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Regulatory Role of Nitric Oxide on Atrial Natriuretic Peptide System in Normotensive and Hypertensive Rats

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The present study was aimed to explore an interaction between endothelium-derived nitric oxide (NO) and atrial natriuretic peptide (ANP) systems in normotensive and hypertensive states. Rats were made two-kidney, one clip (2K1C) hypertensive and supplemented with either N^G-nitro-L-arginine methyl ester (L-NAME, 5 mg/100 ml drinking water) or L-arginine hydrochloride (400 mg/100 ml drinking water). One group supplied with normal tap water served as control. Sham-clipped rats were also divided into the L-NAME, L-arginine, and control groups. The plasma levels and atrial contents of ANP were determined at day 28 following clipping the renal artery. In 2K1C rats, the plasma level of ANP was higher and the atrial content was lower than in the sham-clipped control. L-Arginine increased the atrial content of ANP in association with a decreased plasma ANP, whereas L-NAME significantly affected neither parameter. The increase of blood pressure in 2K1C rats was not affected by L-arginine or L-NAME. In sham-clipped rats, the plasma level of ANP was significantly increased by L-NAME along with an increase in blood pressure. On the contrary, L-arginine did not affect the blood pressure or plasma ANP. The atrial content of ANP was significantly altered neither by L-arginine nor by L-NAME. These results suggest that NO plays a tonic inhibitory role on the ANP release with concomitant increases of the atrial tissue content. In addition, hypertension is suggested to modify the release and tissue storage of ANP.

Key Words: Nitric oxide, Atrial natriuretic peptide, 2-Kidney, 1 clip hypertension.

INTRODUCTION

It has been known that the vascular endothelium subserves a regulatory modulation of the vascular tone through production and release of factors either constricting or dilating the underlying smooth muscle layer. With increasing numbers of the endothelial factors discovered, interactions between these and other hormonal systems have been widely suggested.

Among others, endothelium-derived nitric oxide (NO) has been found to influence the secretion of atrial natriuretic peptide (ANP) (Anderson et al, 1986; De Zeeuw et al, 1992; Goetz, 1988). When bovine aortic endothelial cells were placed in coculture with rat atrial myocytes, the release of ANP was stimulated (Lew & Baertschi, 1989). In addition, circulating ANP levels are increased during infusion of NO precursor, L-arginine (Dell'Omo et al, 1995). In the rat atria, on the contrary, removal of the endocardium or inhibition of NO synthesis augments the release of ANP, indicating that NO inhibits the release of ANP (Sanchez-Ferrer et al, 1990). It was also reported that ANP levels were unchanged after 7 days of treatment with inhibitors of NO synthase (Dananberg et al, 1993).

ANP release may also be modified by hypertension, in which the high blood pressure has been associated with elevated plasma concentrations of ANP (Imada et al, 1985). Furthermore, there is compelling evidence that NO and renin-angiotensin systems are interactive at various levels impinging on

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blood pressure regulation. Taken together, an interaction between NO and ANP may be altered in two-kidney, one clip (2K1C) hypertension, of which development has been primarily attributed to an enhanced renin release of the clipped kidney.

The present study was designed to explore an interaction between NO and ANP systems in normotensive and 2K1C hypertensive states. NO system was either stimulated or inhibited by oral supplementation with either its precursors or inhibitors of NO synthesis in normotensive and 2K1C rats, and the plasma levels and atrial tissue contents of ANP were determined.

METHODS

Two-kidney, one clip hypertension

Male Sprague-Dawley rats (160-200 g) were constricted at the left renal artery with a silver clip having an internal gap of 0.25 mm under ketamine anesthesia; the contralateral kidney was left untouched. The rats were then divided into three groups. The first group was supplemented with N^G-nitro-L-arginine methyl ester (L-NAME, Sigma; 5 mg/100 ml drinking water), the second with L-arginine hydrochloride (Choongwae, 400 mg/100 ml drinking water), and the third group was supplied with tap water to serve as control. Sham-clipped rats were operated as 2K1C rats except for that no clipping was made. They were also divided into the three L-NAME, L-arginine, and control groups, and treated as such.

Three to five rats of the same group were housed together in a cage. Systolic blood pressure (SBP) was measured in a conscious state at days 7, 14, 21, and 28 after clipping the renal artery. Basal blood pressure was taken as an average of three-consecutive-day values before clipping the artery. On day 28, following the measurement of SBP, blood samples were collected by decapitation, and the right atria were immediately taken thereafter.

Radioimmunoassay of ANP

The plasma was extracted with Sep-Pak C18 cartridges (Waters Associates, Milford, MA) for ANP assay. The right atria taken were boiled in 1.0 *N* acetic acid for 10 min, homogenized, and the supernatant was taken after centrifugation. Concentrations of ANP in the aliquots were determined using a commercial radioimmunoassay kit (Research & Diagnostic Antibodies; Berkeley, CA).

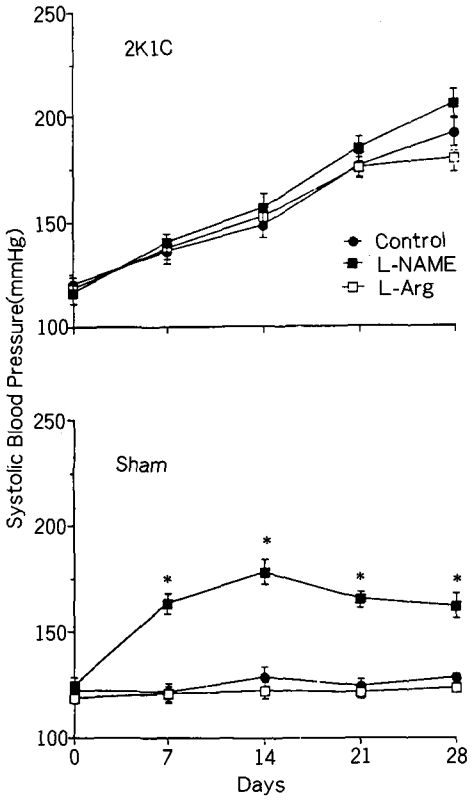

Fig. 1. Effects of oral supplementation with L-NAME and L-arginine on the blood pressure in 2-kidney, 1 clip (2K1C) and sham-clipped rats. Numbers of rats in each group are 8-11. * $p<0.01$, compared with control.

Statistics

Results were expressed as means ± SEM. The statistical significance was determined using analysis of variance with repeated measures.

RESULTS

In 2K1C rats, SBP progressively increased during the period of observation, the magnitude of which was not significantly affected by either L-NAME or L-arginine (Fig. 1). L-Arginine significantly decreased the plasma concentration and increased the atrial content of ANP, whereas L-NAME was without effect on either parameter in these rats (Fig. 2). Daily amounts of L-NAME and L-arginine ingestion did not differ between the 2K1C and sham-clipped rats (data not shown).

In sham-clipped rats, L-NAME significantly in-

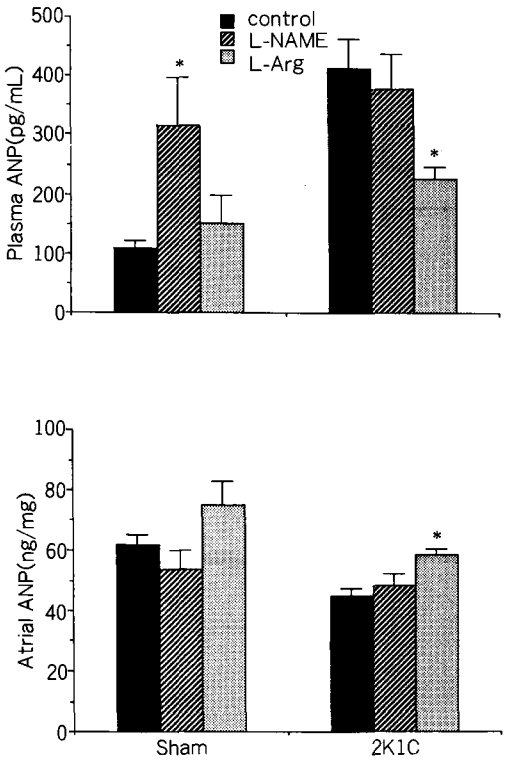

Fig. 2. Plasma concentrations and right atrial tissue contents of ANP in sham-clipped and 2K1C rats. The blood samples were taken following decapitation in a conscious state. L-Arginine significantly increased the atrial contents and decreased the plasma concentrations of ANP in 2K1C rats (*$p<0.01$). L-NAME increased the plasma concentrations of ANP (*$p<0.01$) without significant changes in atrial contents in sham-clipped rats.

creased SBP as well as the plasma ANP (Fig. 1, 2). On the contrary, L-arginine did not significantly affect either the blood pressure or plasma ANP. The atrial content of ANP was significantly altered neither by L-arginine nor by L-NAME.

DISCUSSION

2K1C hypertension was associated with higher plasma levels of ANP than in control, being in accord with previous investigations (Imada et al, 1985; Lee et al, 1992; Sugimoto et al, 1986). The higher plasma level along with lower atrial contents may reflect an increased release from the atria, since the tissue ANP content is determined by the secretory rate (Takayanagi et al, 1985). The mechanism by which an increased blood pressure augments the release of ANP remains to be established, however.

L-Arginine, the precursor of NO synthesis, has been suggested to produce a vasodilating effect via stimulating synthesis and release of NO (Hashikawa et al, 1992). Therefore, we used L-arginine to explore an effect of endogenous NO on ANP system in the present study, and found a conflicting effect between the normotensive and hypertensive rats. L-Arginine did not affect either the plasma level or the atrial content of ANP in sham-clipped rats, whereas it decreased the former and increased the latter in 2K1C rats. This finding may indicate that basal NO secretion is not stimulated strong enough to modify the ANP system by L-arginine in a normal state. L-Arginine has been indeed known not to be a rate-limiting factor in NO production (Gold et al, 1990; Schini & Vanhoutte, 1991). NO may preferentially modulate the release of ANP when enhanced by other factor(s), such as hypertension. It is unlikely that a difference in ingested amount of L-arginine is responsible for the discrepancy, since the ingested amount was comparable between 2K1C and sham-clipped rats.

Providing that NO is inhibitory to the release of ANP, a blockade of NO synthesis by L-arginine analogues such as N^G-monomethyl-L-arginine and L-NAME may be stimulatory. We indeed found that the plasma ANP values were significantly higher in the L-NAME-treated rats than in control. The speculation is substantiated by the previous finding (Sanchez-Ferrer et al, 1990), in which agents inhibiting the release of NO increase the basal release of immunoreactive ANP from the isolated rat atria. Recently, Leskinen et al (1995) also observed an increase of the plasma ANP following an administration of L-NAME.

L-NAME may influence the synthesis and release of ANP not only by directly acting on the secretory cell, but also by acting indirectly via elevating the blood pressure. However, combination of L-NAME and development of 2K1C hypertension was ineffective to result in an additive effect either on ANP or blood pressure. The plasma ANP was also comparable between the 2K1C and sham-clipped rats following L-NAME-supplementation. In addition, neither the blood pressure nor the plasma ANP differed between the L-NAME and control groups of 2K1C rats. It is likely that L-NAME and 2K1C hypertension have the final common pathway in stimulating the secretion of ANP. The mechanism may be primarily

activated by either L-NAME or hypertension, so that no additive effect can be achieved by the other. Further studies will be needed to further explore differential effects of L-NAME and hypertension on the ANP system.

Finally, it should be pointed out that a feedback loop between NO and ANP systems has been suggested (Akiho et al, 1995; Vollmar & Schulz, 1995). The increased secretion of ANP may stimulate NO production, which in turn checks an oversecretion of the peptide. It is also possible, however, that ANP secretion may be automatically shut off at certain limit even in the absence of a functional NO system. It will be of interest to explore whether this loop is altered in the hypertensive state.

In summary, these results suggest that NO has a regulatory role on ANP system. It is also suggested that hypertension may modify the release and tissue storage of ANP. NO may inhibit the release of ANP with a concomitant increase of its tissue storage.

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