The Brain Renin-Angiotensin System Modulates Angiotensin II–Induced Hypertension and Cardiac Hypertrophy

Ovidiu Baltatu, José Antonio Silva, Jr, Detlev Ganten, Michael Bader

Abstract—The potential involvement of the brain renin-angiotensin system in the hypertension induced by subpressor doses of angiotensin II was tested by the use of newly developed transgenic rats with permanent inhibition of brain angiotensinogen synthesis [TGR(ASrAOGEN)]. Basal systolic blood pressure monitored by telemetry was significantly lower in TGR(ASrAOGEN) than in Sprague-Dawley rats (parent strain) (122.5 ± 1.5 versus 128.9 ± 1.9 mm Hg, respectively; P < 0.05). The increase in systolic blood pressure induced by 7 days of chronic angiotensin II infusion was significantly attenuated in TGR(ASrAOGEN) in comparison with control rats (29.8 ± 4.2 versus 46.3 ± 2.5 mm Hg, respectively; P < 0.005). Moreover, an increase in heart/body weight ratio was evident only in Sprague-Dawley (11.1%) but not in TGR(ASrAOGEN) rats (2.8%). In contrast, mRNA levels of atrial natriuretic peptide (ANP) and collagen III in the left ventricle measured by ribonuclease protection assay were similarly increased in both TGR(ASrAOGEN) (ANP, ×2.5; collagen III, ×1.8) and Sprague-Dawley rats (ANP, ×2.4; collagen III, ×2) as a consequence of angiotensin II infusion. Thus, the expression of these genes in the left ventricle seems to be directly stimulated by angiotensin II. However, the hypertensive and hypertrophic effects of subpressor angiotensin II are at least in part mediated by the brain renin-angiotensin system. (Hypertension. 2000;35[part 2]:409-412.)

Key Words: renin-angiotensin system ■ collagen ■ angiotensin II ■ hypertrophy ■ atrial natriuretic peptide

The renin-angiotensin system (RAS) is acknowledged to play an important role in the pathophysiology of hypertension and cardiovascular diseases. Since tissue RASs have been postulated, local formation of angiotensin II (Ang II) is consistently invoked to explain that RAS inhibitors can exert beneficial effects in cardiovascular diseases, even in the absence of Ang II plasma levels that directly increase blood pressure (BP).1 The mechanisms by which increases in plasma Ang II can induce an increase in BP in these situations are still not clearly defined. One experimental animal model designed to mimic human hypertension that is often used to obtain insights regarding its pathophysiological mechanisms is attained by chronic infusion (days to weeks) of subpressor doses of Ang II.2 Infused doses of Ang II up to 250 ng/kg per minute SC that do not produce direct vasoconstriction are described as “subpressor” or “slow pressor” and can induce a gradual increase of BP. Several studies have indicated that the central nervous system is involved in the effects of the subpressor Ang II. Arguments are based on the elimination of the hypertensive effect of Ang II by ablation of area postrema3 or lateral parabrachial nucleus.4 Furthermore, the hypertensive effect of subpressor doses of Ang II can be inhibited by central sympathoinhibitors,5 ganglionic blockers,6 nonselective α-blockade,7 or renal denervation,8 supporting a neurogenic pressor mechanism.

The role of the brain RAS in the central control of cardiovascular homeostasis and pathophysiology is well documented.9 Complementing and interacting with the endocrine RAS, the brain RAS is postulated to contribute to the pathology of hypertension and cardiovascular diseases.10 Although a role of the brain RAS has been taken into consideration for the hypertension induced by subpressor Ang II,11 the study was limited by the fact that a complete blockade of all brain angiotensin receptors cannot be achieved by a pharmacological approach.

In the present study we sought to investigate the relative importance of the brain RAS in the hypertension induced by subpressor doses of Ang II by employing a recently established transgenic rat model with low levels of brain angiotensinogen [TGR(ASrAOGEN)].12 In this study the rate of Ang II infusion (100 ng/kg per minute) was chosen to best mimic renal hypertension.13 The BP evolution was monitored. We then evaluated the Ang II–induced heart hypertrophy and the gene expression of atrial natriuretic peptide (ANP) and collagen III in the left ventricle (LV).

Methods

Animals
Adult (aged 5 months) male transgenic rats [TGR(ASrAOGEN)] (n = 15 rats) and age-matched Hanover Sprague-Dawley rats (parent...
strain used as normal controls) (n=15 rats) were obtained from the animal breeding facilities of the Max-Delbrück Center for Molecular Medicine, Berlin, Germany. The rats were synchronized to a 12-hour light/dark cycle (light: 6 AM to 6 PM, 200 lux; dark: 6 PM to 6 AM, <0.1 lux), at ambient temperature 23°C, 60.1 lux), at ambient temperature 23°C. A standard rat diet (ssniff R-ZUCHT) and tap water were supplied at libitum.

**Experimental Protocols**

The experimental protocols were performed in accordance with the guidelines for the human use of laboratory animals by the Max-Delbrück Center for Molecular Medicine. Studies were designed to compare the effects of slow-pressor-dose Ang II infusion on BP, heart weight/body weight ratio, and specific gene expression between TGR(ASrAOGEN) and Sprague-Dawley rats. BP was continuously monitored by radio telemetry, as described previously.12 After implantation of the telemetry transmitters, the rats were randomly separated into 2 groups: 1 received Ang II by osmotic pump for 7 days, and the other served as control group without Ang II infusion. Osmotic minipumps (Alzet, model 2001, Alza Corp) with an infusion rate of 1 μL/h for 7 days were filled with Ang II (Bachem) dissolved in 0.9% saline and were implanted under light ether anesthesia subcutaneously in the retroscapular area. The concentration of the Ang II solution was calculated to allow an infusion rate of 100 ng Ang II per kilogram per minute. At the end of the Ang II infusion period, the rats were killed and the heart was removed, washed in 0.9% saline and weighed. Then the LV was carefully separated from the right ventricle and atria, weighed, and snap-frozen in liquid nitrogen for RNA extraction and gene expression studies. As marker for the efficiency of the Ang II infusion, plasma renin activity was measured by radioimmunoassay in trunk blood collected at the end of minipump infusion.

**Gene Expression Studies**

Total RNA was isolated from the LVs with TRIzol reagent (Life Technologies) followed by chloroform-isopropanol extraction, according to the protocol of the manufacturer. Specific mRNAs for rat ANP, collagen III, and β-actin were quantified by ribonuclease protection assay (RPA) with the use of the Ambion RPA II kit (AMS Biotechnology), as described previously.14

**Statistical Analysis**

Data were analyzed by independent samples t test for comparison of 2 groups or by the general linear model—general factorial or repeated-measures procedure (software SPSS 8.0) for multigroup and multifactorial analysis. The criterion for significant differences between groups of study was \( P<0.05 \). Results are expressed as mean±SE.

**Results**

**Effect of Ang II Infusion on BP**

The systolic BP of TGR(ASrAOGEN) rats obtained in basal (not stressed) conditions was significantly lower in comparison to Sprague-Dawley rats. Ang II infusion for 7 days at the rate of 100 ng/kg per minute produced a gradual increase of systolic BP, which was significantly less pronounced in TGR(ASrAOGEN) rats (Figure 1). The overall rise of systolic BP (Figure 2), calculated by subtracting 2-day mean basal values from the mean values of the last 2 days at the end of Ang II infusion, was significantly attenuated by subtracting 2-day mean basal values from the mean values of the last 2 days at the end of Ang II infusion, which was significantly less pronounced in TGR(ASrAOGEN) rats (Figure 1). The overall rise of systolic BP (Figure 2), calculated by subtracting 2-day mean basal values from the mean values of the last 2 days at the end of Ang II infusion, was significantly attenuated in TGR(ASrAOGEN) compared with Sprague-Dawley rats. Renin activity in the plasma collected from animals at the end of Ang II infusion reached the lower limits of detection in both strains, indicating that the minipumps worked efficiently (Figure 3).

**Cardiac Alterations Caused by Ang II Infusion**

The heart weight/body weight ratio (heart index, g/100 g body wt) (Figure 4A) and LV/body weight ratio (LV index, g/100 g body wt) (Figure 4B) were significantly increased by Ang II infusion in Sprague-Dawley rats but not in TGR(ASrAOGEN). The heart index increased by 11.1% in Sprague-Dawley rats (\( P<0.05 \)) and 2.8% in TGR(ASrAOGEN) rats (\( P>0.05 \)), and the LV index increased by 11.8% in Sprague-Dawley rats (\( P<0.05 \)) and 4.9% in TGR(ASrAOGEN) rats (\( P>0.05 \)). In unstimulated conditions, there were no differences between the
Determintaions of the LV mRNA levels for ANP and collagen III indicated a significant increase caused by Ang II infusion in both Sprague-Dawley and TGR(ASrAOGEN) rats. LV ANP mRNA levels increased 2.4-fold in Sprague-Dawley rats and 2.5-fold in TGR(ASrAOGEN) rats ($P<0.005$) (Figure 5A). LV collagen III mRNA levels were elevated 2.0-fold in Sprague-Dawley rats and 1.8-fold in TGR(ASrAOGEN) rats ($P<0.005$) (Figure 5B). There were no differences in the mRNA levels of either ANP or collagen III between Sprague-Dawley and TGR(ASrAOGEN) rats.

**Discussion**

A large number of studies have focused on determining the contribution of the brain RAS to the regulation of cardiovascular and fluid-electrolyte homeostasis as classically described functions of the endocrine RAS. Brain Ang II increases BP, thirst, sodium appetite, and vasopressin release, causes sympathetic activation, and modulates the baroreflex control. These functions are partially mediated by interactions of Ang II with neuroendocrine processes, eg, the hypothalamo-pituitary axis, or with classical neurotransmitters, such as noradrenaline, acetylcholine, serotonin, dopamine, and opioids. Although many pharmacological studies indicate multiple roles of Ang II in complex brain physiology, further investigations are necessary to establish the relative significance of the RAS in the proposed processes. Progress in genetic and transgenic technology has yielded meaningful contributions to our understanding of the RAS. In this study we used a recently produced transgenic model expressing an antisense RNA against angiotensinogen mRNA specifically in the brain to test the potential participation of brain RAS on the hypertension induced by slow-pressor dose of Ang II. This rat model is characterized by an up to 90% reduction of angiotensinogen levels throughout the brain, hypotension, and low plasma vasopressin levels.

As previously demonstrated, the basal levels of BP were significantly reduced in TGR(ASrAOGEN) rats in comparison to the parent strain. The analysis of BP showed an increase in systolic BP on the first day after the start of Ang II infusion, which remained elevated during the 7-day infusion period. A major finding of this study is that the TGR(ASrAOGEN) rats failed to reach the same levels of hypertension. This indicates that normal activity of the brain RAS is necessary for the full development of hypertension induced by slow-pressor doses of Ang II. This rat model is characterized by an up to 90% reduction of angiotensinogen levels throughout the brain, hypotension, and low plasma vasopressin levels.

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ity of the central sympathetic nervous system as one possibility for the observed alterations on the BP during Ang II infusion. This possibility is supported by evidence for a role of the RAS in long-term modulation of sympathetic activity

and by the observation that neonatal sympathectomy prevents Ang II–induced hypertension. Thus, further studies are necessary to test this possibility. The fact that the BP increase by subpressor Ang II was not completely abolished in TGR(ASrAOGEN) rats may be due to the incomplete inhibition of brain angiotensinogen synthesis in these rats. Moreover, additional mechanisms could be responsible for the Ang II–induced hypertension, such as activation of the sympathetic nervous system in the periphery,

trropic stimulation of cardiovascular tissues, autotopotivation of vasoconstrictor responses by Ang II, or tissue-specific induction of local RASS.

Ang II infusion induces cardiovascular pathophysiological alterations associated with hypertension. For instance, subpressor doses of Ang II induce cardiac hypertrophy with increased expression of ANP, collagen I, III, and IV, and fibronectin. Our study shows that the reduced hypertensive effect of Ang II infusion in TGR(ASrAOGEN) rats was associated with reduced LV hypertrophy. The lower BP levels may be responsible for this effect. We also determined the LV mRNA levels of ANP to be an early marker of cardiac hypertrophy and the LV mRNA levels of collagen III to be a marker of fibrosis. While we could observe a marked increase of gene expression after 7 days of Ang II infusion, the magnitude of this increase was similar in both TGR(ASrAOGEN) and Sprague-Dawley rats and for both ANP and collagen III mRNAs. The fact that the stimulation of the expression of these genes could not be correlated with the increase of BP and cardiac hypertrophy indicates that Ang II may directly increase ANP and collagen III formation, independently of hemodynamic events, or may precede the hypertrophy.

To summarize, the results of these studies indicate that normal development of the brain RAS is necessary for full development of subpressor-dose Ang II–induced hypertension and LV hypertrophy. The expression of ANP and collagen III genes in the LV seems to be directly stimulated by Ang II.

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References

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