The Feedback Regulation of Angiotensinogen Production by Components of the Renin-Angiotensin System

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SUMMARY. The in vivo generation of angiotensin appears to be dependent on both plasma renin and angiotensinogen concentrations. Much less is known about the control of hepatic angiotensinogen synthesis and release, as compared to that of renin. In this study, we examined the feedback regulation of angiotensinogen synthesis and release by various components of the renin-angiotensin system, using an in vitro rat liver slice system. Livers were removed for study from rats which were subjected to various systemic infusions or physiological perturbations. Infusion of angiotensin II has been reported to increase angiotensinogen release rate. However, infusion of angiotensin I (with simultaneous suppression of plasma renin by antirenin antibody infusion and angiotensin II production by captopril administration) had no effect on the angiotensinogen release rate. Direct infusion of renin in rats treated with captopril resulted in further suppression of the angiotensinogen release rate, compared with those given captopril alone. We postulate that renin (or des-angiotensin I-angiotensinogen) inhibits the angiotensinogen release rate, whereas angiotensin II stimulates it. Angiotensin I has no effect on angiotensinogen release rate. This hypothesis was evaluated further in rats with various physiological states. Indeed, when conditions were such that the renin-angiotensin system was completely suppressed, such as binephrectomy and antirenin antibody infusion, angiotensinogen release rate was markedly stimulated. When angiotensin II production was prevented by captopril treatment, angiotensinogen release rate declined. This appeared to be the case for all states of sodium balance. Thus, angiotensinogen release rate is subject to a complex feedback control by other components of the renin-angiotensin system. (Circ Res 52:328–334, 1983)
or renin infusion), while simultaneously suppressing the other components. The results of these experiments demonstrate that the production of angiotensinogen is subject to feedback regulation by plasma renin and angiotensin II.

Methods

Preparation of Liver Slices

Sprague-Dawley rats (Charles River Laboratory) weighing 150–200 g were used in all experiments. Unless otherwise specified below, they had free access to rodent chow (normal diet containing 0.36% sodium) and tap water. Groups of animals underwent the experimental protocols described below and then were decapitated to allow collection of blood in chilled tubes (Vacutainer) containing EDTA. Livers were removed, rinsed in 0.9% saline, and blotted. Liver slices of about 200 mg were obtained with a Stadie-Riggs microtome, as previously described (Herrmann et al., 1980). Slices were rinsed in saline, blotted, and incubated in RPMI 1640 medium (3 ml) at 37°C for 3 hours in an atmosphere of 95% O2-5% CO2. Previous studies demonstrated that these liver slices produced and released angiotensinogen linearly during a 3-hour period and that the production of angiotensinogen was inhibited by actinomycin (Herrmann et al., 1980).

Assays

Angiotensinogen I was measured by radioimmunoassay according to the method of Haber et al. (1969): 125I-labeled [1-Asp,5-Ile]angiotensin I tracer (Bachem) in 0.1 M Tris-HCl (pH 7.4) buffer was added to the unknown sample and then mixed with anti-angiotensin I antibody. After equilibration, free tracer was separated from tracer bound to the antibody by absorption with dextran-coated charcoal. The charcoal was removed by centrifugation and the 125I-Al remaining in the supernatant extract was determined using a Beckman gamma counter. 125I counts/min (cpm) were converted to nanograms (ng) of Al by comparison to known standards. Results are presented as ng Al (0.6856 pmol Al/ng Al). Plasma-renin concentration (PRC) was determined as the amount of Al generated in 1 hour at 37°C in a reaction mixture (total volume of 350 jul) containing the sample (100 jul of plasma), anephric dog plasma (250 jul), dimercaptopropanol, and 8-hydroxyquinolone (0.34 mm) in 0.1 M Tris-HCL, pH 7.4. The concentration of angiotensinogen of anephric plasma was approximately 3600 ng Al equivalent/ml. These conditions represented substrate excess, since Al generation was linear up to 3 hour. Al concentration was determined as described above, and results are expressed as ng Al/ml per hr.

Angiotensinogen concentration was determined by adding 30 jul of excess dog renin [0.1 Goldblatt unit (GU) in 30 jul] to the sample (50 jul of plasma or 500 jul incubation medium) at pH 5.4 and incubating the mixture at 37°C for 1 hour. Generation was stopped by adding cold water (1.0 ml) and boiling for 2 minutes. Al was measured in the supernatant following centrifugation, and the results are expressed as ng Al equivalent/ml of plasma or ng Al equivalent/g liver per 3 hours of release time. The validity of measurement of angiotensinogen exhaustion was demonstrated by incubating plasma (100 jul) from rats nephrectomized 24 hour previously, with renin (0.1 GU). Al generation reached a plateau after 30 minutes of reaction. Beyond this point, no further generation or degradation of Al was observed up to 2 hours. Since no samples in these experiments had an angiotensinogen concentration as high as nephrectomized plasma, 1 hour incubation was performed in all samples.

For experiments in which animals were injected with antirenin IgG, excess circulating antibodies in the plasma of these animals were demonstrated by adding exogenous dog renin (3 × 10^{-4} GU) to the plasma (100 jul) and incubating at 37°C for 1 hour. Excess angiotensinogen (25 jul anephric dog plasma) was then added and the reaction continued for an additional hour. Residual renin activity, as measured by the generation of AI, is expressed as ng Al/ml plasma.

Experimental Treatments

Sodium Balance

So that we might study the effects of sodium depletion, rats were given furosemide (5 mg intraperitoneally) and then fed a low sodium diet containing 0.05% sodium (Purina) and tap water for 10 days. Sodium-loading was accomplished by feeding rats high sodium diet (1.6%) for 10 days. Daily 24-hour urine samples were collected, and the urinary sodium and potassium concentrations were determined by ion-selective electrode analysis (NOVA Biomedical). Results are expressed as mEq/24 hours after multiplying by the total urine volume. The potassium content of the low sodium diet was lower (0.46%) than the normal or high sodium diets (1.06%).

Captopril Administration

The orally active converting enzyme inhibitor captopril (SQ 14225 from Squibb Pharmaceutical Corp.) was administered by gavage (10 mg/100 g body weight per day) or by intraperitoneal injection (2 mg/100 g body weight per day). Both treatments resulted in similar increases in PRC after 10 days; hence, these data were pooled for analysis.

Nephrectomy

Anesthetized rats (sodium pentobarbital, 50 mg/kg) were bilaterally nephrectomized 20–24 hours prior to sacrifice. Care was taken not to disturb the adrenal glands.

Antirenin Antibody Injection

A non-uremic low renin state was induced by injecting rats with renin-specific antibody obtained from a goat immunized with purified canine renal renin (Dzau et al., 1980). The IgG fraction of the goat antisera (CLN 8–23) was prepared from the serum of DEAE column chromatography (Dzau et al., 1982). This IgG pool (100 jul) inhibited 50% of the activity of purified rat renin (5.6 × 10^{-4} GU) at a titer of 1:7500. Animals received a single intracardiac injection (300 jul) of antibody or saline control 6 hours before they were killed. Plasma was collected at the time of sacrifice and assayed for circulating antirenin antibody as described below.

Angiotensin Infusion

Angiotensin I was infused intravenously using Alzet osmotic minipumps (Alza Corp.). The pumps were implanted subcutaneously in the dorsal vertebral region of the anesthetized animal and the outflow catheters were led subcutaneously to the left jugular vein. Prior to implantation, the pumps and the outflow catheters were filled with angiotensin I (2.6 mg/ml saline), or isotonic saline. The solutions then were infused at the rate of 1 jul/hr (43 ng Al/min) for 3 days.
Renin Infusion

To examine the effect of renin on hepatic angiotensinogen production, semi-purified rat renal renin (specific activity 2 Goldblatt units/mg protein), isolated as described (Dzau et al., 1982), was infused into rats via Alzet osmotic minipumps. Two pumps containing $2.2 \times 10^2$ to $7.5 \times 10^2$ ng AI/hr of renin activity per pump were implanted intraperitoneally, resulting in 4 days of renin infusion at a rate of $2.2 \times 10^2$ to $7.5 \times 10^2$ ng AI/hr (44 to 150 Goldblatt milliunits/hr). All animals received intraperitoneal captopril 6 days prior to and throughout renin infusion in order to avoid the generation of angiotensin II by the infused renin. A second parallel group of rats (control) received captopril and a continuous infusion of isotonic saline (2 µl/hr) instead of renin for the same periods.

Statistical Analysis

Results are presented as mean ± se. Statistical evaluation of differences between means or within an experiment was determined by Student's t-test or by two-way analysis of variance.

Results

Sodium Balance

Sodium balance was assessed by measuring urinary sodium per 24 hours on days 4, 7, and 10 of a 10-day experiment (Table 1). All animals were in sodium balance by day 4, with no changes in sodium excretion occurring by day 10. At equilibrium, urinary sodium excretion was $0.05 ± 0.01$ mEq/24 hours in sodium-restricted animals, $5.2 ± 0.2$ mEq/24 hours in sodium-loaded animals, and $1.1 ± 0.2$ mEq/24 hours in control animals eating regular chow ($P < 0.001$ for both groups relative to control).

In rats maintained on a low sodium diet, plasma renin concentration increased from a control level of $3.6 ± 0.7$ to $22.0 ± 5.5$ ng AI/ml per hour ($P < 0.01$) (Fig. 1). Plasma angiotensinogen concentration in the sodium-depleted rats was significantly lower than in the control animals ($1,142 ± 147$ vs. $753 ± 109$ ng AI/hr, $P < 0.001$). The rate of angiotensinogen release (ARR) by liver slices from these animals was not significantly different from that of the control group (Fig. 1). Although plasma renin concentration decreased during sodium loading to $0.6 ± 0.08$ ng/ml per hour, no significant change in angiotensinogen release or PAC occurred.

Effect of Captopril

Captopril was administered to animals on low (n = 8), unrestricted (n = 10), and high sodium diets (n = 6), as described. Efficacy of converting enzyme inhibition was demonstrated by the increase in plasma renin concentration from $3.6 ± 0.7$ to $29.4 ± 6.3$ ng AI/ml per hour ($P < 0.001$) in animals on the normal sodium diet following captopril treatment. Plasma renin concentration also increased in both the sodium-depleted animals ($22.0 ± 5.5$ to $61.2 ± 4.8$ ng AI/ml per hour $P < 0.001$) and the high sodium animals ($0.6 ± 0.08$ to $5.2 ± 1.9$ ng AI/ml per hour, $P < 0.05$) after captopril administration (Fig. 1). Captopril treatment resulted in a decrease in PAC in all states of sodium balance. Rats with normal sodium intake had a PAC of $1142 ± 147$ ng AI/ml which decreased to $646 ± 106$ ng AI/ml after captopril treatment ($P < 0.02$). The PAC in sodium-depleted rats decreased from $753 ± 109$ to $214 ± 40$ ng AI/ml with captopril administration ($P < 0.001$). Similarly, captopril resulted in a decrease in PAC from $820 ± 81$ to $372 ± 106$ ng AI/ml ($P < 0.01$) in high sodium animals

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**FIGURE 1.** Plasma renin concentration, plasma angiotensinogen concentration, and hepatic angiotensinogen production are compared in groups of rats on normal (n = 15), low sodium (n = 12), and high sodium (n = 5) diets, with and without captopril treatment (n = 10, eight or six for the respective diets with captopril treatment). Values are mean ± se. (*P < 0.05, **P < 0.01 relative to control group).
A decrease in the rate of angiotensinogen release (ARR) from 33.0 ± 2.9 to 19.5 ± 1.6 ng/g per 3 hours (P < 0.001) occurred during captopril treatment of rats on normal diet. The ARR by liver slices of sodium-depleted rats treated with captopril was also lower than from control slices (P < 0.005). In the high salt state, ARR from the liver slices also declined in the captopril-treated animals, but this decrease was not statistically significant, possibly due to the smaller number of animals studied on this diet (Fig. 1).

Effect of Nephrectomy

To study the effects of a low renin state, we performed bilateral nephrectomies in six rats 20-24 hours prior to sacrifice. PRC in these animals decreased from 1.97 ± 0.3 ng/ml per hour (after sham surgery) to barely detectable levels (P < 0.05). A large increase in both PAC and ARR was noted relative to sham-operated controls (n = 6) (Fig. 2): PAC increased 8-fold (P < 0.01), and angiotensinogen release increased more than 3-fold (P < 0.01). These results are expressed as percent of control in Figure 2.

Effect of Antirenin Antibody

To separate the effects of uremia from those of a low renin state, we injected antirenin antibody to suppress renin and AI changed in the same directions with captopril. Hence, we isolated the effect of AI by its infusion into animals pretreated with both renin antibody to suppress renin and captopril to prevent the conversion of AI to All. Twelve animals received captopril for 10 days, continuous AI infusion intravenously (43 ng/min) for 3 days, and were injected with antirenin IgG 6 hours prior to sacrifice. These animals were compared with the controls (n = 14) receiving only captopril and antirenin IgG.

In the animals receiving AI infusions, although the plasma AI levels doubled (P < 0.05), the rate of angiotensinogen release and the plasma angiotensinogen concentration were not significantly altered (Fig. 2). The AI levels measured in the medium of the liver slices prior to angiotensinogen determinations were still evident 6 hours after renin antibody injection, as plasma angiotensinogen concentration decreased by 52 and 44%, respectively, from control (Fig. 2). Be-cause the data were pooled from several separate experiments, each with a small number of animals, results were analyzed by two-way analysis of variance and were highly significant (overall P < 0.002). When rates of angiotensinogen release from the liver slices of all of the 29 rats receiving captopril (and renin or saline infusion) were analyzed in relation to their plasma renin concentrations, a significant inverse correlation (r = -0.64, P < 0.001) was observed (Fig. 3). Similarly, plasma angiotensinogen concentration correlated inversely with plasma renin concentration in these rats (r = -0.68, P < 0.001).

Discussion

The factors which regulate renin release have been extensively studied. Feedback suppression of renin
ability, we treated sodium-deplete and sodium-replete rats with captopril. A decrease in plasma angiotensinogen production was observed. Herrmann and Reid et al., 1980). Therefore postulated that the increment of increase in angiotensinogen production was a significant contributing factor in the decline of PAC during sodium depletion. This decrease in hepatic angiotensinogen production may be due to a direct or indirect effect of increased plasma renin on angiotensinogen synthesis.

The mechanism of decreased substrate production with captopril may also be due to decreased circulating AI, resulting in decreased hepatic stimulation either directly or by a secondary decrease in plasma corticosterone level. However, Reid and colleagues have previously demonstrated that glucocorticoids have an unimportant role in angiotensinogen production in dogs (Reid, 1977; Sernia et al., 1980). Since converting enzyme also degrades bradykinin, the observed response could also be due to increased kinins (Swartz et al., 1979). Finally, prostaglandins have been reported to increase with captopril. The contributions of kinins and prostaglandins to the results we obtained were not assessed in our present investigation.

To examine further the contribution of renin and AI to the regulation of substrate production, we studied the effects of suppression of the RAS by nephrectomy and systemic anti-renin antibody administration. PAC and hepatic production of angiotensinogen both increased significantly, confirming the previous findings of Nasjletti et al. (1972) and of Freeman and Rostorfer (1972) in bilaterally nephrectomized animals. Although Freeman and Rostorfer (1972) suggested that the effect of nephrectomy was mediated by an increase in glucocorticoids occurring as part of a stress reaction, this is unlikely, since bilateral nephrectomy performed 7 days after bilateral adrenalectomy also results in an increased plasma angiotensinogen concentration (Tateishi et al., 1971; Carretero and Gross, 1967).

Thus, either renin or AI appears to exert an inhibitory effect on angiotensinogen production. To address this question further, we isolated the effect of AI by infusing the decapeptide into rats in which both renin and AI production were blocked by the administration of antirenin antibodies and captopril, and observed that AI failed to alter angiotensinogen production. This result differs from that of Nasjletti and Masson, who showed increased substrate production when AI was infused into the isolated perfused liver (Nasjletti et al., 1973). Since converting enzyme is present in the liver (Roth et al., 1969; Huggins and Thampi, 1968), intrahepatic conversion of AI to All may account for the increased angiotensinogen release in their experiments.

Direct evidence that renin suppresses hepatic angiotensinogen production was obtained in the renin infusion experiments. Indeed, angiotensinogen from liver slices appears to be affected by plasma renin concentration in a dose-related fashion (Fig. 3). The

![Graph](http://circres.ahajournals.org/DownloadedFrom.png)

**FIGURE 3.** Inverse relationship of plasma renin concentration and hepatic angiotensinogen release in all rats (n = 29) receiving intraperitoneal renin or saline infusion along with captopril administration (r = -0.64, P < 0.001).
mechanism by which renin suppressed substrate production is unclear. One mechanism could involve des-(angiotensin I)-angiotensinogen, a product of renin-angiotensinogen reaction, whose concentration is dependent on renin activity, (Bouhnik et al., 1981) and might exert feedback control on its precursor, angiotensinogen. Alternatively, renin may have a direct inhibitory action on hepatocytes or may inactivate an endogenous circulating angiotensinogen-stimulating factor. The existence of such a factor was first postulated by Hasegawa et al. (1973), when they noted an increased rate of substrate synthesis by rat liver slices after incubation with plasma of nephrectomized animals in vitro. Indeed, those investigators subsequently isolated this factor and demonstrated that its activity was destroyed upon incubation with renin (Hasegawa et al., 1976).

Finally, it has been suggested that PAC may have a negative feedback effect on angiotensinogen production (Reid et al., 1974). However, based on our data, this effect must be relatively unimportant, since PAC and hepatic ARR were always directly related. In no circumstances did we observe an increased PAC associated with depressed hepatic ARR or vice versa.

The results obtained in this investigation demonstrate that renin decreases PAC by the consumption of plasma angiotensinogen, as well as by exerting an inhibitory effect on hepatic angiotensinogen synthesis and release. Angiotensin II, the final product of the cascade, stimulates hepatic ARR. However, angiotensin I has no effect on the synthesis or consumption of plasma angiotensinogen (Fig. 4). On the basis of these conclusions, the effect of various physiological perturbations in this study can be explained (Table 2):

1. During complete suppression of the RAS by nephrectomy or antirenin antibody, the net effect is increased hepatic ARR. This is consistent with a tonic inhibitory effect of renin on hepatic angiotensinogen synthesis and cannot be explained solely on the basis of induced changes in plasma corticosterone or All level.

2. In sodium-loaded animals, the RAS is less extensively suppressed, and a balanced effect between renin and All on hepatic angiotensinogen production is observed.

3. In sodium-deplete animals, whose RAS is stimulated, a balanced effect on hepatic angiotensinogen production is also observed. However, elevated PRC leads to increased consumption of plasma angiotensinogen, resulting in a net decrease in PAC.

4. Captopril increases PRC and decreases All. Elevated PRC results in decreased production and increased consumption of angiotensinogen. The decrease in All also leads to decreased angiotensinogen production. This appears to be the case for all states of sodium balance.

5. Steroid hormones increase hepatic angiotensinogen production and PAC. Studies of physiological perturbations of angiotensinogen production should, therefore, include the measurement of plasma corticosterone levels.

Thus, the interrelationships between components of the renin-angiotensin system are quite complex, and in this paper we have demonstrated these relationships as they influence angiotensinogen production. Further delineation of the factors which regulate angiotensinogen production may be important in blood pressure control, and continues to be an important area of research.

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References

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**TABLE 2**

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<th>Renin infusion</th>
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<th>All infusion</th>
<th>Nephrectomy</th>
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* Hepatic rate of angiotensinogen release.
† Plasma angiotensinogen concentration.

**FIGURE 4.** Schematic representation of the factors which regulate angiotensinogen production. Stimulating factors are shown by solid lines, inhibitory factors by dashed lines.
INDEX TERMS: Renin-angiotensin system • Angiotensinogen biosynthesis • Regulation of angiotensinogen • Plasma angiotensinogen • Liver slice
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