PLASMA RENIN ACTIVITY AND PLASMA RENIN SUBSTRATE IN HYPERTENSION ASSOCIATED WITH STEROID EXCESS

L. R. KRAKOFF AND M. MENDLOWITZ

Department of Medicine, Mount Sinai School of Medicine of The City University of New York, New York, U.S.A.

SUMMARY

1. Plasma renin activity and plasma renin substrate were measured by radioimmunoassay of generated angiotensin I in patients with steroid excess syndromes. Significant increases in substrate were observed in patients with Cushing’s syndrome, during glucocorticoid therapy and on oral contraceptive agents. Suppression of plasma renin activity occurred only in primary aldosteronism.

2. The Michaelis constant ($K_m$) for the reaction between renin and substrate in plasma at physiological pH (7.4) was also determined. The extent to which elevated plasma renin substrate increases the velocity of angiotensin I formation was then calculated.

3. In patients with Cushing’s syndrome, glucocorticoid therapy or oral contraceptive use, elevated renin substrate coupled with failure of suppression of circulating renin results in increased angiotensin I formation.

Key words: renin, renin substrate, angiotensin, aldosteronism, glucocorticoids, Cushing’s syndrome, oral contraceptive agents.

Prior studies have emphasized characteristic abnormalities in circulating renin in certain forms of secondary hypertension. In primary aldosteronism plasma renin concentration is suppressed. Hypertension in the accelerated phase or associated with renal artery stenosis is frequently accompanied by striking elevation in plasma renin (Brown Davies, Lever & Robertson, 1965). In patients whose hypertension is associated with oral contraceptive agents or oestrogens, the principal abnormality appears to be an elevation in plasma renin substrate, the glycoprotein on which circulating renin acts to produce angiotensin I (Crane, Harris & Winsor, 1971). Hypertension frequently occurs in patients with Cushing’s syndrome due to adrenal hyperplasia, tumour or to therapy with pharmacological doses of glucocorticoids. In experimental animals both adrenocorticotrophic hormone (ACTH) and glucocorticoids produce an elevation in
plasma renin substrate (Helmer & Griffith, 1951; Haynes, Forsham & Hume, 1953; Lazar & Hoobler, 1971). The following studies were undertaken to analyse the role of circulating renin and its homologous substrate in hypertension associated with steroid excess in man. Plasma renin activity and plasma renin substrate were measured by radioimmunoassay of generated angiotensin I in normal subjects and patients with hypertension associated with primary aldosteronism, Cushing's syndrome, glucocorticoid excess and oral contraceptive agents. The role of changes in plasma renin substrate in adjusting the velocity of renin–substrate reaction in circulating plasma was assessed.

METHODS

Angiotensin I was measured by radioimmunoassay (Haber, Köerner, Page, Kliman & Purnode, 1969). Plasma renin activity (PRA) was measured by the rate of generation of angiotensin I in plasma incubated at 37°C at pH 7.4 by using EDTA, 8-hydroxyquinoline (3.4 mM), and di-isopropylfluorophosphate (5.7 mM), to inhibit angiotensinases and converting enzyme. Plasma renin substrate (PRS) was determined by incubation of a 10 μl sample of plasma containing the previously mentioned inhibitors with an excess of partially purified human renal renin (Haas, Goldblatt, Gipson & Lewis, 1966) for 18 h at 37°C.

The Michaelis constant (K_m) of the reaction between circulating renin and substrate was determined at pH 7.4 to assess the effect of changes in PRS in adjusting the velocity of the renin reaction. Plasma containing renin was made free of substrate by using the procedure of Skinner (1967). The substrate source, plasma from a nephrectomized patient, had a substrate concentration of 1100 ng/ml and no detectable renin activity. The velocities of the reaction between a fixed amount of plasma renin and various concentrations of plasma renin substrate were determined and the K_m derived by a double-reciprocal plot (Lee & Wilson, 1971). The approximate K_m value for the reaction of plasma renin and plasma renin substrate at pH 7.4 was 1000 ng/ml. Standardized PRA (PRA_s) was calculated from the measured PRA and PRS by correction to the normal PRS of 1100 ng/ml by using the formula PRA_s = PRA (530/PRS+0.53) derived from the Michaelis equation (Dixon & Webb, 1964). Patients on glucocorticoid therapy were taking dexamethasone (8–16 mg/day) or prednisone (40–80 mg/day) for a minimum of 3 weeks. All subjects were taking diets with unrestricted sodium unless otherwise stated.

RESULTS

The first two columns of Table 1 show the plasma renin substrate concentrations and plasma renin activities observed in normal subjects, and patients with primary aldosteronism, Cushing's syndrome, glucocorticoid therapy, and oral contraceptive use. Patients with primary aldosteronism had the expected suppression of plasma renin activity while renin substrate was slightly elevated. Patients with Cushing's syndrome or glucocorticoid therapy had elevations of plasma renin substrate to twice normal levels. Plasma renin activity was quite variable in patients with Cushing's syndrome but was below the normal range in only one case. Plasma renin activity in patients with glucocorticoid therapy was clearly elevated, but must be interpreted with reservation as the precise state of sodium balance was not defined in these subjects. The most striking elevation of plasma renin substrate was observed in patients taking oral
contraceptive agents. Plasma renin activity in these subjects was slightly, but not significantly, elevated.

The third column of Table 1 shows the standardized PRA (PRA$_s$) in which the velocity of the renin reaction was corrected to normal substrate concentration. Column 4 of Table 1 shows the percentage of the measured PRA (PRA$_m$) which is due to elevated substrate. In primary aldosteronism the slight elevation of PRS is associated with a small but significant enhancement of the velocity of the renin reaction. However, the suppression of circulating renin keeps angiotensin I formation well below normal. In patients with Cushing’s syndrome, with glucocorticoid therapy and those taking oral contraceptive agents there is significant elevation in the velocity of renin reaction in plasma which is due to increased substrate concentrations in the absence of suppression of plasma renin.

**DISCUSSION**

The rate of formation of angiotensin I in circulating plasma has been assessed in prior studies through the use of assays of plasma renin activity. The role of plasma renin substrate in adjusting the velocity of the renin reaction in plasma has not been entirely clear due to conflicting data concerning the kinetic properties of human renin. Estimates of the $K_m$ value for human renin have varied from 150 to 2000 ng/ml by using partially purified human renal renin at pH 5.7–6.0, the pH optima for renin (Rosenthal, Wolff, Weber & Dahlheim, 1971; Gould & Green, 1971; Lee, 1969). In the present report a $K_m$ value of 1000 ng of AI/ml was determined at the physiological pH of human plasma by utilizing an all plasma system. This permits analysis of the effect of changes in plasma renin substrate on the velocity of the reaction of renin and substrate in the circulation. The relative role of circulating renin and substrate in determining the rate of formation of angiotensin I in hypertension associated with various steroid excess syndromes was examined by utilizing the kinetic data described. In mineralocorticoid excess typified by primary aldosteronism, striking suppression of plasma renin activity was associated with slight elevations of plasma renin substrate. This is in accord with the observations of

---

**Table 1.** Plasma renin activity (PRA) and plasma renin substrate (PRS) in normal subjects and patients with steroid excess syndromes. Results are expressed as means ± SEM. PRA$_m$ is the measured plasma renin activity. PRA$_s$ is the calculated plasma renin activity based on correction to a normal substrate concentration as described in the text.

<table>
<thead>
<tr>
<th>No.</th>
<th>PRS (ng/ml)</th>
<th>PRA$_m$ (ng ml$^{-1}$ h$^{-1}$)</th>
<th>PRA$_s$ (ng ml$^{-1}$ h$^{-1}$)</th>
<th>$\left(\frac{PRA_m}{PRA_s}-1\right)\times 100$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24</td>
<td>1082 ± 44</td>
<td>3·4±0·4</td>
<td>3·6±0·5</td>
</tr>
<tr>
<td>Primary aldosteronism</td>
<td>7</td>
<td>1400 ± 82*</td>
<td>0·3±0·1**</td>
<td>0·3±0·1**</td>
</tr>
<tr>
<td>Cushing’s syndrome</td>
<td>6</td>
<td>2275 ± 441*</td>
<td>7·1±4·5</td>
<td>5·6±3·3</td>
</tr>
<tr>
<td>Glucocorticoid therapy</td>
<td>7</td>
<td>2279 ± 213**</td>
<td>11·2±2·8*</td>
<td>8·8±2·3*</td>
</tr>
<tr>
<td>Oral contraceptive agents</td>
<td>8</td>
<td>4290 ± 438**</td>
<td>4·9±1·6</td>
<td>3·3±1·1</td>
</tr>
</tbody>
</table>

* $P<0·05$; ** $P<0·01$, compared with normal.
Rosset & Veyrat (1971), who have found that desoxycorticosterone administration plus high salt intake produces modest increases in renin substrate accompanied by suppression of renin activity in normal man. In contrast, patients with Cushing's syndrome, glucocorticoid therapy and those taking oral contraceptive agents had striking elevations in plasma renin substrate. Plasma renin activity was on the average above normal in these groups as well. When plasma renin activity was corrected to a standard renin substrate concentration (PRA,) the resultant formation rate of angiotensin I was still elevated in patients with glucocorticoid therapy but was in the normal range in patients with Cushing's syndrome or taking oral contraceptive agents. Thus the striking suppression of plasma renin observed with the mineralocorticoid excess of primary aldosteronism is not seen with either glucocorticoid excess or oestrogenic substances. Further, in patients with Cushing's syndrome, all of whom were normokalaemic, suppression of standardized PRA was observed in only one of six subjects. The failure to observe suppression of plasma renin in patients with Cushing's syndrome is in agreement with the findings of Brown et al. (1965), and suggests that mineralocorticoid excess may not account for the hypertension so uniformly observed in these subjects.

Factors other than renin and/or substrate may alter the rate of formation of angiotensin I. Recent studies have suggested the existence of a renal factor which may inhibit the renin-substrate reaction (Kotchen, Rice & Walters, 1972). If this inhibitor proves to be of a competitive type, it will increase the dependence of the renin-substrate reaction on the substrate concentration. Thus, in the present study the assessment of the added rate of formation of angiotensin I due to increases in plasma renin substrate observed in patients with Cushing's syndrome, glucocorticoid excess, and oral contraceptive agents, may be minimum estimates. Nonetheless, elevations in plasma renin substrate clearly enhance the rate of angiotensin I formation in human plasma and may be related to the pathogenesis of hypertension associated with Cushing's syndrome, glucocorticoid therapy and oral contraceptive agents.

ACKNOWLEDGMENT

The expert technical skills of Miss Ellen Elting and Miss Ellen Sutter are gratefully acknowledged. This research was supported by USPHS Grant HL 13595. Dr L. R. Krakoff is the recipient of a Career Development Award KO 4 HL 46495.

REFERENCES


Haynes, F.W., Forsham, P.H. & Hume, D.M. (1953) Effects of ACTH, cortisone, desoxycorticosterone and
Renin and substrate in steroid hypertension


