Changes in Cardiac Adrenomedullin Concentration in Renovascular Hypertensive Rats

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We assessed changes in tissue and plasma adrenomedullin levels in two-kidney, one-clip renovascular hypertensive rats. Four weeks after clipping, adrenomedullin concentrations were significantly higher in the cardiac ventricles and lower in the left atrium than the respective values in sham-operated rats. The left ventricular adrenomedullin concentration significantly correlated with systolic blood pressure and the degree of cardiac hypertrophy. No difference was noted in the adrenomedullin concentrations of the adrenal gland, aorta, lung, kidneys, or plasma between the two groups. These findings indicate possible involvement of cardiac adrenomedullin in this model of hypertension. (Hypertens Res 1997; 20: 113-117)

Key Words: adrenomedullin, cardiac hypertrophy, renovascular hypertension

Cardiac hypertrophy occurs during pressor overload to the heart in two-kidney, one-clip (2K1C) renovascular hypertensive rats. Cardiac hypertrophy is considered a compensatory mechanism for increased wall stress; however it often results in heart failure (1). Increased synthesis of atrial natriuretic peptide (ANP) (2) and endothelin-1 (3) is observed in hypertrophied myocardium, and these substances are considered to be involved in myocardial hypertrophy. In addition, functional abnormalities not only in the clipped kidney but also in the contralateral kidney have been described in 2K1C rats (4). Impaired renal function is of importance in the development and maintenance of hypertension (5).

Adrenomedullin, originally identified from an extract of human pheochromocytoma (6), is a potent vasodilatory peptide (7). Adrenomedullin circulates in the blood, and radioimmunoassay (RIA) and Northern blot analysis have shown that adrenomedullin is widely distributed in various organs, such as the adrenal medulla, heart, kidney, lung, and aorta (8-10). Studies of rat [125I] adrenomedullin, have demonstrated specific binding sites in a range of rat tissues, including the heart (11). A previous study showed positive immunostaining for adrenomedullin within the myocardium in both the atria and ventricles (12), and the level of adrenomedullin gene expression detected in the heart was comparable to that in the adrenal gland (13). These findings, together with a recent study showing that adrenomedullin has natriuretic and diuretic activities (14), suggest that adrenomedullin plays a unique role in cardiovascular and renal regulation. However, its role in pathophysiological states is not been fully understood, and no study has focused on the importance of adrenomedullin in renovascular hypertension. The main aim of the present study was to investigate whether endogenous adrenomedullin, especially in adrenomedullin-rich tissues, is involved in pathophysiologic processes in 2K1C rats.

Methods

Animals and Surgical Procedures
For this study, 4-wk-old male Wistar rats were obtained from Charles River Inc. (Atsugi, Japan). The animals (172-200 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and 2K1C renovascular hypertension (n=9) was produced by placing a silver clip (0.2 mm) on the left renal artery and leaving the contralateral kidney untouched. Sham-operated control rats (n=9) underwent an identical procedure except for clipping. The rats were housed in a temperature- and light-controlled environment and maintained on standard rat chow (CE-2; CLEA Japan, Tokyo, Japan) with free access to tap water. When systolic blood pressure (tail-cuff method) reached a level higher than 150 mmHg within 2 wk after the operation, the animals were regarded as having renovascular hypertension. Systolic blood pressure and body weight were measured on day 28 after the operation, and then the animals were sacrificed by decapitation.
Preparation of Plasma Samples
Whole blood was collected into ice-cooled tubes containing aprotinin (70 μl/ml) and EDTA-2Na (1.0 mg/ml) and centrifuged at 3,000 rpm for 10 min at 4°C. Plasma renin activity was measured by a solid-phase RIA (15). Plasma was diluted two-fold with 0.9% saline and then loaded onto a Sep-Pak C18 cartridge (Waters, Milford, MA, USA), which was pre-equilibrated with 0.9% saline. After the cartridge was washed with saline and 10% acetonitrile in 0.1% trifluoroacetic acid (TFA), the absorbed material was eluted with 4.0 ml of 60% acetonitrile in 0.1% TFA and then submitted to a RIA for rat adrenomedullin.

Tissue Extraction
Cardiac atria, ventricles, adrenal gland, aorta, lung, and kidneys of animals in both groups were resected. After weighing, the tissues were boiled in 10 volumes of 1 M acetic acid for 10 min to inactivate intrinsic proteases. The resulting mixture was homogenized with a Polytron mixer (KINEMATIKA, Littau, Switzerland) for 2 min, and the homogenate was centrifuged at 12,000 rpm for 30 min. The supernatant was applied to Sep-Pak C18 cartridges that had been activated with 0.5 M acetic acid, and the absorbed material was eluted with 4.0 ml of 60% acetonitrile in 0.1% TFA. The extracted samples were subjected to RIA.

RIA for Adrenomedullin
Immunoreactive adrenomedullin in the plasma samples and tissue extracts was determined by RIA with an anti-adrenomedullin antibody (#172CI-7) that recognizes the C-terminal region of rat adrenomedullin (9). The RIA was performed by a method similar to that reported previously (9).

Statistical Analysis
All data are expressed as means ± SEM. Results were compared between 2K1C and sham-operated rats by Student’s t-test. Linear regression analysis was used to assess the correlations between variables. Differences with a p < 0.05 were considered to be significant.

Results
Table 1 summarizes the physiological profiles of 2K1C and sham-operated rats. As expected from systolic blood pressure, the left ventricular weight / body weight and plasma renin activity in 2K1C rats...
were significantly higher than those in sham-operated controls. Body weight was not significantly different between the two groups. The kidney weight / body weight ratio of the clipped kidney was lower than that of the ipsilateral kidney in sham-operated rats. In contrast, the unclipped kidney in 2K1C rats showed compensatory hypertrophy. Consequently, the 2K1C rats could be considered as a model of renovascular hypertension.

A comparison of adrenomedullin concentrations in the heart between 2K1C and sham-operated rats is shown in Fig. 1. The concentrations of adrenomedullin in the right and left ventricles in 2K1C rats were significantly higher than those in sham-operated controls. In contrast, the unclipped kidney in 2K1C rats showed compensatory hypertrophy. Consequently, the 2K1C rats could be considered as a model of renovascular hypertension.

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As shown in Fig. 2, the left ventricular adrenomedullin concentration positively correlated with systolic blood pressure (r = 0.79, p < 0.01, Fig. 2A) and with left ventricular weight / body weight (r = 0.65, p < 0.01, Fig. 2B) in 2K1C and sham-operated rats. In contrast, a negative correlation was observed between left atrial adrenomedullin concentration and left ventricular weight / body weight (r = -0.59, p < 0.05, Fig. 3).

### Discussion

The present study demonstrated that the left ventricular adrenomedullin concentration was increased in 2K1C rats. In addition, the left ventricular adrenomedullin concentration was found to significantly correlate with systolic blood pressure and left ventricular hypertrophy. These findings suggest that adrenomedullin increases in myocardium in association with pressure overload and cardiac hypertrophy.

Increased concentrations of ANP, a vasodilatory and diuretic peptide, also occur in the left ventricle of 2K1C rats (2). Moreover, the left ventricular ANP levels closely correlated with the degree of left ventricular hypertrophy and with the left ventricular end-diastolic pressure in that study (2). Although there is no report on mechanisms regulating cardiac cellular adrenomedullin production and secretion,

The adrenomedullin concentration in the left atrium was about 20 times higher than that in the left ventricle. The right atrial adrenomedullin concentration was observed in the left atria of 2K1C rats. Thus, adrenomedullin seems to play a role in the activation of the left ventricular adrenomedullin, similar to the ANP system.

In conclusion, the present study demonstrated that adrenomedullin concentration was significantly increased in both ventricles and decreased in the left atrium in 2K1C rats. This result is noteworthy since adrenomedullin concentration in the left atrium was about 20 times higher than that in the left ventricle. The right atrial adrenomedullin concentration has also been reported to be decreased in a rat model of pulmonary hypertension (21). Although it remains to be determined whether the reduced adrenomedullin concentration in the present study was the result of suppressed adrenomedullin synthesis or an enhanced rate of atrial adrenomedullin secretion, the atrial adrenomedullin concentration may reflect the pressure overload on the left atrium, since there was a negative correlation between the left atrial adrenomedullin concentration and the degree of left ventricular hypertrophy, which is expected to increase the left ventricular end-diastolic pressure. Hemodynamic alteration may be one of regulators for the left-sided cardiac adrenomedullin system.

The present study revealed that there were no changes in adrenomedullin concentration in the other organs examined, including both the clipped and the unclipped kidney. It is generally recognized that the unclipped kidney exhibits an increase in renal vascular resistance, and the capability to autoregulate renal blood flow and tubular reabsorptive function of this kidney is impaired (4, 5). Although a variety of mechanisms are involved in these functional disturbances, there is evidence to suggest a role of alterations in renal hormone production (22). Given the potent vasodilatory and natriuretic effects of adrenomedullin, the lack of changes in renal adrenomedullin suggests the compromised function of the unclipped kidney. The future application of agents that inhibit the action of adrenomedullin or block the synthesis of adrenomedullin will clarify its role in the kidney.

There was no significant difference in plasma adrenomedullin level between 2K1C and sham-operated rats. We previously reported that plasma adrenomedullin level was elevated in patients with essential hypertension, with a progressive rise proportional to disease severity (23). We have no clear explanation for this discrepancy, but the lack of rise in plasma adrenomedullin in 2K1C rats may indicate that circulating adrenomedullin is of less importance in renin-angiotensin II dependent hypertension. In fact, angiotensin II does not stimulate adrenomedullin production in cultured vascular endothelial cells, which are assumed to be one of the major sources of plasma adrenomedullin (unpublished observations), and the adrenomedullin concentration in the aorta did not differ between the two groups in the present study.

In conclusion, the present study demonstrated that adrenomedullin concentration was significantly increased in both ventricles and decreased in the left atrium in 2K1C rats. Thus, adrenomedullin might be involved in the pathophysiologic processes in this model of renovascular hypertension, especially in the heart.

References


