The Role of the Renin-Angiotensin and Cardiac Sympathetic Nervous Systems in the Development of Hypertension and Left Ventricular Hypertrophy in Spontaneously Hypertensive Rats

Aimin Dang, Deyu Zheng, Bing Wang, Yuqing Zhang, Penghua Zhang, Minfu Xu, Guozhang Liu, and Lisheng Liu

To elucidate the relationship between the development of left ventricular hypertrophy (LVH) in hypertension and the development of both the cardiac sympathetic nervous and renin-angiotensin systems, as measured by norepinephrine and angiotensin II levels, respectively. In this longitudinal study, we compared blood pressure (BP), left ventricular weight, and norepinephrine (NE) and angiotensin II (Ang II) concentrations, in Spontaneously Hypertensive Rats (SHR) and age-matched Wistar-Kyoto (WKY) rats at 5, 10, 15, 20, and 28 wk of age. Blood pressure, plasma and ventricular Ang II and tissue NE were measured by the tail-cuff method, radioimmunoassay, and high-performance liquid chromatography (HPLC), respectively. At 5 wk, systolic blood pressure was the same in both strains. But the left ventricular plus septum weight to body weight (LVSW/BW) ratio was higher in SHR than in WKY rats (p < 0.01), which finding may have been related to the increased cardiac tissue NE concentration, and this increase tended to parallel the rise in blood pressure. Both left ventricle and forelimb muscle NE concentrations were significantly higher in SHR than in WKY rats at 5, 10, and 15 wk of age (p < 0.01, respectively), and were similar at 20 and 28 wk of age. The heart and plasma Ang II levels decreased with age, which results were in keeping with the known developmental tendencies of the biological aging process. There was no significant difference in plasma Ang II levels between the two strains from 5 to 20 wk, whereas these levels were remarkably higher in WKY than in SHR rats at 28 wk (p < 0.01). Otherwise, the left ventricular tissue Ang II concentrations were significantly higher in SHR than in WKY rats at the late stage (from 15 to 28 wk), which may have contributed to the late-stage cardiac hypertrophy. These results suggested that the sympathetic nervous system (SNS) and the renin-angiotensin-system (RAS) in SHR may contribute to the pathogenesis of hypertension and LVH at the early and late stages, respectively. (Hypertens Res 1999; 22: 217-221)

Key Words: spontaneously hypertensive rats, left ventricular hypertrophy, norepinephrine, angiotensin II

The Left ventricular hypertrophy (LVH) occurring in hypertension is known to be an independent risk factor for cardiovascular diseases. In addition to increased arterial pressure, such neurohormonal factors as norepinephrine (NE) and angiotensin II (Ang II) are well known to play important roles in the development of LVH. Although the mechanism for this effect is uncertain, NE and Ang II might promote cell hypertrophy by directly stimulating the synthesis of myocyte cell protein. It has been demonstrated the Sympathetic Nervous System (SNS) and Renin Angiotensin System (RAS) may contribute to the development of LVH and may be involved in the regression of LVH by antihypertensive drugs (1, 2), with the cardiac tissue RAS playing the more important role in this progression.

Spontaneously hypertensive rats (SHR) have been studied by numerous investigators interested in their hypertension and cardiovascular hypertrophy, which are very similar to primary hypertension in humans. Imai (3) reported that an increase in a 1-adrenergic receptors might be involved in cardiac hypertrophy in the early phase of hypertension in SHR. Current evidences suggest that the SNS be important in the early stage of development of hypertension and LVH in SHR (4-8). It remains uncertain whether the SNS and RAS exert their influences on hypertension and LVH over the same age-span of SHR. In the present longitudinal study, we therefore compared blood pressure, left ventri-
Materials and Methods

Animals and Blood Pressure Measurements

Five-wk-old male SHR and WKY rats, obtained from the Animal Center of the Fu Wai Hospital in Beijing, were housed in groups of three rats to a cage in a room with a 12-h light/dark cycle and an ambient temperature of 22-24°C, and provided food and water ad libitum. The two strains were randomly divided into 5 groups, i.e., those to be examined at 5, 10, 15, 20, and 28 wk of age, with 5-7 rats in each group.

Systolic blood pressure (SBP) and heart rate were measured in conscious rats with an automated multichannel system that used tail cuffs and photoelectric sensors to detect the tail pulses. A test chamber maintained at 27-28°C was used to place rats in holders appropriate for their body weight. Over the last week of each time period, there were a minimum of 3 SBP recording sessions: the first data were discarded, and the SBP was taken as the mean of the last two sessions (4-6 sets of measurements) recorded over the last 2-3 d.

Preparation of Left Ventricle and Forelimb Muscle, and Collection of Blood Samples

The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and the lower abdominal aorta was exposed through a midline abdominal incision. Blood was collected from the aorta in chilled polyethylene tubes containing ethylenediamine tetraacetic acid disodium (EDTA·2Na). Blood was centrifuged at 3,000 g for 15 min and plasma was applied to assay of Ang II as described below. The rats were then killed by exsanguination. The left ventricle plus septum, and the forelimb muscle (caput longum of the triceps brachii) were quickly dissected from the other tissues, washed repeatedly with saline, blotted dry, weighted, frozen and stored at -70°C for the NE and Ang II assays.

Measurements of Norepinephrine and Angiotensin II

NE was extracted from the tissue by a slight modification of a procedure described previously (9). Briefly, the tissue was homogenized by Polytron in ice-cold 0.4 M perchloric acid. The homogenate was stored at -70°C for the NE and Ang II assays.

Statistical Analysis

All data were expressed as mean ± SEM. The various age and strain differences were compared by one-way analysis of variance. A level of p < 0.05 was taken to indicate statistical significance.

Results

Relation of Age to Systolic Blood Pressure

Between 5 and 15 wk of age in both strains, SBP was found to increase with age (Fig. 1). The difference in SBP between the two strains increased progressively during the period of rapid growth from 5 to 15 wk. The difference was small and not significant at 5 wk (n = 6 in each strain). At 10 wk and all time points thereafter, the SBP was higher in SHR than in WKY rats (p < 0.01). In WKY rats the “adult” BP value was reached by about 10 wk of age, whereas the corresponding value in SHR was reached by about 15 wk (Fig. 1). There was a progressive increase in the difference in SBP throughout the period of rapid growth, culminating in a difference of about 70 mmHg between WKY and SHR rats in adulthood.

Relation of Age to Left Ventricle and Septum Weight

The left ventricle plus septum weight/body weight (LVSW/BW) ratio declined in both SHR and WKY rats from 5 wk to reach stable values at about 10 wk (Fig. 1). At 5 wk the LVSW/BW ratio was only 8% higher in SHR than in WKY rats (p > 0.05), but this ratio was ≥30% higher in SHR at 28 wk (p < 0.001, Fig. 1).

Relation of Age to Left Ventricle and Forelimb Muscle Tissue Norepinephrine Concentrations

In the left ventricle and septum, NE concentrations ([NE]) were nearly twice as great in SHR as in WKY rats from 5 to 15 wk of age (Fig. 2, p < 0.001). However, [NE] in the left ventricle and septum declined rapidly from 15 to 20 wk of age in SHR, such that [NE] was highly similar in both strains at 20 and 28 wk.

Forelimb muscle [NE] was about 30-50% higher in SHR than in age-matched WKY rats during the rapid growth period from 5 to 15 wk (p < 0.001, Fig. 2), but thereafter the difference in tissue concentration was much smaller and not significant. A similar developmental tendency was seen for heart tissue [NE]. In the left ventricle and septum, there was a substantial sympathetic innervation of the myocardium. In skeletal muscle, however, most of the innervation goes to blood vessels, so that the NE measured from the forelimb muscles could be taken as the NE from blood vessels (12).

Relation of Age to Plasma and Left Ventricle Tissue Angiotensin II

Both the left ventricle plus septum tissue and plasma angiotensin II concentrations ([Ang II]) de-
creased with age in both strains. From 5 to 20 wk of age, the plasma [Ang II] was similar in both strains ($p > 0.05$, Fig. 3). However, at 28 wk, the plasma [Ang II] was significantly higher in WKY than in SHR rats ($p < 0.05$).

There was no significant difference in the left ventricle plus septum [Ang II] between the two strains at the early stage (from 5–10 wk). At the late stage (15–28 wk), the left ventricle and septum [Ang II] was significantly greater in SHR than in age-matched WKY rats ($p < 0.05$).

Discussion

The present studies provided a detailed analysis of the time course of LVH in SHR and examined the sequential changes of regional tissue [NE] and [Ang II] over this time course. We found that: 1) LVH proceeded the development of hypertension in SHR; 2) [NE] was increased in the left ventricle and septum, and in the skeletal muscle vessels of young SHR (5–15 wk); 3) Plasma [Ang II] was similar in both strains during the early life span, but the left ventricle and septum [Ang II] was significantly higher in SHR than in age-matched WKY rats from 15 to 28 wk.

At 5 wk, before “hypertensive” pressure levels were reached in SHR, there was a significant increase in the LVSW/BW ratio, which was related to the increased left ventricle and septum [NE]. Similar results were reported by Sen (1) and Michael (2).

Tissue [NE] was raised in the LVS and forelimb
muscles up to 15 wk of age in SHR, which finding agreed with those of Michael (8). It has been reported that the levels of both tyrosine hydroxylase and dopamine β-hydroxylase are higher in the mesenteric vessels of SHR than in those of WKY rats (13). However, another mechanism capable of increasing tissue [NE] may be increased innervation density in the vasculature and heart of young SHR, as recently demonstrated by Head et al. (14) and Lee et al. (15) reported that hypertension and hypertrophy are completely prevented by sympathetic ablation, although the mechanism responsible for this effect remains unknown.

Increased [NE] may have an additional trophic growth-promoting role at the early stage in SHR and WKY rats, which would be independent of the level of neural activity (16). The trophic effects of sympathetic innervation have been found to contribute to the development of arterial smooth muscle in normal rabbits in vivo (6), as well as to the development of medial hypertrophy of cerebral vessels in SHR (17). Previous tissue culture studies have indicated that catecholamines stimulate growth through adrenergic receptor-mediated mechanisms (7, 18), but this process is complex and appears to involve interactions with numerous growth factors (2, 19). Our findings of high [NE] at 5 wk in the skeletal muscle bed suggest a possible trophic role of the SNS in early vascular hypertrophy. We might speculate that the transiently increased density of sympathetic vascular innervation elevates the amount of NE released in the tissue, and that this increased NE may interact with local growth factors released by immature vascular smooth muscle to promote the hypertrophy of vascular smooth muscle cells (18, 19).

In the present study, both LVS-tissue and circulating [Ang II] decreased with age, which results were in keeping with the known developmental tendencies of the biological aging progress (20). Moreover, at the prehypertensive stage (5 wk), development stage (5-15 wk), and maintenance stage (15-28 wk), plasma [Ang II] was similar in both strains, but it was lower in SHR than in WKY rats at 28 wk, which decrease was in contrast to the increased hypertension in SHR. These results indicated that circulating RAS [Ang II] may not play an important role in the development and maintenance of hypertension and LVH.

In the present study of SHR, during the development and maintenance stages (15-28 wk) of hypertension and LVH, left ventricle tissue [Ang II] was increased and it was significant higher than that in age-matched WKY rats. Ang II, the active component of the RAS, acts directly on cultured myocytes and activates cell proliferation (21, 22). In a previous study in which 15-wk-old SHR were treated with ACEI-enalapril for 5 wk, LVH was found to regress (23). We therefore postulated that Cardiac RAS (Ang II) might contribute to the development and maintenance of LVH in SHR at the late stage. Long-term α1 blockade does not reverse cardiac hypertrophy in SHR rats (24). This may be because the treatments initiated at the late stage (after 15 wk of age) when the regional Ang II plays an important role instead of NE. Thus cardiac tissue regional NE contributes to the development of LVH at the early stage, whereas Ang II plays an important role in the maintenance of LVH at the late stage in SHR. The circulating Ang II may not be attributable to the LVH. Our results suggest that the α-receptor blocker should be used at the early stage, and that ACEI should be used at the late stage to reverse the LVH.

References
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