Physiological elevation in plasma angiotensinogen increases blood pressure

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Received 16 January 2001; accepted in final form 21 June 2001

Klett, Christoph P. R., and Joey P. Granger. Physiological elevation in plasma angiotensinogen increases blood pressure. Am J Physiol Regulatory Integrative Comp Physiol 281: R1437–R1441, 2001.—Hepatic angiotensinogen secretion is controlled by a complex pattern of physiological or pathophysiological mediators. Because plasma concentrations of angiotensinogen are close to the Michaelis-Menten constant, it was hypothesized that changes in circulating angiotensinogen affect the formation rate of ANG I and ANG II and, therefore, blood pressure. To further test this hypothesis, we injected purified rat angiotensinogen intravenously in Sprague-Dawley rats via the femoral vein and measured mean arterial blood pressure after arterial catheterization. In controls, mean arterial pressure was 131 ± 2 mmHg before and after the injection of vehicle (sterile saline). The injection of 0.8, 1.2, and 2.9 mg/kg angiotensinogen caused a dose-dependent increase in mean arterial blood pressure of 8 ± 0.4, 19.3 ± 2.1, and 32 ± 2.4 mmHg, respectively. In contrast, the injection of a purified rabbit anti-rat angiotensinogen antibody (1.4 mg/kg) resulted in a significant decrease in mean arterial pressure (−33 ± 3.2 mmHg). Plasma angiotensinogen increased to 769 ± 32, 953 ± 42, and 1,289 ± 79 pmol/ml, respectively, after substrate and decreased by 361 ± 28 pmol/ml after antibody administration. Alterations in plasma angiotensinogen correlated well with changes in plasma renin activity. In summary, variations in circulating angiotensinogen can result in changes in blood pressure. In contrast to renin, which is known as a tonic regulator for the generation of ANG I, angiotensinogen may be a factor rather important for long-term control of the basal activity of the renin-angiotensin system.

renin-angiotensin system; Sprague-Dawley rat; hypertension; plasma renin activity; anti-rat angiotensinogen antibody

THE RENIN-ANGIOTENSIN SYSTEMS both in blood and tissues (15, 20) are recognized as important effector systems for the regulation of blood pressure and salt and water homeostasis (8, 19). They have been implicated as a pathogenic factor in selected forms of primary or secondary hypertension (10, 16, 21). In the circulation, plasma concentrations of angiotensinogen are believed to directly influence the system’s activity, because substrate concentrations are within the concentration range of the $K_m$ value of the substrate-enzyme reaction, which is, under most physiological or pathophysiological conditions, the rate-limiting step of the enzymatic cascade of the circulating renin-angiotensin system (1). The subsequent conversion of ANG I to active ANG II occurs rapidly and is generally not limiting because the angiotensin-converting enzyme is ubiquitously distributed. Several experimental findings confirmed the relationship between substrate and ANG I/ANG II formation. For example, selective knockout of angiotensinogen in mice (9) has been shown to decrease resting blood pressure in proportion to the number of alleles present in mice, and transfection of antisense oligonucleotides directed against exon 1 of the angiotensinogen gene induced a transient decrease in blood pressure in rats (24). In clinical studies of all components of the renin-angiotensin system, only mutations of the angiotensinogen gene have been reported to be associated with elevated blood pressure in humans, including the variants M235T and T174M (7, 14). These published findings on the genomic DNA, mRNA, or protein level support the hypothesis of a functional linkage between the expression of the substrate for renin and changes in blood pressure. However, until the present it was not clear how direct changes of substrate in the circulation on the protein level affect blood pressure. We have, therefore, in the current study injected physiological amounts of angiotensinogen in the Sprague-Dawley rat and monitored blood pressure changes. In addition, we analyzed how the observed changes in blood pressure are related to alterations in plasma concentrations of angiotensinogen and plasma renin activity.

METHODS

Animals. Male Sprague-Dawley rats, body weight 300–350 g, were used in the experiments. Animals were kept under 24-h dark-light cycles, fed a standard rat chow, and had free access to water.

Animal instrumentation and blood pressure measurement. Blood pressure was measured in conscious chronically instrumented rats after catheterization of the femoral vein and carotid artery. Five days before experimentation, animals were anesthetized with isoflurane (4%) and surgically instrumented with a femoral venous (drug application) and carotid...
arterial (measurement of mean arterial blood pressure and blood withdrawal) catheter (PE-50) under aseptic conditions. Catheters were exteriorized at the nape of the neck and filled with heparin. The arterial line was connected to a pressure transducer and an oscillographic recorder (Grass, Quincy, MA), which records pulsatile and mean arterial pressure (MAP).

Experimental protocol for intravenous injections. The injection protocol was started after stabilization of blood pressure for 20–30 min. Subsequently, three different dosages (0.8, 1.2, and 2.9 mg/kg) of angiotensinogen were administered intravenously. Blood pressure was monitored for 45 min followed by the injection of 1.4 mg/kg of an anti-rat angiotensinogen antibody. Plasma angiotensinogen concentrations increased to 10.2 ± 0.3 mmHg on average. Times of injections are indicated by arrows.

Determination of plasma angiotensinogen. Angiotensinogen plasma concentrations were measured indirectly by quantitative conversion of angiotensinogen to ANG I with an antibody obtained from Chemicon (Los Angeles, CA). To analyze whether the observed changes in blood pressure were associated with alterations in circulating substrate, we determined plasma angiotensinogen concentrations.

Isolation of rat angiotensinogen. Rat angiotensinogen was isolated from plasma of nephrectomized rats according to a protocol described by Hackenthal and Hilgenfeldt in 1979 and 1980 (5). For improvement of the purity of the angiotensinogen preparation, an HPLC molecular weight column (GF-250, Zorbax) was added as an additional separation step. Angiotensinogen-containing fractions were detected by an indirect radioimmunoassay of ANG I (as described above) and by SDS-PAGE.

Anti-rat angiotensinogen antibody. Polyclonal antibodies against angiotensinogen were generated following a protocol of Vaitukaitis and colleagues (25). Antisera obtained after the last booster injection were subjected to protein A-Sepharose (Pharmacia, Piskataway, NJ) chromatography for isolation of the IgG fractions.

RESULTS

To clarify the importance of substrate concentrations for the generation of ANG I/ANG II, we first injected varying amounts of angiotensinogen intravenously into Sprague-Dawley rats subsequent to a 20- to 30-min control and adaptation period. The administration of the vehicle, 0.3 ml sterile saline, did not significantly change MAP (data not shown). The injection of 0.8, 1.2, or 2.9 mg/kg angiotensinogen resulted in dose-related increases of MAP of 8 ± 1.4 (P ≤ 0.05), 19.3 ± 2.1 (P ≤ 0.01), and 32 ± 2.4 mmHg (P ≤ 0.005), respectively (Fig. 1). Maximum effects were obtained 30–40 min after intravenous administration of renin substrate. Subsequent to the clearance period, 45 min after substrate injection, blood pressure started to decrease in all experimental groups. At this time rats of all three experimental groups were intravenously injected with 1.4 mg/kg purified anti-rat angiotensinogen antibody. The administration of the antibody resulted in a decrease of MAP by 28 ± 2.9, 37 ± 3.2, and 32 ± 3.9 mmHg in the experimental groups previously injected with 0.8, 1.2, or 2.9 mg/kg angiotensinogen, respectively. No significant differences were apparent between the experimental groups with regard to the net decrease in blood pressure after the injection of the same amounts of antibody (Fig. 1).

To analyze whether the observed changes in blood pressure were associated with alterations in circulating substrate, we determined plasma angiotensinogen concentrations. Before the injection of angiotensinogen, rats had plasma angiotensinogen concentrations of 683 ± 33, 695 ± 49, and 651 ± 53 pmol/ml in the three experimental groups (Fig. 2) selected to, subsequently, receive intravenous injections of 0.8, 1.2, or 2.9 mg/kg angiotensinogen. After the injection of substrate, plasma angiotensinogen concentrations increased to 1500 ± 150, 1500 ± 150, and 1500 ± 150 pmol/ml, respectively.
administration of antibody (1.4 mg/kg). The injection of antibody resulted in a significant (P = 0.005) decrease in plasma renin activity to 14.7 ± 1.3 (P = 0.1), 28.0 ± 3.5 (P = 0.005), and 46.8 ± 3.1 ng ANG I·mL⁻¹·h⁻¹ (P = 0.001), respectively, and the administration of antibody resulted in a significant decrease (P = 0.005) to 6.2 ± 1.4, 12.9 ± 2.0, and 26.4 ± 2.9 ng ANG I·mL⁻¹·h⁻¹, respectively (Fig. 3).

**DISCUSSION**

Renin-angiotensin systems in tissues and in the circulation play a critical role in blood pressure regulation and salt and water homeostasis (8, 15, 16, 19, 20). They have been discussed as a pathogenic factor in many forms of primary or secondary forms of hypertension. The starting point of the endocrine system is the prohormone of ANG I, angiotensinogen (23). Plasma renin activities were 11.7 ± 1.2 (0.8 mg/kg angiotensinogen), 14.5 ± 1.8 (1.2 mg/kg angiotensinogen), and 13.9 ± 2.1 ng ANG I·mL⁻¹·h⁻¹ (2.9 mg/kg angiotensinogen) before the injection of substrate. The intravenous administration of angiotensinogen increased plasma renin activity to 14.7 ± 1.3 (P = 0.1), 28.0 ± 3.5 (P = 0.005), and 46.8 ± 3.1 ng ANG I·mL⁻¹·h⁻¹ (P = 0.001), respectively, and the administration of antibody resulted in a significant decrease (P = 0.005) to 6.2 ± 1.4, 12.9 ± 2.0, and 26.4 ± 2.9 ng ANG I·mL⁻¹·h⁻¹, respectively (Fig. 3).

In accordance with these experimental studies are clinical observations that report an association of mutations in the angiotensinogen gene with elevated blood pressure (7, 14) or preeclampsia (6). With regard to blood pressure regulation, at present it is not clear how these identified mutations interfere with the kinetics of the cleavage reaction through renin, the rate-limiting step of the enzymatic cascade of the circulating renin-angiotensin system. However, in preeclampsia, the identified mutation (L10F) is part of the cleavage site identified by renin and causes changes in the kinetics of the cleavage reaction. Taken together, all the observed changes in angiotensinogen at the genomic or mRNA level in human and rat seem to support the hypothesis of a functional link between the expression of the substrate for renin and changes in blood pressure and its potential implication in the pathogenesis of hypertension in the spontaneously hypertensive rat and in humans. However, up to now nobody has addressed or confirmed the importance of circulating angiotensinogen protein for the adjustment of ANG II formation rate. This would be of importance because many physiological and pathophysiological factors are known to interfere with hepatic angiotensinogen secretion at the transcriptional or posttranscriptional level (1, 12). In a letter to the Editor, Mendard and colleagues (13) mentioned that they observed an increase in blood pressure in rats on a low-salt diet. In rats on a regular diet they did not see a significant change in blood pressure after administration of the same dosage of angiotensinogen. The brief comment did not provide data on plasma renin activity. The dosage of 250 μg/rat used in their study may correspond to our lowest dosage, which resulted in very moderate increases in blood pressure, plasma concentrations of angiotensinogen, and plasma renin activity in rats on a normal diet. Because of the lack of critical information and because of the experimental and clinical evidence for an important role of substrate in the production of the vasoactive effector peptide ANG II, we have, therefore, in our current study addressed this.

![Fig. 3. Plasma renin activity before and after the administration of angiotensinogen or anti-rat angiotensinogen antibody. Plasma renin activity was analyzed after a self-incubation protocol. ANG I generated during this time period was measured by a radioimmunoassay specific for ANG I. Blood samples were taken before (20 min) and 30 min after the injection of angiotensinogen, as well as 10 min after the administration of antibody (1.4 mg/kg).](http://ajpregu.physiology.org/)
issue in a greater detail. We injected angiotensinogen in various concentrations and monitored blood pressure changes, angiotensinogen plasma concentrations, and, most importantly, plasma renin activity. The injection of angiotensinogen dose dependently increased blood pressure in the Sprague-Dawley rat. Analysis of plasma angiotensinogen concentrations and plasma renin activity clearly indicated that the dosages used in the experiment correspond to plasma concentrations observed during various pathophysiological situations, such as nephrectomy or inflammation (11), or elevated glucocorticoid or estrogen plasma levels (10). In these studies plasma concentrations of angiotensinogen increased approximately two- to threefold subsequent to the administration of estradiol or dexamethasone, respectively. Nephrectomy usually results in 2- to 2.5-fold increases in plasma concentrations of angiotensinogen, and the administration of interleukin-6 led to a 2.5-fold increase. The highest amounts of angiotensinogen administered in our current experiments resulted in 1.8- to 1.9-fold increases in angiotensinogen plasma concentrations. These increases in circulating angiotensinogen are within the range of the changes observed in vivo. Results from our current study indicate a clear dose-response relationship between circulating angiotensinogen concentrations and mean arterial blood pressure. In addition, the administration of constant amounts of anti-rat angiotensinogen antibody resulted in similar decreases of blood pressure in all experimental groups independently of the amount of angiotensinogen injected before the administration of antibody. Our current data, therefore, indicate that changes (increase or decrease) in the amount of circulating angiotensinogen can indeed affect plasma renin activity and, therefore, blood pressure in the Sprague-Dawley rat.

Angiotensinogen secretion is sensitive to glucocorticoids, estrogens, mineralocorticoids, insulin, and glucagon (1, 10, 21). Additionally, inflammatory mediators, e.g., interleukin-1 or -6, as well as tumor necrosis factor, have been identified as regulatory elements (11, 22), although the role of angiotensinogen as an acute-phase protein is not clear at present. An important and well-recognized (12) positive-feedback regulator of angiotensinogen formation is the active peptide product, ANG II. This mechanism is believed to restore the plasma pool of angiotensinogen during pathophysiological situations of increased consumption of substrate and depends on a posttranscriptional (RNA stabilization) mechanism. Abnormalities of this important feedback mechanism may be involved in the pathophysiology of certain forms of primary or secondary hypertension. In the mouse, Kim and colleagues (9) reported an increase in blood pressure of ~8 mmHg per angiotensinogen gene copy, resulting in a difference of 16 mmHg between the two-copy and four-copy mouse while angiotensinogen plasma concentrations are elevated 1.45-fold. No data on plasma renin activity are available from this study. In our study we observed an increase in mean arterial blood pressure of 19 mmHg while plasma concentrations are elevated 1.4-fold after the injection of 1.2 mg/kg substrate. Our data seem to be comparable, at least for this dosage, to the effects observed in the mouse, although one has to anticipate different kinetics for the cleavage reaction in mice. The biochemical characteristics of both systems indicate that angiotensinogen is the rate-limiting factor in the mouse, while in the rat both renin and angiotensinogen are expected to be rate limiting.

In the rat as in the mouse, angiotensinogen has an influence on the formation rate of ANG I and ANG II. However, regulatory mechanisms for the generation of ANG II signaling through angiotensinogen production cannot be considered a very sensitive and tonic control of the system’s activity because of the relatively long time lag needed for angiotensinogen secretion to respond to regulatory input. Such a role is recognized for renin, which is rapidly and effectively released from secretory granula. Angiotensinogen may rather play a role in mechanisms for long-term adjustment of the system’s basic activity. Data from our current study demonstrate that acute changes in circulating angiotensinogen indeed have an effect on blood pressure regulation. Because, however, blood pressure regulation is a complex multifactorial process with considerable counterregulation to be anticipated, it is difficult to extrapolate long-term effects of elevated angiotensinogen plasma concentrations at this time. Future studies, including continuous infusions of angiotensinogen, remain to be done to address this important issue.

**Perspectives**

ANG II has been implicated in the etiology of genetic hypertension in humans and animals. Initial studies on the importance of the renin-angiotensin system, mostly conducted in dogs, led to the conclusion that renin is the only rate-limiting component in the enzymatic cascade. However, more recent studies on the genetic level indicated a strong association between mutations of the angiotensinogen gene and the pathogenesis of hypertension in rats and humans. In addition, cleavage kinetics for the enzyme-substrate reaction have been proven to be different in dogs compared with humans and rats. Our current study in rats demonstrated that the rate of formation of ANG I and ANG II is regulated in part by the availability of angiotensinogen, which is in accordance with the biochemical characteristics of the enzymatic cascade of the renin-angiotensin system. The current study used bolus injections of angiotensinogen to demonstrate its effect on blood pressure. Future studies remain to be done to address long-term effects of elevated angiotensinogen plasma concentrations on cardiovascular parameters to assess the importance of angiotensinogen for physiological blood pressure regulation and for the pathophysiology of several forms of primary and secondary hypertension.

This study was supported in part by Grant-in-Aid 9950874V from the American Heart Association, Southeast Affiliate (to C. P. R. Klett), by National Heart, Lung, and Blood Institute (NHLBI) Grant HL-33947 (to J. Granger), and by NHLBI Program Project Grant HL-51971.
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