Role of the Central Nervous System in the Development of Hypertension Produced by Chronic Nitric Oxide Blockade in Rats


We examined the role of the central nervous system, and particularly the renin-angiotensin (RA) system, in the development of hypertension produced by chronic inhibition of NO synthesis. In experiment 1, Wistar rats drank either nitro-L-arginine-methyl ester (L-NAME) or tap water. Before L-NAME treatment rats were divided into 6 groups. Four of them were administered either losartan or artificial cerebroventricular fluid (a-CSF) intracerebroventricularly (i.c.v.) for 1 week using an osmotic mini pump. The other two groups were administered the same amount of losartan intravenously (i.v.). In experiment 2, cardiovascular responses to acute i.v. losartan and muscimol, a GABA_A agonist, were examined in conscious L-NAME-treated rats. Finally, in experiment 3, effects of ablation of the AV3V (anteroventral third ventricle) area, known to be one of the centers of cardiovascular control, were tested in the development of L-NAME hypertension. The development of hypertension by L-NAME treatment was attenuated with chronic i.c.v. losartan in a dose-dependent manner, while i.v. losartan had no effect. One week after cessation of i.c.v. losartan, blood pressure was elevated to the same level as in a-CSF-infused, L-NAME-treated rats. Acute i.v. losartan produced no cardiovascular changes in either L-NAME-treated or control rats. On the other hand, although i.c.v. muscimol elicited depressor effects in both groups, these responses were significantly larger in L-NAME-treated rats. Cardiovascular responses to i.v. hexamethonium were similar in both groups. The existence of prior lesions in the AV3V area significantly attenuated the development of L-NAME-induced hypertension. These results indicate that the central RA system plays an important role in the development of hypertension produced by chronic inhibition of NO synthase. Moreover, disorder of the central GABA system, rather than that of the RA system, might be important in the maintenance of hypertension in this model. (Hypertens Res 2001; 24: 39-45)

Key Words: nitric oxide, losartan, central nervous system, GABA, AV3V

Introduction

Although it is well known that long-term inhibition of nitric oxide (NO) synthase will produce sustained hypertension in normotensive animals (1-3) in association with mild renal damage (4), the precise mechanisms are still unclear (5). Recently, Ribeiro et al. (6) reported that continuous i.v. administration of losartan, an angiotensin II (ATII) receptor antagonist, prevented the development of hypertension produced by chronic NO blockade. Pollock et al. (7) also reported that, in the rat, inhibition of the
RA system not only prevents nitro-l-arginine-methyl-ester (L-NAME)-induced hypertension but also reverses established hypertension. Long-term NO-synthase inhibition might induce changes in such vasconstrictrict agents as AII, resulting in blood pressure elevation (8). In contrast, Sigmon et al. (9) reported that inhibition of NO synthase decreases plasma renin activity (PRA) as a result of the elevation in renal plasma flow and a decrease in β-adrenergic activity. NO is also produced in the brain upon activation of glutamate receptors, which are thought to mediate central autonomic modulation (10). Sakuma et al. (11) reported that NO might play a role in the central regulation of sympathetic tone, since increases in blood pressure and renal nerve activity elicited by bolus i.v. injection of L-NAME were markedly altered by baroreceptor deafferentation and spinal transection in rats.

GABA and its receptors are widely distributed throughout the mammalian central nervous system (12, 13), and it has been repeatedly reported that the central GABA system plays an important role in regulating sympathetic and cardiovascular activities (14). We previously reported that decreased GABAergic activity in the central presynaptic area could contribute to the augmented sympathetic discharge to thereby elevate blood pressure in spontaneously hypertensive rats (15, 16). And it has recently been reported that NO regulates NMDA-driven GABAergic inputs in the hypothalamus (17). In this study, we hypothesized that the central RA system, rather than the peripheral one, plays an important role in the development of hypertension produced by chronic NO synthase inhibition. The central GABAergic role in the maintenance of hypertension in this model was also examined.

Methods

Experiment 1

Forty-two male Wistar rats weighing 300 g (Shimizu Lab. Animals Supply, Kyoto, Japan) were housed in a room under constant-temperature and a 12-h light/12-h dark cycle. The animals were cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institute of Health (NIH Publication No. 85-23, revised 1985). All animals had free access to drinking water and rat chow. The rats drank either L-NAME (Sigma, St. Louis, USA), dissolved in tap water at a concentration of 0.7 g/l (LNAME-icv- or LNAME-iv groups) or tap water (CONT and CONT-icv-H groups). Three days prior to L-NAME treatment, 7 groups of rats were anesthetized with pentobarbital (35 mg/kg, i.p.). Mini-osmotic pumps (model 2002, Alzet Co., Palo Alto, USA), filled with losartan or a-CSF, were implanted and connected to cannulas stereotaxically placed in the lateral ventricle for i.c.v. infusions. They received either 10 mg/day (LNAME-icv-L, L: low dose) or 100 mg/day (LNAME-icv-H and CONT-icv-H, H: high dose) of losartan or a-CSF (LNAME-icv-C and CONT) i.c.v. The same doses of losartan were administered i.v. to the other two groups (LNAME-iv-H and LNAME-iv-L) to exclude the effect of leakage of losartan to the periphery. Rats were anesthetized with pentobarbital for implantation of a catheter, which was attached to a mini-osmotic pump, in the femoral vein (see below). Rats in CONT and CONT-icv-H (100 mg/day losartan, i.c.v.) groups drank tap water. Other groups received L-NAME to drink for 2 weeks and the i.c.v. or i.v. treatments were discontinued after the first week. Systolic blood pressures were measured by the tail cuff method (UR5000, Ueda Co., Ltd., Kyoto, Japan) once a week.

Experiment 2

Sixteen additional male Wistar rats weighing about 300 g were divided into two groups. One group drank the L-NAME solution (see above) (L-NAME group, n=8) and the other, tap water (CONT group, n=8) for 2 weeks. Two days before the end of the treatment rats were anesthetized with sodium pentobarbital, and catheters (PE10; filled with 500 U/ml heparinized saline) were implanted into the femoral artery and vein for subsequent measurement of mean arterial pressure (MAP), heart rate (HR) and drug administration. At the same time, 24G stainless steel cannulas were also inserted into the lateral ventricle according to the coordinates of Paxinos (18). Two days later cardiovascular responses to i.c.v. losartan (100 μg/10 μl) and muscimol (500 ng, 1 μg/10 μl), an agonist of GABA, were examined in conscious, freely moving rats. Cardiovascular responses to i.v. norepinephrine (25, 50, or 100 ng/rat; Narai Teshque, Kyoto, Japan) and hexamethonium (35 mg/kg; Narai Teshque) were also tested on the next day. Each drug was administered after MAP and HR had returned to the baseline level. Responses were quantified as the maximum MAP value following that recorded immediately before the drug was administered. In our previous experiments (19), the effectiveness of the ganglionic blockade was assessed by monitoring HR responses to bolus injections of phenylephrine.

Experiment 3

Twenty-four additional male Wistar rats weighing about 300 g were anesthetized with pentobarbital and mounted on a stereotaxic apparatus (David Kopf, Tujunga, USA). Then, a monopolar stainless steel microelectrode (tip diameter: 0.25 mm) was inserted into the middle of the anteroventral third ventricle (AV3V) area according to the coordinates of Paxinos (18). Lesions (lesioned group, n=8; control group, n=8) were made using 10-s DC currents of 2 mA delivered by a stimulator (SEN1100, Nihon Kohden, Tokyo, Japan) through a constant isolator
were evaluated using a light microscope. Lesioned animals shared a common area of damage to the periventricular tissue surrounding the optic recess. The lesions consistently destroyed the preoptic anterior hypothalamic nuclei, the organum vasculosum of the lamina terminalis, and the median preoptic nucleus. Some bilateral damage was always present in the medial portion of the median preoptic nucleus, immediately caudal to the lamina terminalis. Only one out of sixteen lesioned rats that did not sustain tissue damage in these areas was excluded from the data analysis (i.e., lesioned group; n=7).

Statistical Analysis

Data were analyzed with appropriate single or repeated measures of ANOVA and are presented as the means±SEM. Post hoc comparisons were made with Fisher's least significant difference test. Values of p less than 0.05 were considered to indicate statistical significance.

Results

Experiment 1

Although the development of hypertension by L-NAME treatment was attenuated by chronic i.c.v. losartan in a dose-dependent manner, i.v. losartan had no effect. One week after the cessation of i.c.v. losartan, blood pressure was elevated to the same level as in a-CSF-infused. L-NAME-treated rats. There were no differences in the effects of i.c.v. losartan administration of 100 mg/day between CONT and control rats (CONT-iv-c) (Fig. 1). Although HR measured by the tail-cuff method showed a transient slight decrease at week 1 in the L-NAME treated groups (LNAME-iv-c and LNAME-iv-h), there were no significant differences in body weight among these groups (Fig. 2).

Experiment 2

In conscious rats, mean AP values were significantly higher in L-NAME-treated rats than control (CONT) rats. Acute i.c.v. losartan produced no cardiovascular changes in neither L-NAME treated or CONT rats (Table 1). On the other hand, although i.c.v. muscimol elicited depressor effects in both groups in a dose-dependent manner, these responses were significantly larger in L-NAME treated rats than in CONT rats (Fig. 3). Vascular responses to i.v. norepinephrine were slightly smaller in L-NAME rats, but this difference was not statistically significant. Depressor responses to i.v. hexamethonium were similar in both groups (Table 1).
Fig. 2. Line graphs showing the changes in heart rate and body weight. LNAME-icv-H indicates an i.c.v. high dose of losartan (100 mg/day); LNAME-icv-L, an i.c.v. low dose of losartan (10 mg/day); LNAME-icv-C, i.c.v. aCSF; LNAME-iv-H, an i.v. high dose of losartan (100 mg/day); LNAME-iv-L, an i.v. low dose of losartan (10 mg/day) with L-NAME treatment. CONT, control rats without L-NAME treatment; and CONT-icv-H, an i.c.v. high dose of losartan (100 mg/day) without L-NAME treatment.

Experiment 3

One week after AV3V-lesioning, systolic blood pressures were the same among the lesioned, control and sham groups. On the other hand, the development of L-NAME hypertension was significantly attenuated in lesioned rats compared to that in control or sham rats (Fig. 4).

Discussion

Central NO is considered to play an important role in the regulation of blood pressure, based on the finding that acute central administration of a low dose of L-NAME increases blood pressure in rats, but i.v. infusion of the same dose of L-NAME does not (20). There is increasing evidence that NO plays a role within the central nervous system as a novel messenger. Experimental evidence indicates that, in the brain, NO is produced enzymatically (by a calcium-dependent mechanism) in response to stimulation of postsynaptic excitatory amino acid receptor (10). Immunoreactivity to NO synthase has been shown to have a widespread distribution in the brain. In the diencephalon, such immunoreactivity has been shown to be prevalent in both the hypothalamus and thalamus (21). Recently Toda et al. (22) reported that hypertension caused by the NO synthase inhibitor is associated with an elimination of nitrooxidergic neural function rather than an impairment of the basal release of NO from the endothelium. Neuronal culture work suggests that NO is specifically involved in mediating the actions of AII (13). In the present study we examined the central role of the RA system in rats, and found that relatively low doses of chronic i.c.v. losartan attenuated the development of hypertension in a dose-dependent manner in this model.
There is evidence that chronic inhibition of AT1 by chronic i.v. administration of AT1 type 1 (AT1) receptor antagonists or angiotensin converting enzyme inhibitor attenuates or prevents the hypertension, cardiac hypertrophy and renal damage in chronically NO-blocked rats (6, 7, 23). One possible explanation for the efficacy of AT1 antagonists is that long-term NO-blockade may result in hypertensivity to vasoconstrictors, such as AII, through the removal of the modulation of endothelium-derived NO, since PRA has been shown to be decreased by L-NAME treatment (7). In our study, leakage of losartan into the periphery is unlikely to have been the reason for attenuation, since the same amount of losartan i.v. had no effects on blood pressure elevation.

One of the action sites of centrally administered losartan might be the circumventricular regions of organs, including the AV3V area, since the access of AII is limited to a few discrete areas without blood-brain barriers, and it has been reported that AT1 receptors are densely located in these areas (24). Bains and Ferguson reported that NO may be acting within the paraventricular nucleus, a hypothalamic structure involved in mediating the cardiovascular changes initiated by activation of the subfornical organ (SFO), to inhibit further release of AII, thereby attenuating the cardiovascular response to stimulation of SFO (25). The AV3V area plays a critical role in the pathogenesis of many forms of experimental hypertension. Electrolytic ablation of the AV3V region has been demonstrated to attenuate the blood pressure elevation in SHR (26). DOCA-salt (27), Grollman (28) and Goldblatt (29) rat models of hypertension. In the present study, AV3V lesions also attenuated the development of hypertension elicited by chronic NO-blockade. Although the precise mechanisms are still unclear, one possible explanation is that this attenuation occurred via blockade of the central RA system due to the destruction of AT1 receptors. In light of the exaggerated sympathetic drive that characterizes most types of experimental hypertension, it is reasonable to posit that AV3V ablation may prevent hypertension, at least in part, by reducing autonomic outflow, since it has been reported that in the sustained phase of chronic NO-blockade, hypertension is largely due to the combined activities of α1-adrenoceptors and AT1 stimulation. Here, we can exclude the differences in L-NAME intake between AV3V-lesioned and sham rats, since the present metabolic study showed no differences in water intake between these groups (data not shown). At 1 week of experiment 1, the HR values in the L-NAME-treated group were lower than those in the control group. This may have been due to activation of the central baroreflex.

It is likely that, once established, the central RA system is not particularly crucial for maintaining this type of hypertension, since acute i.c.v. losartan produced no cardiovascular changes in conscious rats. Our preliminary study revealed that this amount of losartan was enough to produce depressor responses in SHR and Dahl salt-sensitive rats when it was i.c.v. injected. In addition, in another preliminary study, continuous i.c.v. losartan for a week also failed to normalize hypertension produced by chronic NOS inhibition (data not shown). These results are consistent with those of Qiu et al., who reported that acute blockade of the AT1 receptor with losartan had little effect on blood pressure or renal vascular resistance in either chronically NO-blocked or normal conscious rats (30). On the other hand, i.c.v. muscimol, a GABA_A agonist, elicited significantly large depressor responses in chronically treated L-NAME rats, indicating central GABAergic dysfunction or increased GABA_A receptor sensitivity in this model of hypertension. The brain GABAergic system has been shown to influence blood pressure maintenance in rats, and this influence may, in part, be accomplished by disruption of the central RA.
system. Although i.c.v. infusion of the GABA receptor antagonist bicuculline produced increases in blood pressure both in SHR and WKY rats, SHR are much more sensitive to GABA (31). We previously reported that chronic GABAergic stimulation attenuates hypothalamic hyperactivity and development of hypertension in SHR (32). One could argue that the depressor response by i.c.v. muscimol in L-NAME-treated rats might not be specific in this model of hypertension, since it is well known that i.c.v. muscimol elicits depressor responses even in SHR and Dahl salt-sensitive rats. Nonetheless, specificity should be present, based on the finding that only half this amount of muscimol was sufficient to produce the same level of depressor response in this model of hypertension. In addition, a trend was previously shown toward attenuation of bicuculline-induced increases in blood pressure by sarothan, an all receptor antagonist (31). These results suggest that cardiovascular responses produced by central application of GABA are mediated, at least in part, by the brain RA system. The interactions between central GABAergic neurons and NOergic neurons are still unclear. Immunohistochemical evidence reveals that neostriatal GABAergic interneurons contain NO synthase (33). Segovia et al. (34) reported that NO acts as a modulator of glutamate and GABA release in the brain via a continuous push-pull method in conscious rats.

In this study, GABAergic inhibitory effects on blood pressure may not have been mediated by sympathetic nerve activity in L-NAME treated rats, since the relative percentage changes in depressor responses to i.v. hexamethonium were similar in both groups. But we cannot completely exclude the effects of the sympathetic nervous system, since the absolute value of decrease in MAP was still larger in L-NAME-treated rats. Another possible mechanism for the depressor effect produced by i.c.v. muscimol is the attenuation of vasopressin secretion. We previously reported that central GABAergic stimulation reduced the pressor responses to hypertonic saline by inhibiting sympathetic activity and AVP secretion (35). In addition, the central action sites of hypertonic saline are thought to be AV3V and area postrema. Further examination is needed to clarify the precise mechanism.

We conclude that the central RA system plays an important role in the development of hypertension produced by chronic inhibition of NO synthesis. The AV3V area might be one of the central action sites in this model of hypertension. Moreover, once established, the central GABA system may be more important in the maintenance of hypertension than the RA system.

References


17. Bains JS, Ferguson AV: Nitric oxide regulates NMDA-
driven GABAergic inputs to type I neurones of the rat paraventricular nucleus. *J Physiol (Lond)* 1997; 499: 733-746.


