Increased Plasma Renin During Renin Inhibition Studies with a Novel Immunoassay

KARL G. HOFBAUER, JEANETTE M. WOOD, NEELAM GULATI, CHRISTOPH HEUSSER, AND JOEL MÉNARD

SUMMARY The response of renin release to the administration of renin inhibitors cannot be studied with conventional enzymatic methods used to measure plasma renin. In the present experiments, a novel multirange enzyme-linked immunosorbent assay for human and primate renin was used to investigate the changes in plasma immunoreactive renin after renin inhibition. A potent and long-acting statine-containing renin inhibitor, CGP 29,287, was injected in conscious marmosets after mild or severe sodium depletion. In mildly sodium-depleted marmosets, CGP 29,287 (0.1 mg/kg i.v.) reduced mean arterial blood pressure and completely inhibited plasma renin activity for up to 30 minutes. This response was associated with a transient increase in plasma immunoreactive renin concentration. After a dose of 1.0 mg/kg i.v., the reduction of mean arterial pressure and the complete inhibition of plasma renin activity persisted for up to 120 minutes. These effects were accompanied by a sustained increase in plasma immunoreactive renin concentration. In severely sodium-depleted marmosets, CGP 29,287 (1.0 mg/kg i.v.) induced a marked fall in systolic blood pressure and complete inhibition of plasma renin activity within 30 minutes of injection. Plasma immunoreactive renin levels increased to 257% of pretreatment values. The converting-enzyme inhibitor enalaprilat (2 mg/kg i.v.) induced a fall in systolic blood pressure of similar magnitude, which was accompanied by an increase in plasma renin activity. Levels of plasma immunoreactive renin increased to 210% of pretreatment values. Hydralazine (0.2 mg/kg i.v.) did not increase plasma renin activity or plasma immunoreactive renin levels despite a comparable hypotensive effect. These results indicate that inhibition of renin or converting enzyme induces a similar increase in renin release that probably is mediated by a common mechanism — the withdrawal of the negative feedback suppression by circulating angiotensin II. (Hypertension 7 [Suppl I]: I-61-I-65, 1985)

KEY WORDS • marmosets • enalaprilat • hydralazine • renin activity • immunoreactive renin • renin release • blood pressure • sodium depletion

THE release of renin from the kidney is under the negative feedback control of circulating angiotensin II. Pharmacological blockade of the formation of angiotensin II by inhibiting converting enzyme eliminates this inhibitory signal and raises plasma renin levels. The response of renin release to inhibitors of renin is not known as conventional enzymatic methods for the determination of renin cannot be used to estimate the amount of renin after administration of renin inhibitors. In the present experiments we used a novel multirange enzyme-linked immunosorbent assay (ELISA) for the determination of renin in human and primate plasma to study the changes in plasma immunoreactive renin (PIR) levels after renin inhibition. A potent and long-acting renin inhibitor, CGP 29,287, was administered in conscious marmosets after various degrees of sodium depletion. In one series of experiments, the relationship between the effects of the renin inhibitor on blood pressure and the concomitant changes in plasma renin activity (PRA) and PIR levels were investigated. In another series of experiments, the changes in PIR levels after renin inhibition were compared with those after converting enzyme inhibition or systemic vasodilatation.

Materials and Methods

Male and female marmosets (Callithrix jacchus) weighing approximately 300 g were used in all experiments. They were fed a pellet diet (NAFAG, Gossau, Switzerland) supplemented with fruit. Mild sodium
depletion was induced by a single injection of furosemide (5 mg/kg i.v.) given before an experiment. Severe sodium depletion was induced by administering a low sodium diet for 10 days and daily injections of furosemide (2 mg/kg i.m.) for 3 days before an experiment.

Mean arterial pressure (MAP) was measured as described previously. Catheters were inserted into a femoral artery and a tail vein and exteriorized at the tail. One to two days after the operation, the marmosets were placed into restraining tubes, the arterial catheter was connected to a pressure transducer (Gould P 23 ID, Oxnard, CA), and blood pressure was continuously recorded (Servomed 130T, Hellige, Freiburg, FRG). Systolic blood pressure (SBP) was determined using the tail-cuff method (W & W 8005, Ugo Basile, Comero, Italy). Marmosets were kept in restraining tubes, and a pneumatic cuff (inner diameter between 7 and 10 mm) and a piezoelectric pressure sensor were positioned around their tail. After blood pressure had stabilized, several readings were taken and the mean values calculated.

Levels of PIR were determined with a solid phase sandwich ELISA as described previously. Microtiter plates were coated with a monoclonal antibody (R-3-36-16 or R-3-27-6) against human kidney renin. Both antibodies showed complete cross-reaction with marmoset renin (Table 1) but did not react with mouse or rat renin (unpublished observations, 1984). After quenching the plates for 1 hour at 37°C with phosphate buffered saline (pH 7.4) containing 1% bovine serum albumin, serial dilutions of a plasma sample were added and incubated in the same buffer for 1 hour at 37°C. Thereafter, the plates were incubated (1 hour at 37°C) with an appropriate dilution of rabbit anti-human renin antiserum (see Table 1), followed by alkaline phosphatase conjugated goat anti-rabbit IgG serum. The plates were thoroughly washed between each of these steps. The reaction was revealed by the substrate p-nitrophenyl phosphate. The optical density, measured at 405 nm (Titertek Multiskan Flow Laboratories, Meckenheim, FRG), is proportional to the amount of renin in the sample. A purified preparation of human kidney renin was used as a standard, and PIR values are expressed as picograms of renin per milliliter of plasma.

The PRA was measured by the rate of angiotensin I formation after incubation (37°C) of plasma at pH 7.2 (TRIS-acetate buffer) in the presence of an angiotensinase inhibitor (2,3-dimercaptopropanol). The angiotensin I formed was determined by radioimmunoassay. Results are expressed as nanograms of angiotensin I per milliliter of plasma per hour of incubation.

All values in the text and the figures are means ± SEM. The statistical significance of differences between mean values was calculated by Student's t test or, where appropriate, paired t test.

Mildly sodium-depleted marmosets received a bolus injection of saline (0.5 ml/kg) or CGP 29 287 (0.1 or 1.0 mg/kg) into the venous catheter. The MAP was continuously recorded for 45 minutes before and for 180 minutes after the injection. At various time intervals, blood samples were collected from the arterial catheter into EDTA-coated tubes (Eppendorf, Hamburg, FRG) for the determination of PRA and PIR levels.

Severely sodium-depleted marmosets received a bolus injection of saline (1.0 ml/kg), CGP 29 287 (1.0 mg/kg), enalaprilat (MK 422; 2.0 mg/kg), or hydralazine (0.2 mg/kg) into a lateral tail vein. Measurements of SBP were taken before and 30 and 60 minutes after injection of compounds. At the same time intervals, blood samples were collected in EDTA-coated tubes after direct puncture of the femoral arteries.

Results

Effects of CGP 29 287

The marmosets that received saline showed a slight, progressive decrease in MAP and PRA during the first 2 hours of the experiment (Figure 1). Levels of PIR remained stable over the entire period of observation (see Figure 1). The renin inhibitor CGP 29 287 in a dose of 0.1 mg/kg i.v. induced a prompt fall in MAP that was accompanied by complete inhibition of PRA 30 minutes after injection (Figure 2). At that time, PIR levels showed a slight increase to 125% of initial values (see Figure 2). Subsequently, MAP and PRA increased and PIR concentration decreased toward control values (see Figure 2).

In a dose of 1.0 mg/kg i.v., the renin inhibitor CGP 29 287 induced a fall in MAP, which was similar to that observed after administration of 0.1 mg/kg, and a complete inhibition of PRA (Figure 3). These changes persisted for up to 120 minutes after the injection. Levels of PIR increased to 147% of initial values 30 minutes after injection and remained elevated (see Figure 3).

Effects of CGP 29 287, Enalaprilat, and Hydralazine

In severely sodium-depleted marmosets PRA was 4.7-fold higher than in the mildly sodium-depleted

---

**Table 1** Characteristics of Antibodies Used in a Multicentre Enzyme-Linked Immunosorbent Assay for Human and Marmoset Renin

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Human (final dilution)</th>
<th>Marmoset (final dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 3-27-6 (M)</td>
<td>5.7 × 10^10</td>
<td>1.0 × 10^10</td>
</tr>
<tr>
<td>R 3-36-16 (M)</td>
<td>1.7 × 10^10</td>
<td>1.3 × 10^10</td>
</tr>
<tr>
<td>Rabbit antiserum</td>
<td>120,000</td>
<td>190,000</td>
</tr>
</tbody>
</table>

\[ IC_{50} = \text{concentration/dilution required to achieve 50% of maximal inhibition of renin activity in human (renin-free plasma with the addition of 250 pg/ml of purified human kidney renin) or marmoset (1.50 dilution) plasma. Plasma renin activity was similar in human and marmoset incubation mixtures (35 and 32 ng of angiotensin I/ml/hr).} \]

BC = concentration/dilution required to achieve 50% of maximal inhibition of renin activity in human (renin-free plasma with the addition of 250 pg/ml of purified human kidney renin) or marmoset (1.50 dilution) plasma. Plasma renin activity was similar in human and marmoset incubation mixtures (35 and 32 ng of angiotensin I/ml/hr).
marmosets described above (147 ± 16, n = 30, versus 31 ± 5 ng of Ang l/m/hr, n = 19; p < 0.001). The PIR concentration was also higher (1658 ± 201, n = 22, versus 936 ± 132 pg/ml, n = 13; p < 0.02), but the difference was only 1.8-fold.

In the severely sodium-depleted marmosets that received saline SBP remained essentially unchanged. The PRA decreased slightly, while PIR levels remained constant (Figure 4). Hydralazine induced a marked and sustained fall in blood pressure. The PRA decreased slightly but consistently. No changes in PIR concentration were observed (see Figure 4). CGP 29 287 induced a fall in SBP that was accompanied by complete inhibition of PRA. Levels of PIR increased to 257 and 276% of initial values 30 and 60 minutes after injection (Figure 5). Enalaprilat induced a hypotensive effect comparable to that found after hydralazine or CGP 29 287 administration. The PRA increased to 193 and 246% of initial values; PIR showed a parallel rise to 210 and 305% 30 and 60 minutes after injection.

**Discussion**

In the present studies a novel ELISA for human and primate renin was used to investigate the changes in renin release after renin inhibition in marmosets. Renin inhibition was induced with CGP 29 287, a statine-containing peptide analogue of the N-terminal sequence of human angiotensinogen. This transition state inhibitor has protection groups at its C-terminal and N-terminal that prolong its biological half-life. The efficacy and specificity of CGP 29 287 and its long duration of action have been demonstrated previously. In the present experiments the i.v. bolus injection of CGP 29 287 in doses of 0.1 and 1.0 mg/kg induced a fall in MAP of comparable magnitude and complete inhibition of PRA. The duration of action of CGP 29 287 was dose dependent. After the low dose, MAP and PRA started to return toward the initial values within 30 minutes of injection, whereas both parameters remained reduced for up to 120 minutes after the high dose. The parallel changes in MAP and PRA indicate that the hypotensive effect of CGP 29 287 was medi-
ed by a reduced formation of angiotensin II in plasma or tissues. Our findings are in contrast to those of Blaue et al.\textsuperscript{11} who observed a marked dissociation between the effects of another statine-containing renin inhibitory peptide, SCRIP, on blood pressure and PRA.

In our experiments PIR concentration increased after administration of the renin inhibitor CGP 29 287. The increase in PIR levels was transient after the low dose of CGP 29 287, which corresponds to the short-lived inhibition of PRA. The increase in PIR levels was sustained after the high dose of CGP 29 287, which corresponds to the persistent inhibition of PRA. An increase in renin release after renin inhibition has been demonstrated by Blaue et al.\textsuperscript{11} in dogs. In their studies, plasma samples were dialyzed before incubation to remove the inhibitor and PRA was determined. These observations are consistent with the assumption that the negative feedback suppression of renin release was eliminated after inhibition of the formation of angiotensin II; however, the fall in blood pressure induced by the renin inhibitor might have been the more important stimulus. Moreover, the increase in renin release might have been the consequence of a direct effect of the renin inhibitor. A negative feedback inhibition by renin itself or by angiotensin I cannot be ruled out by these experiments.

Our comparative studies with a systemic vasodilator and an inhibitor of converting enzyme were done to differentiate between the possible mechanisms involved in the response of renin release to inhibition of renin. The results obtained with hydralazine do not support the assumption that the fall in blood pressure was an important factor in the experiments with the renin inhibitor CGP 29 287. Hydralazine induced a hypotensive effect of similar magnitude to that of CGP.
29 287, but PIR levels remained unchanged and PRA actually decreased. The absence of an increase in both parameters after hydralazine might be due to the fact that the signals by which this vasodilator increases renin release under normal conditions were already maximal after severe sodium depletion. It is also conceivable that the hydralazine-induced fall in blood pressure was too small to stimulate renin release. It has been shown that renin release is more responsive to changes in blood pressure in the lower than in the higher pressure range. The effects of the converting-enzyme inhibitor enalaprilat indicate that withdrawal of the negative feedback suppression of renin release by angiotensin II is the most important factor for the response of renin release to inhibition of renin. Both modes of interference with the renin-angiotensin system induced identical changes in PIR concentration. Since inhibition of renin and converting enzyme induced opposite changes in PRA and plasma angiotensin I concentrations, a contribution of these factors is unlikely.

The increase in PIR levels after administration of CGP 29 287 was more pronounced in marmosets after severe than after mild sodium depletion. This finding might indicate that negative feedback suppression of renin release by angiotensin II is more important after stimulation of the renin-angiotensin system than under normal conditions. Basal PRA values were about five times higher in severely than in mildly sodium-depleted marmosets, but basal PIR values were only increased about twofold. This finding suggests that sodium depletion not only stimulates the overall release of renin from the kidney but also affects the ratio between active and total renin. The precise ratio between active and inactive renin in marmoset plasma has to be determined after standardization of the PRA and the PIR assay with pure marmoset renin. A possible interference of enzymes related to renin in the ELISA cannot be excluded but seems unlikely as the monoclonal antibodies used in the ELISA do not even recognize renin from other species such as mouse and rat.

In summary, the results of the present studies suggest that renin release increases after renin inhibition to the same degree as after converting enzyme inhibition. This response appears to be entirely attributable to the withdrawal of the negative feedback suppression of renin release by circulating angiotensin II. The data obtained in our experiments also demonstrates the value of the direct measurement of plasma renin for studies on the renin-angiotensin system. This novel ELISA, or modifications thereof, should prove valuable for various experimental and clinical applications.

Acknowledgments
We gratefully acknowledge the excellent technical assistance of Mr J Bews, Mr P Forguirini, Mr H P Baum, Mrs M Lartigot, and Mrs E Scheidegger. We thank Miss C Metzger for her expert secretarial assistance.

References
1 Davis JO, Freeman RH. Mechanisms regulating renin release. Physiol Rev 1976;56:1–56
3 Heel RC, Brodgen RN, Speight TM, Avery GS. Captopril: a preliminary review of its pharmacological properties and therapeutic efficacy. Drugs 1980;20:409–452
Increased plasma renin during renin inhibition. Studies with a novel immunoassay.
K G Hofbauer, J M Wood, N Gulati, C Heusser and J Ménard

*Hypertension*. 1985;7:I61
doi: 10.1161/01.HYP.7.3_Pt_2.I61
*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/7/3_Pt_2/I61