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# BIOLOGICAL SIGNIFICANCE OF ACTIVE AND INACTIVE RENIN IN MAN

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## SUMMARY

The biological significance of active and inactive renin was investigated by comparison of an in-vitro assay of active, total and inactive plasma renin concentration (PRC), plasma renin activity (PRA) and plasma concentrations of angiotensin I and II with an in-vivo change in mean arterial blood pressure (MAP) produced by antagonism of angiotensin with treatment with saralasin and by blockade of angiotensin-converting enzyme by treatment with captopril.

A significant relationship between the changes in MAP during treatment with saralasin and captopril with the pretreatment levels of PRA, active and total PRC and angiotensin II were found; while the pre-existing level of inactive renin was not a predictor for the hypotensive effect of saralasin and captopril. During treatment with saralasin and captopril significant increases in PRA, plasma angiotensin I concentration and total and active PRC were found and no change in inactive PRC was observed.

## INTRODUCTION

Inactive forms of renin in human plasma can be converted in-vitro into active renin by the acidification of plasma (Lumbers, 1971; Morris & Lumbers, 1972; Leckie & McConnell, 1975; Skinner, Cran, Gibson, Taylor, Walters & Catt, 1975; Boyd, 1977; Derckx, Wenting, Man in 't Veld, Verhoeven & Schalekamp, 1978), or by treatment with trypsin (Cooper, Murray & Osmond, 1977), cold (Sealey, Moon, Laragh & Alderman, 1976; Sealey, Moon, Laragh & Atlas, 1977) or kallikrein (Millar, Clappison & Johnston, 1978; Osmond, Lo, Loh, Zingy & Hedlin, 1978; Derckx, Man in 't Veld & Schalekamp, 1979; Rumpf, Lankisch, Koop, Becker, Schmidt & Scheler, 1979). Many in-vitro studies have investigated the proportions in human plasma of active and inactive renin and the correlations between angiotensin II and both forms of renin (Leckie, McConnell, Grant, Morton, Tree & Brown, 1977; Lijnen, Amery & Fagard, 1979*a*).

However, no direct in-vivo evidence of the relative biological significance of active and inactive renin is available. We have investigated, therefore, the in-vivo significance of active and inactive renin by comparing an in-vitro measurement of active, inactive and total plasma renin concentrations (PRC), concentrations of angiotensin I and II in plasma and plasma renin activity (PRA) with an in-vivo change in mean arterial blood pressure (MAP) produced by antagonism of angiotensin by treatment with saralasin and by blockade of angiotensin-converting enzyme by treatment with captopril.

## MATERIALS AND METHODS

### *Subjects*

Twelve hypertensive patients (eight men, four women) with an average age of $39{\cdot}9 \pm 3{\cdot}1$ (S.E.M.) years and weighing $71{\cdot}0 \pm 2{\cdot}7$ kg were studied, their informed consent

having been obtained. Six had essential hypertension and six had hypertension with renal artery stenosis. The severity of hypertension was assessed by the criteria of the World Health Organization: six were at stage I, five at stage II and one at stage III because of an eye fundus, grade 3.

Inclusion and exclusion criteria have been reported for similar studies (Amery, Billiet, Boel, Fagard, Reybrouck & Willems, 1976).

*Experimental design*

The patients were followed on an outpatient basis for about 1 month whilst they were taking three placebo tablets/day and they were instructed to follow a low-sodium diet; four of these patients were also treated with chlorthalidone (50 mg/day; Hygroton; Ciba–Geigy, Basle, Switzerland). The urinary sodium excretion over 24 h in the patients not on a diuretic averaged 72 mequiv. In two patients with high blood pressure, the placebo period was shortened because it was considered to be unethical to delay treatment for these patients. This study was performed in salt-deplete hypertensive patients because no correlation existed between decrease of blood pressure during treatment with saralasin (Eaton Laboratories, a division of Morton–Norwich, New York, U.S.A.) and the pre-existing level of total PRC in salt-replete hypertensive patients (Fagard, Lijnen, Amery & Reybrouck, 1978).

The patients were studied in the recumbent position after a light breakfast in the morning and in the laboratory where room temperature was 18–22 °C and humidity 40–60%. A small catheter (Vygon, 115·09) was introduced into the brachial artery for sampling of blood and for recording the intra-arterial pressure using an Elema Schonander EMT pressure transducer; pressure was recorded continuously on a Mingograph 81 recorder and MAP was obtained by electrical damping every 5 min.

After the technical procedures, including insertion of a needle into an arm vein, a control period of 30 min was observed while 5% glucose was given intravenously. Saralasin was infused at a rate of 10 μg/kg per min for 45 min. At the end of the infusion of the drug the patient was allowed to recover for 90 min before being given one tablet of captopril (25 mg; Squibb Institute for Medical Research, New Jersey, U.S.A.) and the blood pressure was monitored for another 75 min. Arterial blood (20 ml) was drawn before and at the end of the infusion of saralasin and also before the administration of captopril and at the end of the observation period.

*Assay of plasma renin activity*

Plasma renin activity was measured by radioimmunoassay of the angiotensin I which was generated during incubation of the plasma samples for 1 h at 37 °C with the endogenous renin substrate at pH 6·0 according to the method of Fyhrquist & Puutula (1978).

*Determination of plasma renin concentration*

The total PRC was measured as the rate of angiotensin I which was generated during incubation for 1 h with renin substrate (pH 7·4) from a bilaterally nephrectomized sheep under zero order kinetic conditions, according to the method of Skinner (1967) which was adapted for radioimmunoassay by Lijnen, Amery & Fagard (1976). This assay consists first of denaturation of endogenous substrate by dialysis against a 0·05 M-amino-acetic acid buffer (pH 3·3) containing 5 mM-EDTA and 90 mM-NaCl and of an inactivation of the angiotensinases by heating at 32 °C for 1 h.

The active PRC was determined after dialysing the plasma against a 0·05 M-amino-acetic buffer (pH 4·5) and after heating the diffusate at 32 °C for 1 h to inhibit the angiotensinases adequately. The plasma was then incubated with excess sheep renin substrate at pH 7·4 for 1 h. The difference between total and active PRC is described as the inactive PRC.

*Assay of angiotensin I and II and of converting enzyme in the plasma*

A radioimmunoassay method was used for the measurement of plasma concentrations of angiotensin I and II as described previously by Lijnen, Amery & Fagard (1978*a*) and Lijnen, Amery, Fagard & Katz (1978*b*). Plasma angiotensin-converting enzyme (ACE) activity was measured spectrophotometrically as described by Lijnen & Amery (1978). Only 0·33% cross-reaction of $^{1}$Asp-$^{5}$Ile-angiotensin I with the angiotensin II antiserum was found. Semple, Boyd, Dawes & Morton (1976) found a higher cross-reaction of the angiotensin II antiserum with angiotensin I (0·6%).

*Statistical analysis*

The statistical methods used were regression analysis and Student's two-tailed *t*-test for paired data. The dispersion of the data is given by S.E.M. Except for ACE activity, the biochemical parameters were transformed to logarithms since only the log distribution was Gaussian.

*Blood sampling*

All the blood samples were withdrawn from the brachial artery into an inhibitor solution (0·161 M-EDTA + 0·025 M-*o*-phenantroline in distilled water) and processed as described before (Lijnen *et al.* 1976, 1978*a*).

## RESULTS

*Acute changes in the plasma renin-angiotensin system during treatment with saralasin and captopril*

As shown in Table 1 treatment with saralasin and captopril produced significant ($P<0·001$) two- to threefold increases of PRA, angiotensin II, total and active PRC and no change in inactive PRC. Angiotensin II in the plasma increased significantly during treatment with saralasin while it dropped ($P<0·001$) during treatment with captopril. No significant change was found in ACE activity during infusion with saralasin, whereas during treatment with captopril ACE activity was reduced significantly ($P<0·001$).

*Relationship between plasma concentrations of angiotensin II and the other biochemical variables during treatment with glucose and saralasin*

Concentrations of angiotensin II in plasma were significantly ($P<0·001$) related to simultaneously determined PRA, total PRC, active PRC and angiotensin I. The correlation coefficients were of the same size (between 0·79 and 0·92) except for inactive PRC where a small coefficient was noted ($r = 0·55$). No relationship between concentrations of angiotensin II and activity of angiotensin-converting enzyme was found ($r = 0·01$; $P>0·10$).

*Relationship of the acute changes of MAP to pre-existing PRA, plasma angiotensin II, active, total and inactive PRC*

The changes in MAP during treatment with saralasin were closely related to the log of the PRA (Fig. 1*a*), of the plasma angiotensin II concentration (33·9 − 28·7 log plasma angiotensin II; $r = -0·74$; $P<0·001$; $n = 11$), of the total PRC (40·91 − 30·81 log total PRC; $r = -0·81$; $P<0·001$; $n = 12$) and of the active PRC (15·63 − 20·47 log active PRC; $r = -0·82$; $P<0·001$; $n = 12$) before its infusion but not to the log of the inactive PRC (Fig. 1*b*).

The changes induced in MAP by treatment with captopril were significantly related to the log of PRA (Fig. 1*a*), of the plasma angiotensin II concentration (26·1 − 28·6 log plasma angiotensin II; $r = -0·72$; $P<0·01$; $n = 12$), of the total PRC (17·10 − 22·20 log total PRC;

Table 1. *Acute (mean ± S.E.M.) changes in plasma renin activity (PRA), plasma angiotensin I and II (PA I, II), active, total and inactive plasma renin concentrations (PRC) and angiotensin-converting enzyme (ACE) before and during treatment of 12 patients with saralasin and captopril*

| | Saralasin (10 μg/kg per min) | | Captopril (25 mg by mouth) | |
|---|---|---|---|---|
| | Before | During | Before | During |
| Log PRA (log ng/ml per h) | 0·31 ± 0·18 | 0·67 ± 0·25* | 0·35 ± 0·21 | 0·91 ± 0·22** |
| Antilog of mean | 2·04 | 4·68 | 2·24 | 8·13 |
| Log PA I (log pg per ml) | 2·19 ± 0·16 | 2·63 ± 0·22*** | 2·22 ± 0·17 | 2·82 ± 0·19*** |
| Antilog of mean | 156 | 431 | 167 | 666 |
| Log PA II (log pg per ml) | 1·61 ± 0·11 | 1·96 ± 0·18*** | 1·66 ± 0·11 | 1·21 ± 0·10*** |
| Antilog of mean | 41·0 | 90·6 | 45·6 | 16·3 |
| Log total PRC (log ng/ml per h) | 1·66 ± 0·11 | 1·86 ± 0·16* | 1·74 ± 0·12 | 1·98 ± 0·15*** |
| Antilog of mean | 45·7 | 72·4 | 55·0 | 95·5 |
| Log active PRC (log ng/ml per h) | 1·26 ± 0·16 | 1·49 ± 0·22* | 1·34 ± 0·17 | 1·70 ± 0·20*** |
| Antilog of mean | 18·2 | 30·9 | 21·9 | 50·1 |
| Log inactive PRC (log ng/ml per h) | 1·26 ± 0·08 | 1·48 ± 0·11 | 1·38 ± 0·13 | 1·47 ± 0·14 |
| Antilog of mean | 18·2 | 30·2 | 24·0 | 29·5 |
| ACE activity (u./ml) | 32·3 ± 2·9 | 32·4 ± 3·3 | 32·7 ± 3·4 | 18·1 ± 2·8*** |

*$P<0·05$, **$P<0·01$, ***$P<0·001$ when compared with the values before treatment with the same drug (Student's two-tailed $t$-test).

$r = -0·62$; $P<0·05$; $n = 12$) and of the active PRC ($1·52 - 17·14$ log active PRC; $r = -0·67$; $P<0·02$, $n = 12$) before its ingestion but not to the log of the inactive PRC (Fig. 1*b*).

## DISCUSSION

Human plasma consists of at least two types of renin. One is enzymatically active at neutral pH and is termed active renin (Skinner *et al.* 1975). The other, called inactive renin, is not enzymatically active in its native form but has renin-like activity after in-vitro exposure to low pH (Skinner *et al.* 1975), to proteolytic enzymes (Morris & Lumbers, 1972) or to the cold (Sealey *et al.* 1976).

An optimal acid activation of plasma renin is obtained at pH 3·3–3·5 (Semple *et al.* 1976; Leckie *et al.* 1977; Lijnen & Amery, 1977). The greatest cryoactivation of inactive renin is obtained between pH 7 and 9; no activation has been found in the frozen state (Sealey *et al.* 1976). Exogenous trypsin activates inactive renin in plasma at normal pH and at 4, 23 or 37 °C proving that neither low pH nor cold are essential (Cooper *et al.* 1977).

Two-thirds of the total renin in normal human plasma is made up of an inactive, acid-activatable form. Skinner *et al.* (1975) and Lijnen, Amery, Fagard, Reybrouck, Moerman & De Schaepdryver (1979*b*) found 34% active renin on average. According to Leckie *et al.*

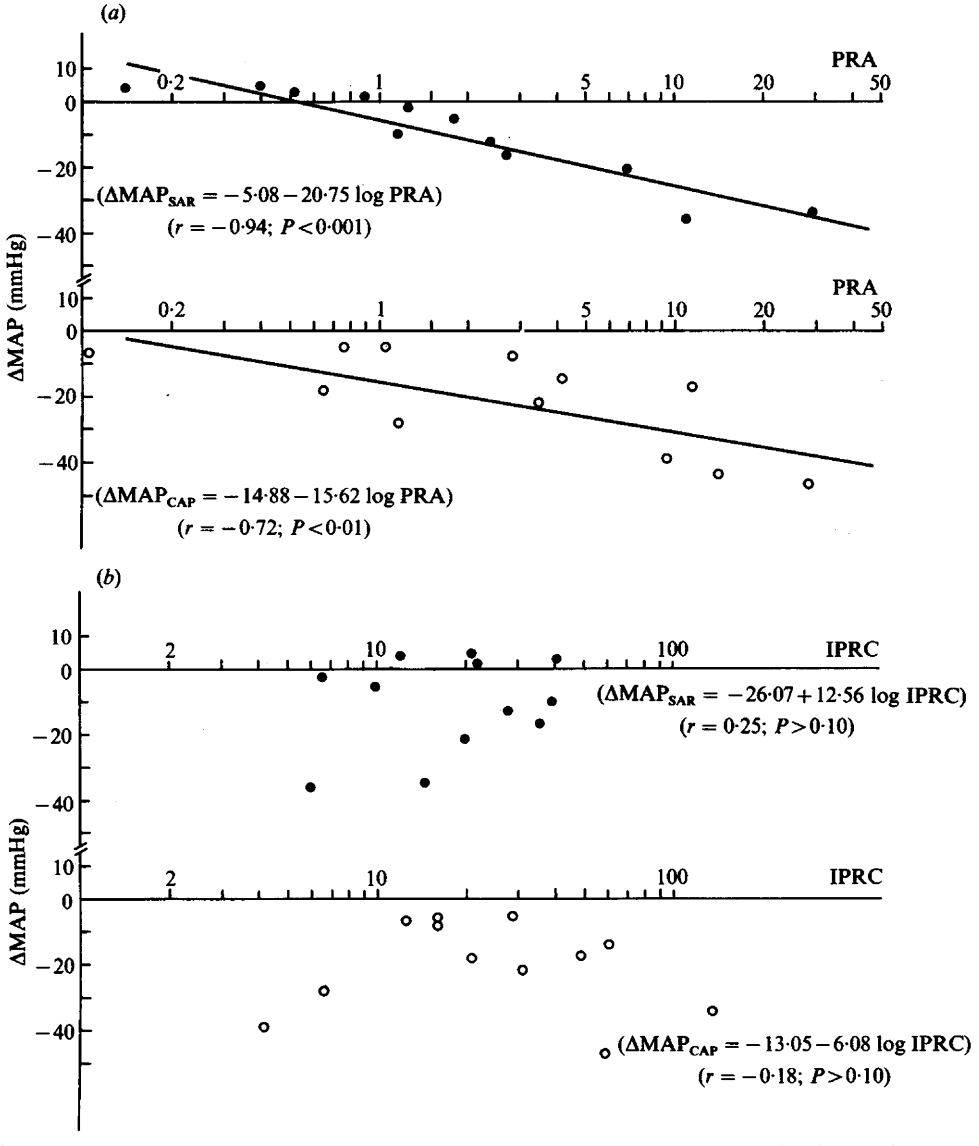


Fig. 1. Relationship of changes in mean arterial blood pressure of 12 patients (ΔMAP) with the log of (*a*) control plasma renin activity (PRA; ng/ml per h) and (*b*) control inactive plasma renin concentration (IPRC; ng/ml per h) during treatment with saralasin (SAR; ●) and captopril (CAP; ○).

(1977) the percentage of inactive renin varied from 36 to 71% of the total renin concentration in normal subjects on an unrestricted diet. Boyd (1977) also reported that active renin represents about 50% of the total renin concentration and that inactive renin is present in all subjects. Inactive renin comprises a larger fraction (Hsueh, Luetscher, Carlson & Grislis, 1978) of total renin in plasma of salt-loaded healthy subjects (82%) than of salt-depleted subjects (62%).

The in-vivo activation of inactive renin is not yet fully understood, although a link between renin and kallikrein-kinin has been proposed (Sealey, Atlas & Laragh, 1978). Derckx *et al.* (1979) found that in plasma from patients with an inherited deficiency of Fletcher factor

(prekallikrein), only a small amount of inactive renin was activated by acid, thus indicating that the acid activation of renin appears to be prekallikrein-dependent.

On the contrary, Millar *et al.* (1978) found appreciable acid activation of inactive renin in plasma from two patients with Fletcher-factor deficiency and suggested, therefore, that plasma kallikrein is not the activator of renin *in vitro*. Osmond *et al.* (1978) also reported that inactive renin makes up a higher than normal proportion of total renin in plasma of patients with Fletcher-factor deficiency whether estimated by cryoactivation (93·3%) or by tryptic activation (95·6%), whereas a low basal PRA (active renin) was found. This therefore suggests that endogenous plasma prekallikrein–kallikrein is required for a normal level of active renin and a normal ratio of active to inactive renin but is not necessary for cryoactivation, tryptic activation or acid activation of inactive renin.

Our in-vitro studies have shown that during treatment with saralasin no change occurred in levels of inactive renin and a two- to threefold increase in active renin (PRA or PRC) and concentrations of angiotensin II and I was observed (Table 1). During blockade of converting enyzme with captopril there was also no change in inactive PRC and an increase in active renin and angiotensin I as well as reductions in the concentration of angiotensin II and in the activity of angiotensin-converting enzyme. These in-vitro results clearly show a distinction between the active and inactive renin response during infusion of saralasin and administration of captopril. When considering the correlation studies between simultaneously determined angiotensin II and inactive or active renin levels, however, a significant correlation between inactive renin and concentration of angiotensin II was found, indicating that a purely in-vitro comparison can be misleading, especially in correlation studies where the range is very important. Restricting the range to normal subjects on an unrestricted diet Lijnen *et al.* (1979*a*) found no significant correlation between inactive PRC and the concentration of angiotensin II, whereas the concentration of angiotensin II was significantly related to active renin (PRC or PRA). Similar results were reported by Leckie *et al.* (1977) who suggested that inactive renin does not produce angiotensin II *in vivo*. However, it is difficult to draw such a conclusion from in-vitro comparisons.

In addition to these in-vitro studies, another approach has been used to investigate the physiological significance of the various forms of renin. We have studied the correlation between in-vitro measurements of active and inactive renin and changes in MAP determined *in vivo*.

The changes in MAP during antagonism of angiotensin with infusion of saralasin and blockade of converting enzyme by treatment with captopril were closely related to active renin (PRA or PRC), to plasma angiotensin II and to total PRC, but not to inactive renin (inactive PRC). These results also indicate that inactive renin is not a predictor of the in-vivo hypotensive effect of saralasin and captopril.

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