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Role of Endogenous Nitric Oxide in the Control of Renin Release

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=ABSTRACT=

The present study was undertaken to investigate the role of endogenous nitric oxide in renin release under different physiological conditions. In the first series of experiments, renin release was either inhibited by acute volume-expansion (VE) or stimulated by clipping one renal artery in the rat. VE was induced by intravenous infusion of saline (0.9% NaCl) up to 5% of the body weight over 45 min under thiopental (50 mg/kg, IP) anesthesia. VE caused a decrease of plasma renin concentration (PRC). With N^G-nitro-L-arginine methyl ester (L-NAME, 5 μg/kg per min) superadded to VE, PRC decreased further. The magnitude of increase in plasma atrial natriuretic peptide levels following VE was not affected by the L-NAME. In two-kidney, one clip rats, L-NAME- supplementation resulted in a decrease, and L-arginine-supplementation an increase of PRC. Plasma atrial natriuretic peptide levels were significantly lower in the L-arginine group than in the control. Blood pressure did not differ among the L-NAME, L-arginine, and control groups. In another series of experiments, the renin response to a blockade of NO synthesis was examined using *in vitro* preparations from isolated renal cortex. L-NAME significantly increased basal renin release, although it was without effect on the isoproterenol-stimulated release. These findings suggest that endogenous nitric oxide significantly contributes to the renin release. Since many factors may affect the renin release *in vivo*, an interaction between NO and renin under various pathophysiological states is to be further defined.

Key Words: Nitric oxide, renin, N^G-nitro-L-arginine methyl ester, volume-expansion, Atrial natriuretic peptide, Two-kidney, One clip hypertension, *In vitro* preparations

INTRODUCTION

Over the last decade, the vascular endothelium has been found to produce factors which may modulate vascular tone. With increasing number of endothelial factors involved in the vascular regulatory mechanisms, interactions between the endothelial mediators and other hormonal systems have been subjects of much interest. Endothelium-derived relaxing factor, among others, has been characterized mainly as nitric oxide (NO) derived from the guanidino nitrogen of L-arginine.

NO is now known to be also involved in the regulation of endocrine secretion. For instance, the secretion of insulin (Corbett et al, 1993) and growth hormone (Kato, 1992) is altered by

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stimulants or inhibitors of NO synthesis. Recent evidence, mostly done *in vitro*, suggests that NO also plays a role in the control of renin secretion. It has not been established, however, how and to what extent NO regulates the renin release under various pathophysiological states.

Vidal et al (1988) have shown that superfusates of aortic preparations treated with acetylcholine inhibited renin release in a kidney slice and concluded that NO is inhibitory. Henrich et al (1988) further provided evidence indicating that the inhibitory effect of NO is mediated by the formation of cGMP in juxtaglomerular cells. Others, however, failed to observe an NO-induced inhibition of renin release but found a stimulation (Munter & Hackenthal, 1991; Persson et al, 1993). Johnson and Freeman (1992a) also observed a decrease in renin release following the inhibition of NO synthesis, even when the renal perfusion pressure was maintained constant.

The present study was aimed to further investigate the role of endogenous NO in the control of renin release. In the first series of experiments, effects of blockade of NO synthesis with N^G-nitro-L-arginine methyl ester (L-NAME) on plasma renin concentration (PRC) were examined under two different conditions. The rat was acutely volume-expanded to inhibit the release of renin, or was made two-kidney, one clip (2K1C) hypertensive to stimulate the release. In another series of experiments, the renin response to a blockade of NO synthesis was examined using *in vitro* preparations from the isolated renal cortex.

METHODS

Acute volume expansion

Male Sprague-Dawley rats (220-260 g) were anesthetized with thiopental (50 mg/kg, IP). The right femoral artery was cannulated to measure arterial blood pressure and the vein to serve as an infusion route. A 30 to 60-min equilibration period was allowed to elapse until volume-expansion (VE) started. L-NAME was used to inhibit the endogenous NO system. Following different groups were provided.

1. The [Control] group was without VE.
2. The [VE] group received intravenous infusion of saline (0.9 % NaCl) over 45 min. The total volume infused was 5 % of the body weight.
3. The [VE+NAME] group was volume expanded as in the [VE] group on the ongoing infusion of L-NAME (5 μg/kg per min) which was started 90 min before initiating VE.
4. The [NAME] group received L-NAME (5 μg/kg per min) for 135 min. To minimize a volume effect, the total volume of infusion amounted up to 137 μL/h in each rat.

A blood sample was taken from the femoral artery at the end of the experiment. PRC was determined by radioimmunoassay as described previously (Cho et al, 1987). Concentrations of atrial natriuretic peptide (ANP) were determined in the C18 sep-pak extracted plasma using a radioimmunoassay kit purchased from Research & Diagnostic Antibodies (Berkeley, CA). The determined values were corrected with the extraction ratio (68.5±2.4%).

Two-kidney, one clip hypertension

In male Sprague-Dawley rats (150-200 g), the left renal artery was constricted with a silver clip having an internal gap of 0.2 mm under ketamine anesthesia. The contralateral kidney was left untouched. The rats were then divided into three groups. One group was supplemented with L-NAME (5 mg/100 mL), and another with L-arginine (400 mg/100 mL) in the drinking water. The other was used as a control group, supplied with normal tap water.

On day 28 after the clipping, systolic blood pressure was indirectly measured by means of a tail cuff method. The animals were then decapitated in a conscious state and the trunk blood was collected for measurement of PRC and

ANP levels.

Isolated renal cortical slices

The kidneys were removed under thiopental (50 mg/kg, IP) anesthesia and decapsulated. The 0.4-mm thick cortical slices were made parallel to the capsular surface with a Staddie-Riggs microtome. The initial slice was discarded, and the next was taken from one hemisphere, so that two slices were obtained from each kidney.

Each slice was placed in an oscillating incubation bath at 37 °C while the incubation medium was maintained with 95 % O_2-5 % CO_2. After 30 min equilibration, the medium was replaced with fresh one. The slice was then incubated for one hour, during which some specimens were incubated with L-NAME (10^{-4} mol/L). A 200-μL aliquot was taken for assay of renin concentration, and the experimental agent (isoproterenol or sodium nitroprusside) or its vehicle was then added. After an additional 30 min, a second 200-μL aliquot was sampled. Samples were centrifuged and frozen for later analysis. The slice was weighed to determine results by milligrams of wet weight. Renin release was measured as the difference in concentrations between the two collections. The composition of the physiological salt solution was as follows (in mmol/L): NaCl 125, $NaHCO_3$ 19, KCl 4, $CaCl_2$ 2.6, NaH_2PO_4 1.2, $MgSO_4$ 0.8, and glucose 0.2 g/100 mL. The drugs used were all purchased from Sigma Chemical Company (St. Louis, MO), except sodium nitroprusside (Roche; Basel, Switzerland).

Statistics

Results are expressed as means±SEM. Univariate analysis of variance with the Scheffe multiple-comparison adjustment was used to test the significance for differences among the groups.

RESULTS

Plasma concentrations of renin following VE are shown in Fig. 1. VE resulted in a decrease of PRC. Although L-NAME alone was without a significant effect on PRC, it caused a further decrease of PRC. Plasma ANP values increased following VE, ie, they were 46.4±5.1 pg/mL in the [VE+NAME], 41.0±9.6 pg/mL in the [VE], and 12.9±1.8 pg/mL in the control.

In 2K1C rats, systolic blood pressure measured on day 28 were 192±10 in [control] (n=6), 210±9 in [L-NAME] (n=7), and 189±6 mmHg in [L-arginine] group (n=6), not being significantly different among the groups. The [L-NAME] group was associated with a significantly lower PRC, and the [L-arginine] group with a higher PRC than the [control] (Fig. 2). Plasma ANP levels were 29.8±6.8 in the [L-NAME] group (n=4), 13.9±2.7 in the [L-arginine] (n=4), and 31.0±6.9 pg/mL in the [control] (n=6). The [L-arginine] group

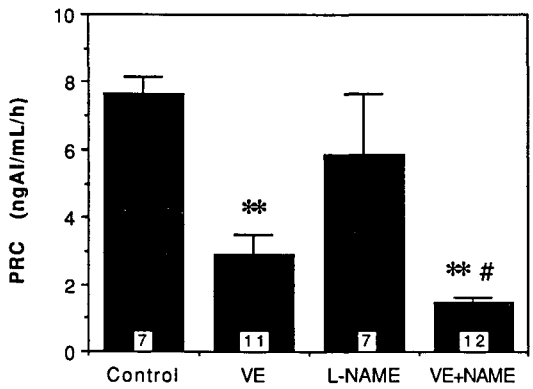

*Fig. 1. Plasma renin concentrations (PRC) following the volume expansion (VE). [L-NAME] denotes the group infused with L-NAME (5 μg/kg per min) without VE. [VE+NAME] group was volume-expanded with L-NAME superadded. Values are means±SEM. Each numeral in the bar represents the number of rats. **p<0.01, compared with control. *p<0.05, compared with VE group.*

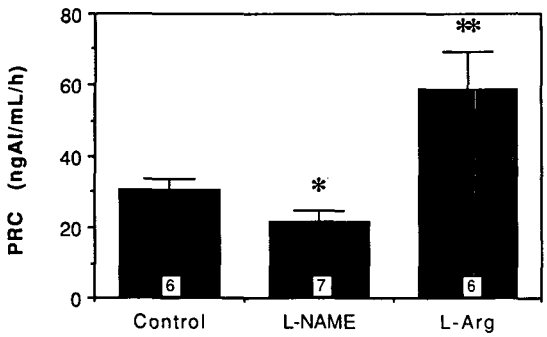

*Fig. 2. Plasma renin concentrations in control, L-NAME or L-arginine (L-Arg)-supplemented groups of two-kidney, one clip rats. Legends as in Fig. 1. *p< 0.05, **p< 0.01; compared with control.*

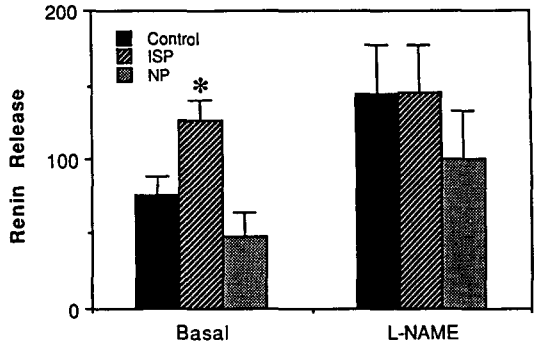

*Fig. 3. Renin release from the isolated renal cortical slice treated without (basal) and with L-NAME. [ISP] and [NP] denote the groups incubated with isoproterenol (10^{-5} mol/L) and sodium nitroprusside (10^{-5} mol/L), respectively. L-NAME was treated (10^{-4} mol/L) 60 min before isoproterenol or sodium nitroprusside was administered. Values of renin release are means ± SEM from six experiments each, expressed in nanograms angiotensin I per hour per milligram per 30 min incubation. *p< 0.05, compared with control.*

showed a significantly lower plasma ANP than the [control] ($p<0.05$).

Fig. 3 shows the renin released by the isolated cortical slice in the incubation media. L-NAME significantly increased basal renin release, although it was without effect on the isoproterenol-stimulated release. Sodium nitroprusside did not significantly alter the rate of either the basal or stimulated-release.

DISCUSSION

The enzyme responsible for the synthesis of NO in the endothelial cell is the constitutive type of NO synthase. It is competitively inhibited by L-arginine analogues such as N^G-monomethyl-L-arginine (L-NMMA) and L-NAME (Gardiner et al, 1990; Palmer et al, 1988). These compounds have been a useful tool in investigating the biological significance of endogenous L-arginine-NO pathway. We used L-NAME in the present study to examine the role of endogenous NO system on renin release.

The results obtained using the *in vitro* preparations of renal cortical slices were consistent with the hypothesis that endogenous NO inhibits renin release, ie, L-NAME significantly increased basal release of renin. This finding is in agreement with many previous investigations, mostly conducted *in vitro*. Vidal et al (1988) showed that the rate of renin secretion from cortical slices was decreased by a perfusion obtained from acetylcholine-stimulated arterial strips. More recently, incubation of rat kidney slices with L-NMMA was found to enhance renin release by more than 50 % compared with control (Beierwaltes & Carretero, 1992). Conversely, sodium nitroprusside has been known to inhibit renin release (Henrich et al, 1988). The lack of significance in the inhibitory effect of sodium nitroprusside on either the basal or stimulated-release in the present study may be attributed to different experimental conditions.

Despite the increased release of renin by L-NAME *in vitro*, however, the isoproterenol stimulated-release was not potentiated by L-NAME. On the contrary, Reid et al (1994)

found that inhibition of NO synthesis decreased resting plasma renin activity as well as an isoproterenol-stimulated release of renin *in vivo*. In fact, results shown in the *in vitro* experiments are not always consistent with those in the *in vivo* studies. Following the inhibition of NO, Sigmon et al (1992) found an elevated plasma renin activity, Majid & Navar (1992) no changes in PRC, and Persson et al (1993) an attenuated renin release. Johnson & Freeman (1992a) also found a reduction of basal renin release following an NO blockade while maintaining renal perfusion pressure constant at around 115 mmHg.

In the present study, the results obtained in the *in vivo* experiments suggested NO being stimulatory to renin release. L-NAME caused a further decrease of PRC which was decreased by VE. This is in line with previous studies (Gardes et al, 1992; Johnson & Freeman, 1992b; Kurtz et al, 1991). In addition, in 2K1C rats, PRC was also lower in the [L-NAME] group than in the [control], although the blood pressure was comparable. The lower PRC in the [L-NAME] group may have resulted from blocking a direct stimulatory effect of NO on renin release. Taken together, L-NAME causes a decrease of renin release regardless of whether its release is under inhibition or stimulation *in vivo*.

Since L-NAME may increase blood pressure (Lahera et al, 1991), its effect on renin release has to be considered in association with the changes in systemic blood pressure. A combination of increased renal perfusion pressure activating the renal baroreceptor mechanism and a reflex reduction in renal sympathetic activity may decrease the secretion of renin. Reid et al (1994) showed a decreased renin release due to L-NAME, which was associated with an increased arterial pressure. Whether the removal of a direct stimulatory effect on renin-secreting cells or the elevated blood pressure caused the inhibition of renin release cannot be differentiated in their study. The suppression of plasma renin occurred independently of the blood pressure changes in the present study, however, and the renin response may be attributed to a direct effect on the renin-secreting cells.

The effects of NO blockade on renin-angiotensin system could be highly complex *in vivo* due to an interplay between the primary and secondary factors affecting renin release. The blockers of NO synthesis would remove any direct influence of NO on renin release. However, an increase in renal perfusion pressure subsequent to removal of vasodilatory effect of NO is likely to suppress renin release on one hand, and its stimulatory effect on prostacyclin (Doni et al, 1988) would enhance the release on the other. The contradictory results among studies may be the consequence of the opposing mechanisms participating in renin secretion. The levels of renin would then depend on the equilibrium between the opposing effects.

L-Arginine also changed PRC in 2K1C rats. PRC was higher in the [L-arginine] group than in the [control]. Hashikawa et al (1992) suggested that exogenous L-arginine could stimulate synthesis and release of NO in hypertension. The stimulatory effect of L-arginine on PRC may be attributed to an increased synthesis of NO via activation of L-arginine-NO pathway by providing the excess substrate. The slightly lower PRC in [L-NAME] group may substantiate such a hypothesis.

An interaction between ANP and renin is also worth considering. NO is known to inhibit the release of ANP (Sanchez-Ferrer et al, 1990), and ANP inhibits the renin release (Burnett et al, 1984). The higher PRC and lower ANP in L-arginine-supplemented 2K1C rats may be in part accounted for by such an interaction. In contrast, however, L-NAME altered plasma ANP levels neither in VE study nor in 2K1C rats. This finding suggests that the inhibitory effect of L-NAME on PRC is not secondary to an altered plasma ANP, but results from a direct

effect on the renin-secreting cells.

Changes in cytosolic free calcium concentration constitute an important element of signal transduction in various cells. At the cellular level, the signal for renin release is associated with a reduction of intracellular calcium in juxtaglomerular cells (Park & Malvin, 1978), metaplastic smooth muscle cells of the afferent arterioles, whereas calcium is used as a transduction mediator between a stimulatory signal and synthesis of NO in endothelial cells (Busse & Mulsch, 1990). Calcium may activate the afferent arteriolar endothelial L-arginine-NO pathway, of which final product NO permeates the adjacent juxtaglomerular cells. In this context, it may be hypothesized that NO decreases intracellular free calcium levels in the juxtaglomerular cells through stimulating the formation of cGMP as in the vascular smooth muscle (Waldman & Murad, 1987), culminating in an enhanced renin secretion. One may also hypothesize that the stimuli causing an increase of calcium in endothelial cells simultaneously increase the calcium in renin-secreting cells, resulting in an inhibition of renin release. At the cellular level, whether NO is acting in a stimulatory or inhibitory manner in renin release may thus be complicated.

In summary, the present study suggests that endogenous NO *per se* has a direct stimulatory effect on renin release. Since many factors may affect the renin release *in vivo*, however, an interaction between NO and renin under various pathophysiological states remains to be further clarified.

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