Kor J Physiol (1994) 28(2), 197-202

A Central Pressor Response to Endogenous Nitric Oxide Synthesis Inhibition in Anesthetized Rats

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=ABSTRACT=

The present study was aimed to determine if endogenous L-arginine-nitric oxide (NO) pathway has central, rather than peripheral, mechanisms in blood pressure regulation. Arterial blood pressure and heart rate responses to acute inhibition of the L-arginine-NO pathway were examined in rats anesthetized with thiopental (50 mg/kg, IP). An intracerebroventricular (ICV) cannula was placed in the left lateral ventricle. The right femoral artery was cannulated to measure arterial blood pressure and the vein to serve as an infusion route. N^G-nitro-L-arginine methyl ester (L-NAME) was infused either intracerebroventricularly or intravenously. ICV infusion (1.25 μL/min) of L-NAME (20 or 100 μg/kg per minute for 60 min) increased the mean arterial pressure and heart rate. Plasma renin concentrations(PRC) were significantly lower in L-NAME-infused group than in the control. L-Arginine (60 μg/min, ICV) prevented the pressor response to ICV L-NAME. The pressor response was not affected by simultaneous intravenous infusion of saralasin, but was abolished by hexamethonium treatment. Intravenous infusion (40 μL/min, 10~100 μg/kg per minute for 60 min) also increased blood pressure, while it decreased heart rate. These results indicate that endogenous L-arginine-NO pathway has separate central and peripheral mechanisms in regulating the cardiovascular function. The central effect may not be mediated via activation of renin-angiotensin system, but via, at least in part, activation of the sympathetic outflow.

Key Words: L-Arginine-nitric oxide pathway, Plasam renin concentration, Sympathetic activation

INTRODUCTION

It is now well established that a number of vasoactive agents induce vascular smooth muscle relaxation by acting on the endothelium to stimulate generation and release of endothelium-derived relaxing factors. This factor is now characterized mainly as nitric oxide (NO) synthesized from the amino acid L-arginine by a family of enzymes, NO synthases (Moncada et al, 1989). They may be inhibited by L-arginine analogues such as N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA) and N^G-nitro-L-arginine methyl ester (L-NAME). These compounds have been a useful tool in the investigation of the biological significance of the endogenous L-arginine-NO pathway in the cardiovascular

function.

L-NMMA induces constriction of aortic rings, indicating that there is a continuous release of NO to maintain a dilator tone (Palmer et al, 1988). A single intravenous injection of L-NAME causes hypertension (Persson et al, 1992), and an intravenous bolus administration or continuous infusion of L-NMMA causes a marked and a sustained rise in blood pressure (Gardiner et al, 1992). These findings point out that endogenous L-arginine-NO pathway plays a role in the regulation of blood pressure.

On the other hand recent immunocytochemical studies have detected varying amounts of NO synthase in all areas of animal and human brain (Forstermann et al, 1990; Bredt et al, 1990). NO has been found to play a physiological role in memory (Schumann & Madison, 1991), vision (Venturini et al, 1991), feeding behavior (Morley & Flood, 1991), nociception (Moore et al, 1991) and olfaction (Breer & Shepherd, 1991). Furthermore, a central role for NO in blood pressure regulation has also been suggested (Horn et al, 1994). Administration of NO donors into the nucleus tractus solitarius has been reported to elicit a significant decrease in blood pressure (Lewis et al, 1991).

The present study was aimed to investigate whether L-arginine-NO pathway in the brain has separate mechanisms from the peripheral pathway in blood pressure regulation. Blood pressure and heart rate responses to acute inhibition of the L-arginine-NO pathway were examined following intracerebroventricular (ICV) infusion of L-NAME.

METHODS

Male rats (Sprague-Dawley), weighing 200 ~250 g, were used. They were maintained in accordance with standards of care and use recommended by the American Physiological Society. They had free access to food and water until used.

On the experimental day, the animals were anesthetized with thiopental (50 mg/kg, i.p.). The right femoral artery was cannulated to measure arterial blood pressure and heart rate, and the vein to serve as an infusion route. An ICV cannula was placed in the left lateral ventricle to serve as a central infusion route.

A 30~60 min equilibration period was allowed to elapse until the protocol started. Basal data (blood pressure and heart rate) were obtained by averaging three values, recorded at least 5 min apart each, before the infusion of L-NAME was started.

In one group of rats, L-NAME was infused into the ventricle (1.25 μL/min for 60 min). The amount of L-NAME infused was 20 or 100 μg/kg per minute. In another, L-arginine (60 μg/min) was given combined with L-NAME (100 μg/kg per minute) to assess whether the effect of L-NAME was due to a specific inhibition of L-arginine-NO pathway. The control group was infused with artificial cerebrospinal fluid (ACSF) only.

To explore whether the central action of L-NAME is mediated by activating sympathetic outflow, effects of hexamethonium on the responses to ICV L-NAME were examined. Hexamethonium was given intravenously (IV) as a single bolus (5 mg/kg), followed by continuous infusion (0.5 mg/kg per minute, 40 μL/min). Whether activation of peripheral renin-angiotensin system is involved was also investigated. Saralasin (20 μg/kg per minute), an antagonist to the angiotensin II receptor, was infused IV at 40 μL/min. ICV infusion of L-NAME was not started until the blood pressure was stabilized at a new low steady state following the hexamethonium or saralasin treatment.

For comparison, L-NAME was infused IV (40 μL/min). The control group was infused IV with the vehicle (ACSF) only. Blood samples were taken from the artery at the end of the experiment in ICV L-NAME and control

groups. Their plasma renin concentrations (PRC) were determined by radioimmunoassay.

Drugs used were purchased from Sigma Chemical Company. Data are expresssed as mean±SEM. Statistical significance was examined using either t-test or analysis of variance with repeated measures on time.

RESULTS

ICV infusion of L-NAME increased mean arterial pressure (MAP) and heart rate (Fig. 1, Table 1). PRC was significantly lower in L-NAME-infused (100 μg/kg per minute) group than in the control (28.7±4.8 vs 41.3±2.7 ngAI/mL per hour, $p<0.05$). L-Arginine prevented the pressor response to L-NAME (Fig. 2).

MAP before hexamethonium treatment was 128±3 mmHg (n=6). When the blood pressure was stabilized at new low steady state (108±7 mmHg), infusion of ICV L-NAME was begun. As shown in Fig. 3, hexamethonium treatment

Table 1. Heart rates (beats/min) in rats infused with L-NAME intracerebroventricularly (ICV)

	Control (n=8)	ICV 20 (n=6)	ICV 100 (n=5)
0 min	359±30	350±15	354±20
15 min	363±37	357±11	369±22
30 min	367±31	373±19	406±28*
45 min	364±38	378±23*	447±37**
60 min	369±36	376±24*	466±36**

ICV 20 and 100 denote the doses of L-NAME, 20 and 100 μg/kg per minute, respectively.
* $p<0.05$, ** $p<0.01$; compared with 0 min value.

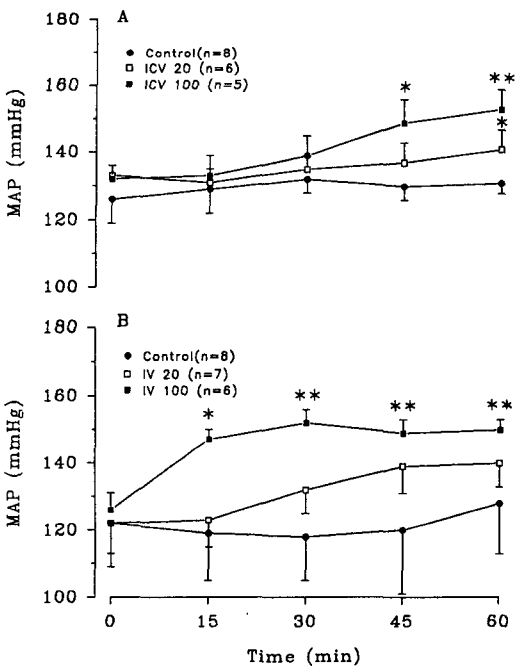

*Fig. 1. Mean arterial pressure (MAP) during intracerebroventricular (ICV, A) and intravenous (IV, B) infusion of L-NAME. Control group was infused with vehicle (artificial cerebrospinal fluid). Numerals 20 and 100 denote the doses of L-NAME infused in μg/kg per minute from 0 to 60 min. n= number of animals. * $p<0.05$, ** $p<0.01$; compared with basal value in each group.*

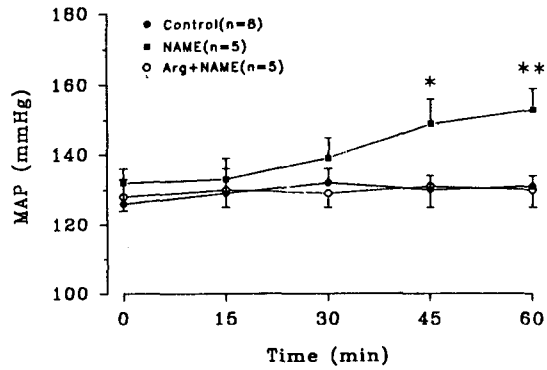

Fig. 2. Effects of L-arginine on the pressor response to L-NAME. Mean arterial pressure during intracerebroventricular infusion of L-NAME. [Arg +NAME] depicts the group infused with L-arginine (60 μg/min) and L-NAME (100 μg/kg per minute) simultaneously. Other legends as in Fig. 1.

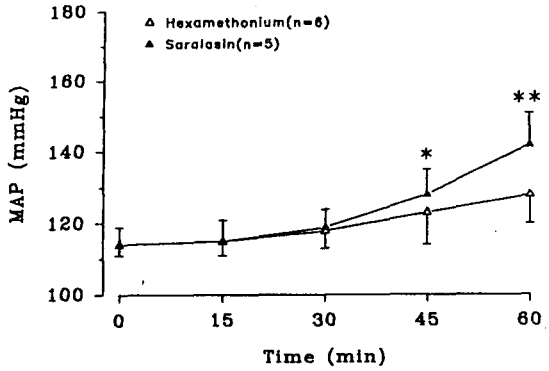

Fig. 3. Mean arterial pressure in responses to L-NAME (ICV, 100 μg/kg per minute) in rats treated with hexamethonium or saralasin. Basal arterial pressures before hexamethonium and saralasin treatment were 128±3 and 126±7 mmHg, respectively. Hexamethonium treatment significantly attenuated the pressor response to L-NAME.

significantly attenuated the pressor response to ICV L-NAME. The maximum increase of MAP during ICV infusion of L-NAME in the hexamethonium-treated was 10±4, being less than the control increase of 23±5 mmHg in the absence of hexamethonium (Fig. 3).

MAP before saralasin-treatment was 126±7 mmHg (n=5). When the pressure was stabilized at 114±5 mmHg, L-NAME was started. Saralasin treatment did not significantly affect the magnitude of the pressor response to ICV L-NAME, ie, 30±9 in the saralasin-treated and 23±5 mmHg in the control.

IV infusion also increased the blood pressure accompanied with a decrease in heart rate (Fig. 1, Table 2).

Table 2. Heart rates (beats/min) in rats infused with L-NAME intravenously (IV)

	Control (n=8)	IV 20 (n=7)	IV 100 (n=6)
0 min	368±29	361±14	342±17
15 min	363±32	349±13*	306±11
30 min	361±28	324±10**	283±13**
45 min	364±35	312±11**	284±13*
60 min	369±27	317± 9**	301±13*

IV 20 and 100 denote the doses of L-NAME, 20 and 100 μg/kg per minute, respectively.
* $p<0.05$, ** $p<0.01$; compared with 0 min value.

DISCUSSION

As has been observed by other investigators (Johnson & Freeman, 1992; Du et al, 1992), IV L-NAME increased MAP and decreased heart rate. This finding suggests that the cardiovascular system is in a state of constant active vasodilation dependent on an endogenous generation of NO. Therefore, the pressor response presumably reflects removal of the vasodilator action of endogenous NO system. The decrease in heart rate may be atrributed to a baroreceptor reflex response to the increased pressure, as suggested previously (Du et al, 1992). An ICV infusion also increased MAP. In addition, increasing the doses of L-NAME resulted in a greater rise of MAP. Whether the hypertension induced by central inhibition of NO synthesis is due specifically to decreases in NO synthesis depends on the specificity of the inhibitor. Two approaches have been used to test the specificity (Manning et al, 1993; Ribeiro et al, 1992). One is to test the ability of the NO precursor L-arginine to prevent or reverse the hypertension, and the other to test the ability to block the depressor or hyperemic response to a NO agonist such as acetylcholine. The effect of L-NAME in the present study is most probably due to a specific inhibition of L-arginine-NO pathway, since the pressor effect of L-NAME was prevented by the simultaneous infusion of L-arginine.

It has been found that intraperitoneal administration of a low dose of L-NNA markedly inhibits NO synthesis in the brain (Dwyer et al, 1991). This finding suggests a central action of

peripherally administered NO synthase inhibitors. One may argue that L-NAME infused ICV conversely leaks into the peripheral circulation and causes a pressor effect by a peripheral mechanism rather than by its direct action in the central nervous system. However, in association with the pressor response to ICV L-NAME, the heart rate was increased. The discrepancy in heart rate responses to ICV and IV L-NAME may rule out a peripheral leakage of ICV-infused L-NAME and further suggest separate peripheral and central cardiovascular regulatory mechanisms of the L-arginine-NO pathway. The parallel changes in heart rate and arterial pressure may indicate a increase of vasomotor tone in the central nervous system.

It has been found that sympathectomy by spinal cord transection eliminates the effect of central L-NMMA on the blood pressure (Togashi et al, 1992), suggesting an increase in sympathetic outflow. An involvement of the sympathetic activation was also suggested in the present study, since hexamethonium significantly attenuated the pressor and tachycardiac responses to ICV L-NAME. El Karib et al (1993) could not observe, however, significant changes in heart rate by ICV L-NNA despite the increased arterial pressure, concluding that the pressor effect was due to activation of the sympathetic nervous system within the central nervous system. A parallel change of both parameters, as in the present study, would be more logical if the sympathetic nervous system is activated.

Furthermore, we also examined if the renin-angiotensin system is involved in the centrally-mediated mechanism leading to the increased arterial pressure. The pressor response to ICV L-NAME was associated with a lower PRC compared with the control. The change in PRC may not be a cause of the pressor response, therefore, but may be accounted for by a reflex decrease in response to the increased blood pressure. In addition, saralasin could not block the pressor effect of ICV L-NAME. These findings are in line with those obtained by El Karib et al (1993) who observed an identical degree of pressor response to ICV L-NNA in nephrectomized rats. Taken together, a renin-dependent mechanism is unlikely to be involved in the central pressor effect of L-NAME.

On the other hand, a number of investigators have demonstrated that long-term increases in arterial pressure due to NO synthesis inhibition are associated with changes in either sodium and water intake or renal function. Lahera et al (1991) have provided evidence that L-NAME shifts the pressure-natriuresis relation, decreasing renal excretory capability. An increase in renal vascular resistance as in other resistive vasculature due to NO synthesis inhibition may be responsible for the decreased renal function. However, since an increased arterial pressure subsequent to the inhibition of NO synthesis may enhance glomerular filtration, the explanation may be complicated. In addition, renal mechanism may be less effective for an acute change in blood pressure. Further studies will be needed to clarify a possible contribution of the renal mechanism.

In summary, the present study indicates that the endogenous L-arginine-NO pathway has a central mechanism in the regulation of cardiovascular function. It may be, at least in part, mediated by activation of the sympathetic outflow.

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