzyme inhibitor (SQ 20881) which inhibits conversion of angiotensin I to angiotensin II (Haber, Sanch, Barger & Barger, 1975).

The fall in standing blood pressure was related to the amount of P113 infused and was not evident when P113 was infused at a dose of 5-10 $\mu g\ kg^{-1}\ min^{-1}$ either starting at this dose or when using the incremental rate of infusion. This

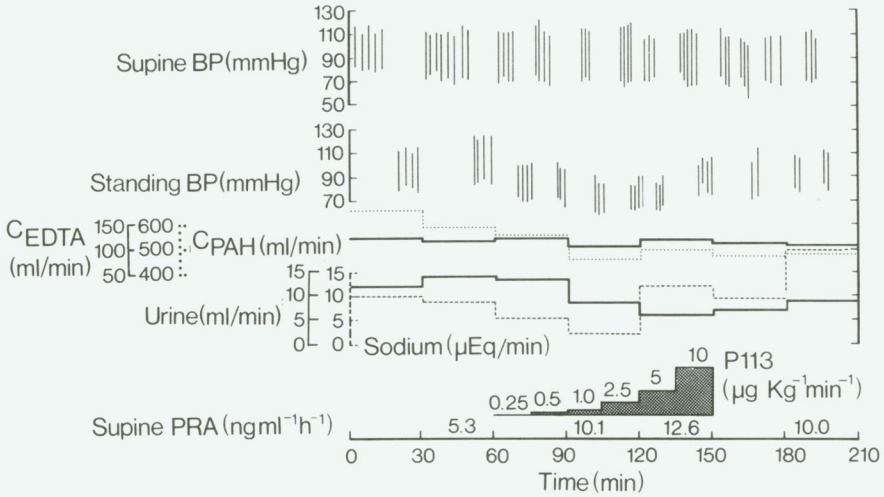


**Figure 2** Effect of P113, given in an incremental dosage, on a sodium-depleted subject.

**Table 3** Effect of P113, given in an incremental dose, on supine, standing BP and PRA (± 1 s.d.) in three subjects after low sodium intake

| | P113 ($\mu g\ kg^{-1}\ min^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Control* | *0.25* | *0.5* | *1.0* | *2.5* | *5* | *10* | *Control* |
| Supine BP (mm Hg) | 108/75 | 108/75 | 111/74 | 109/71 | 113/74 | 107/73 | 110/72 | 111/71 |
| Standing BP (mm Hg) | 110/79 | 107/71 | 103/71 | 92/63 | 97/67 | 100/69 | 105/70 | 109/75 |
| Supine PRA ($ng\ ml^{-1}\ h^{-1}$) | 8.20 (5.1) | | | | 15.84 (8.5) | | 17.03 (6.58) | 12.6 (3.5) |

may be due to a partial agonist effect that P113 may have at larger doses, but could be due when P113 was given in an incremental dose either to a change in plasma volume as there was a reduction in urine flow with the fall in standing blood pressure or to a change in autonomic homeostatic response due to the previous fall in standing blood pressure. When the same normals were salt loaded, P113 infusion (5-10 $\mu g\ kg^{-1}\ min^{-1}$) caused a slight rise in lying and standing blood pressure. This effect has been reported in some hypertensives whose blood pressure did not fall during P113 infusion (Streeten *et al.*, 1975). This rise in blood pressure cannot be due to release of renin as PRA decreased during infusion when normals were sodium loaded. These results indicate that P113 can have a dose related agonist effect on the blood pressure in certain circumstances.

After sodium loading P113 infusion caused a reduction in urine flow and sodium excretion, GFR and ERPF. These results are the same as those of angiotensin II when infused in sub-pressor doses into normals (Brown & Peart, 1962; Laragh, Cannon, Bentlel, Sicinski & Meltzer, 1963). P113 therefore has an agonist effect on the renal vasculature and the mechanisms controlling urine flow and sodium excretion in sodium loaded normals.

After sodium depletion P113 infusion at a rate of 5-10 $\mu g\ kg^{-1}\ min^{-1}$ caused no change in urine flow or sodium excretion. However, using the incremental rate of infusion (0.25-10 $\mu g\ kg^{-1}\ min^{-1}$) urine flow and sodium excretion GFR and ERPF decreased, but individual clearances varied considerably. Interpretation of this data is more difficult as the fall in standing blood pressure may have influenced the results and also as the action of sub-pressor doses of angiotensin II in salt depleted normals is not known.

PRA did not increase, and if anything, decreased during infusion of P113 when endogenous levels were suppressed by sodium loading. When PRA was stimulated by sodium depletion infusion of P113 caused a rise in PRA. These results can be explained by invoking the hypothesis of negative feedback of angiotensin II on the juxta glomerular apparatus inhibiting release of renin. With low levels of angiotensin II, P113 has no effect or may even have an angiotensin II-like action reducing release of renin but with high levels of angiotensin II, P113 may block the negative feedback resulting in greater release of renin, and higher levels of PRA.

P113 has antagonist and agonist activity in normal man, the effect appearing to depend on the state of sodium balance and endogenous levels of angiotensin II. When looking for angiotensin II mediated hypertension it may be important to use an incremental dose of P113 as the agonist activity of larger doses may mask its hypotensive action.

We would like to thank the volunteers who participated in this study. We would also like to thank Mrs N. Markandu, Mr D.C. Faulkner and Mr J.R. Keddie for invaluable technical assistance, the department of nuclear medicine for measurement of GFR and ERPF, and Dr R. Keenan of Eaton Laboratories for supplies of P113.

## References

BROWN, J.J. & PEART, W.S. (1962). The effect of angiotensin on urine flow and electrolyte excretion in hypertensive patients. *Clin. Sci.*, **22**, 1-17.

BRUNNER, H.R., GAVRAS, H., LARAGH, J.H. & KEENAN, R. (1973). Angiotensin II blockade in man by $sar^1$-$ala^8$-Angiotensin II for understanding and treatment of high blood pressure. *Lancet*, **ii**, 1045-1048.

DÜSTERDIECK, G. & McELWEE, G. (1971). Estimation of angiotensin II concentration in human plasma by radioimmunoassay. Some applications to physiological and clinical states. *Eur. J. clin. Invest.*, **2**, 32-38.

HABER, E., SANCH, R., BARGER, R.E. & BARGER, A.C. (1976). The role of the renin-angiotensin system in cardiovascular homeostasis in normal man. *Clin. Sci. mol. Med.* (in press).

LARAGH, J.H., CANNON, P.J., BENTLEL, E.J., SICINSKI, A.M. & MELTZER, J.I. (1963). Angiotensin II, norepinephrine, and renal transport of electrolytes and water in normal man and in cirrhosis with ascites. *J. clin. Invest.*, **42**, 1179-1192.

MIMRAN, A., HINRICHS, K.J. & HOLLENBERG, N.K. (1974). Characterization of smooth muscle receptors for angiotensin: studies with an antagonist. *Am. J. Physiol.*, **226**, 185-190.

STREETEN, D.H.P., ANDERSON, G.H., FREIBERG, J.M. & DALAKOS, T.G. (1975). Use of an Angiotensin II antagonist (Saralasin) in the recognition of 'angiotensinogenic' hypertension. *New Eng. J. Med.*, **292**, 657-662.

*(Received July 15, 1975)*