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A nitric oxide-dopamine link pathway in organum vasculosum laminae terminalis of rat brain exerts control over blood pressure

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1 Experiments were carried out to explore the possible role played by the nitric oxide (NO) and dopamine (DA) system in the organum vasculosum laminae terminalis (OVLT) of rat brain in arterial pressure regulation.

2 Intracerebroventricular (ICV) administration of NO donors such as hydroxylamine or sodium nitro-prusside (SNP) caused an up to 59 mmHg decrease in blood pressure (BP) and a decrease in DA release (measured by nafion coated carbon fibre electrodes in combination with voltammetry) in the OVLT. In contrast, ICV administration of N^G-nitro-L-arginine methyl ester (L-NAME; a constitutive NO synthase inhibitor) or 7-nitroindazol (a neuronal NO synthase inhibitor) caused an up to 98 mmHg increase in BP and an increase in DA release in the OVLT.

3 Intra-OVLT injection of amphetamine (0.1–0.3 mg), SKF 38393 (a DA D_1 receptor agonist; 0.01–0.03 mg), or apomorphine (a DA $D_{2,3}$ receptor agonist; 0.01–0.03 mg) caused an increase in BP. On the other hand, intra-OVLT injection of SCH23390 (a DA D_1 receptor antagonist; 0.005–0.020 mg) or haloperidol (0.005–0.020 mg) caused a decrease in BP.

4 The pressor effects induced by intra-OVLT administration of L-NAME were attenuated by pretreatment with intra-OVLT injection of haloperidol, SCF23390, or 6-hydroxydopamine. In the contrast, the hydroxylamine-, 8-Br-cGMP- or SNP-induced depressor effects were attenuated by pretreatment with intra-OVLT injection of amphetamine, SKF 38393 or apomorphine.

5 The data suggest that activation of a NO-DA link pathway within the OVLT of rat brain exerts control over blood pressure.

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Abbreviations: BP, blood pressure; DA, dopamine; ICV, intracerebroventricular; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; OVLT, organum vasculosum laminae terminalis; SNP, sodium nitroprusside

Introduction

Evidence has indicated that the organum vasculosum laminae terminalis (OVLT) of the brain is a major forebrain area for maintaining the homeostasis of blood pressure (Johnson & Gross, 1993). Immunocytochemical studies have reported that high concentration of nitric oxide (NO) (Jurzak *et al*., 1994; Lin & Lin, 2000) and dopamine (DA) (Jennes *et al*., 1982) within OVLT. Indeed, intracerebral administration of NO donors such as hydroxylamine, sodium nitroprusside (SNP) or S-nitro-acetyl-penicillamine caused an up to 55 mmHg decrease in blood pressure but an increase in NO release in the OVLT (Lin *et al*., 1999). In contrast, intracerebral administration of N^G-nitro-L-arginine methyl ester (L-NAME; a constitutive NO synthase inhibitor) caused an up to 45 mmHg increase in blood pressure but a fall in NO release in the OVLT (Lin *et al*., 1999).

Other lines of evidence has suggested that the dopaminergic systems in the central nervous system (CNS) possess pressor effects in rats. For example, the pressor effects produced by systemic injection of guinpirole (a dopamine D_2 receptor agonist) were antagonized by metoclopramide (a dopamine D_2 receptor antagonist which is able to pass through blood-brain barrier), but not by domperidone (a dopamine D_2 receptor antagonist which is unable to pass through blood-brain barrier) (Nagahama *et al*., 1986; Van den Buuse, 1992). Physical or chemical stimulation of mesolimbic, mesocortical or nigrostriatal dopamine pathway was also shown to produce pressor effects (Spring & Winkelmuller, 1975; Tan *et al*., 1983; Cornish & Van den Buuse, 1994; Lin & Yang, 1994).

Recently, Horn and his associates (1994) have provided the first evidence that NO may play significant roles in regulating central nervous system control over the cardiovascular system actions within the paraventricular nuclei (PVN) and NO may exert significant control over endogenous release of amino acid neurotransmitters within this region of the brain. In addition, the pressor effects induced by intracerebral injection of angiotensin were attenuated by intra-OVLT administration of 6-hydroxydopamine (a neurotoxin which is able to deplete central DA) (Bellin *et al*., 1987; 1988). These observations have led to the suggestion that the NO within OVLT acts through the endogenous release of DA to induce blood pressure responses.

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Therefore, to deal with the question, present experiments were undertaken to determine whether central administration of NO donors, NO synthase (NOS) inhibitors, evoked changes in both the DA release within the OVLT and cardiovascular function in rats. In addition, experiments were carried out to determine whether the cardiovascular response can be produced by intra-OVLT administration of DA receptor agonists or antagonists.

Methods

Experimental animals

Three hundred and two male Sprague-Dawley rats (253–352 g) were used in the entire series of experiments. Upon receipt from the supplier (Animal Resource Center, National Yang-Ming University Medical College, Taipei, Taiwan, Republic of China), the animals were housed in a temperature-regulated ($22\pm1^{\circ}C$) room on 12/12 h light/dark cycle with food and water *ad libitum* for at least 2 weeks before experiments. The light was turned on at 0600 h and turned off at 1800 h.

Surgical preparation

The animals were anaesthetized with urethane (1.4 g kg^{-1}, i.p.) and placed in a Kopf stereotaxic apparatus. For direct injection of drugs into the lateral cerebral ventricle or the OVLT, a stainless-steel cannula which consisted of a guide tube (0.81 mm outer diameter) with a snugly fitting trocar was implanted into the lateral cerebral ventricle (AP, −0.8 mm; LAT, −1.5 mm and DV, −3.5 mm) or the OVLT (AP, −0.5 mm; LAT, −0.1 mm and DV, −8.5 mm) according to the atlas and the coordinates of Paxinos & Watson (1982). Microinjection was made into the OVLT through a 26 gauge cannula connecting to a 10 μl Hamilton microsyringe. The volume of fluid injected over 5 s was 5.0 or 0.5 μl for intracerebroventricular (ICV) or intra-OVLT injection, respectively. For measurement of DA release, a nafion-coated carbon fibre electrode was implanted stereotaxically into the OVLT. Auxiliary (silver wire) and reference (Ag/AgCl) electrodes were placed on the dura surface of the parietal skull. Differential pulse voltammograms were then recorded automatically every 0.5 s (Lin & Yang, 1994). For assessment of cardiovascular functions, a fine catheter was inserted into the femoral artery and was connected *via* a Statham blood pressure transducer to a Gould 4-channel polygraph for recording mean and pulsatile arterial blood pressure. Both the heart rate and blood pressure were measured.

Drugs

Drugs, administered into the OVLT included hydroxylamine (Sigma, 0.01–0.1 mg), sodium nitroprusside (SNP; sigma; 0.01–0.1 mg), N^G-nitro-L-arginine methyl ester (L-NAME; RBI; 0.05–0.2 mg), 7-nitroindazol (7-NI; RBI; 0.1 mg) and aminoguanidine (RBI; 0.10 mg). All compounds were dissolved in saline. The drugs administered intracerebroventricularly included hydroxylamine (0.05–0.50 mg), L-NAME (0.1–1.0 mg), aminoguanidine (1.0 mg), SNP (0.10–0.50 mg), or 7-NI (1.0 mg).

DA monitoring

A multiple carbon fibre (28 μm in diameter, AVCO, Lowell, MA, U.S.A.) was inserted into the pulled glass micropipette (20–25 mm in length). The tip was cut, and then carbon fibre was pushed out of the pipette tip. Electrical contact with the fibre was made using silver paste. The tip and blunt end of the pipette were sealed with cyanoacrylate adhesive (super glue). The entire surface of a pyrolytic carbon fibre was 12 μm thick and 100 ± 25 μm long. To improve the sensitivity and selectivity of carbon fibre for DA, the electrodes were electrically pretreated as described previously (Lin & Yang, 1994). This treatment consisted of a DC current applied in two stages, 2.2 V for 30 s in 0.1 M H_2SO_4, and 2.2 V for 30 s in 0.1 M HCl. The carbon fibre electrode was washed with distilled water. The tip of the carbon fibre electrode was coated with 1% nafion solution (Aldrich Chemical Company, Inc, Milwaukee, WI, U.S.A.). The nafion-coated electrode was then dried at 60°C for 20 s and used immediately for *in vitro* followed by *in vivo* measurements. Differential pulse amperometry was performed *in vitro* and *in vivo* with a Biopulse (Solea Tacussel Co., France) using the following scan parameters: imposed initial potential = −220 mV; imposed final potential = −70 mV; prepulse = 70 ms; measuring pulse = 40 ms; measuring potential = 40 mV; and pulse cycle = 25 s. The sensitivity of the nafion-coated carbon fibre electrode to catecholamines in the concentration range of 200–800 nm was determined using differential pulse amperometry in a temperature-controlled (37°C) water bath. Phosphate-buffered saline was used as blank and solvent for the test solutions. Our electrodes were 300–1200 times more sensitive to DA than to 3,4-dihydroxyphenylacetic acid, ascorbic acid, or uric acid. Our electrodes are insensitive to serotonin.

Histology

After the completion of the experiments, an aliquot of 0.5 μl of methylene blue was injected down the cannula to measure the spread of the injected solution. The head of each animal was perfused with PBS solution, followed by 4% paraformaldehyde (PFA) fixative solution. After perfusion, the brain was removed and placed in a well labelled glass vial filled with 4% PFA fixative, and fixed at 4°C for 0.5–1 h after which the solution was changed to 15% sucrose in PBS until the brain sank in the vial. Later, the fixed brains were cut in 50 μm sections so that stereotaxic coordinates of injection site in each animal were verified. It was found that the stained cross-sectional area in the OVLT was approximately 0.5 mm in diameter.

Statistics

Data obtained from 302 animals were included after successful experiments including histological verification of the stereotaxic target. Blood pressure and DA release responses were assessed as changes from pre-injection values (mmHg or nM). Results are expressed as the mean ± s.e.mean for an experiment. A two-way analysis of variance (ANOVA) with repeated measures was used to evaluate the difference between groups. Differences between individual means were assessed by *post hoc* Scheff's multiple range tests. $P<0.05$ was taken to indicate statistical significance.

Results

Studies with NO donors

Intracerebroventricular administration of SNP (0.10–0.50 mg) or hydroxylamine (0.125–0.50 mg) caused a dose-dependent decrease in both the mean arterial pressure and DA release from the OVLT of rat brain. The data are summarized in Table 1. Figure 1 depicts tracings from a representative experiment showing the effect of ICV injection of SNP on pulsatile BP, MAP and DA release of the OVLT. Direct administration of SNP or hydroxylamine into the OVLT of rat brain (Table 2) also caused a decrease in MAP. As shown in Table 2, the depressor effects produced by intra-OVLT injection of hydroxylamine, SNP or 8-Br-cGMP were attenuated by pretreatment with intra-OVLT injection of either amphetamine, SKF38393 or apomorphine. An i.v. dose of 5 mg kg^{-1} of SNP caused an insignificant effect on either MAP or DA concentration in OVLT.

Studies with NOS inhibitors

In contrast, intracerebroventricular administration of L-NAME caused a dose-dependent increase in both MAP and DA release in the OVLT of rat brain. The data are summarized in Table 1. Figure 2 depicts tracings from a typical experiment showing the effect of ICV injection of L-NAME on the pulsatile BP, MAP and DA release of the OVLT. Direct administration of L-NAME (0.05–0.2 mg), AG (0.05–0.2 mg) or 7-NI (0.05–0.2 mg) into the OVLT also caused an increase in MAP (Table 3). AS compared with those of AG, intracerebral administration of either L-NAME or 7-NI had a greater pressor effect and a greater release of OVLT DA. Pretreatment of rats with intra-OVLT injection of haloperidol, SCH23390 or 6-OHDA significantly attenuated the pressor responses induced by intra-OVLT injection of L-NAME, AG or 7-NI (Table 3). Neither the MAP nor DA release in OVLT was affected by an i.v. dose of L-NAME (Table 1). Furthermore, ICV (Table 1) or intra-OVLT (Table 2) administration of a hypertonic solution such as 4.5% NaCl had an insignificant effect on MAP.

Studies with dopaminergic drugs

Intra-OVLT administration of either amphetamine (0.1–0.3 mg), SKF38393 (0.01–0.03 mg) or apomorphine (0.01–0.03 mg) caused pressor effects in rats (Table 4). On the other hand, direct injection of either SCH23390 (0.005–0.020 mg) or haloperidol (0.005–0.020 mg) into the OVLT of rat brain caused depressor effects (Table 4). However, systemic

Table 1 Maximal changes in MAP and DA concentration in the OVLT produced by injections of NOS inhibitors and NO donors in rats

Treatment	*ΔMAP* (mmHg)	*ΔDA release* (nM)
0.9% NaCl, i.c.v.	3±2	5±2
4.5% NaCl, i.c.v.	3±2†	4±2†
L-NAME 0.1 mg, i.c.v.	26±4*	88±12*
L-NAME 0.5 mg, i.c.v.	45±7*	172±23*
L-NAME 1.0 mg, i.c.v.	98±8*	230±31*
AG 0.1 mg, i.c.v.	6±2*	4±2*
AG 0.5 mg, i.c.v.	27±3*	77±8*
AG 1.0 mg, i.c.v.	45±4*	151±19*
7-NI 0.1 mg, i.c.v.	22±3*	75±9*
7-NI 0.5 mg, i.c.v.	50±7*	163±19*
7-NI 1.0 mg, i.c.v.	91±7*	211±26*
SNP 0.10 mg, i.c.v.	−30±3*	−63±5*
SNP 0.25 mg, i.c.v.	−44±4*	−87±14*
SNP 0.50 mg, i.c.v.	−59±3*	−124±22*
Hydroxylamine 0.125 mg	−22±2*	−58±4*
Hydroxylamine 0.25 mg	−39±3*	−87±6*
Hydroxylamine 0.50 mg	−52±4*	−125±12*
0.9% NaCl, i.v.	−3±2	4±2
L-NAME 10 mg kg^{-1}, i.v.	−4±2	−5±2
SNP 5 mg kg^{-1}, i.v.	5±2	3±1

Values are means±s.e.mean for six rats per group. Δ, difference between the control values before and 30 min after the start of drug injection. DA, dopamine; i.c.v., intracerebroventricular; i.v., intravenous. *Significantly different from corresponding control values (saline group), $P<0.05$ (ANOVA). †Insignificantly different from control values (0.9% NaCl group), $P>0.05$ (ANOVA).

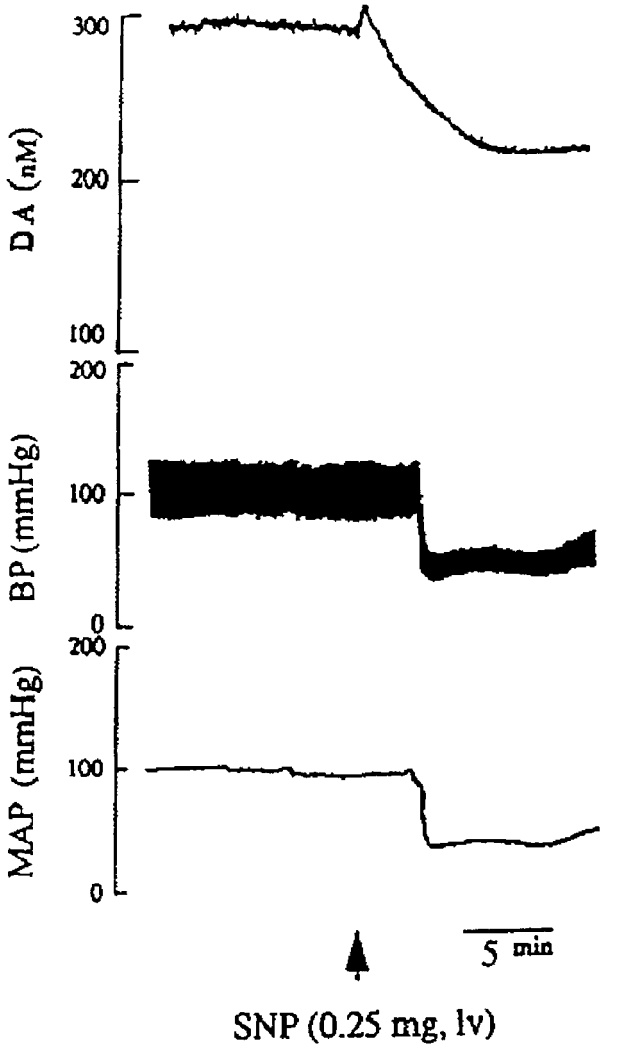

Figure 1 Representative tracings showing the effects of administration of SNP into the lateral cerebral ventricle (lv) on DA release recorded in the OVLT area, blood pressure (BP) and mean arterial pressure (MAP) in a rat. The basal level of DA in the OVLT is 298 nM.

Table 2 Maximal changes in MAP produced by intra-OVLT injection in NO donors in saline-, amphetamine-, SKF38393-, or apomorphine-treated rats

Treatment	*ΔMAP, mmHg*
Saline-treated rats	
Hydroxylamine 0.01 mg	−10±2
Hydroxylamine 0.05 mg	−26±3
Hydroxylamine 0.10 mg	−43±4
SNP 0.01 mg	−15±2
SNP 0.05 mg	−27±3
SNP 0.10 mg	−50±4
8-Br-cyclic GMP 0.05 mg	−21±2
Amphetamine (0.1 mg)-treated rats	
Hydroxylamine 0.01 mg	2±1*
Hydroxylamine 0.05 mg	−11±3*
Hydroxylamine 0.10 mg	−23±4*
SNP 0.01 mg	−2±1*
SNP 0.05 mg	−13±2*
SNP 0.10 mg	−24±3*
8-Br-cyclic GMP 0.05 mg	−10±2*
SKF38393 (0.01 mg)-treated rats	
Hydroxylamine 0.01 mg	2±1*
Hydroxylamine 0.05 mg	−9±2*
Hydroxylamine 0.10 mg	−18±3*
SNP 0.01 mg	−3±2*
SNP 0.05 mg	−12±3*
SNP 0.10 mg	−22±3*
8-Br-cyclic GMP 0.05 mg	−9±2*
Apomorphine (0.01 mg)-treated rats	
Hydroxylamine 0.01 mg	−2±1*
Hydroxylamine 0.05 mg	−14±2*
Hydroxylamine 0.10 mg	−28±3*
SNP 0.01 mg	−4±2*
SNP 0.05 mg	−14±2*
SNP 0.10 mg	−28±3*
8-Br-cyclic GMP 0.05 mg	−11±2*

Values are means±s.e.mean for four rats per group. Δ, difference between the control values before and 20 min after the start of second injection; the second injection was given 1 h after the first injection. *Significantly different from corresponding control values (saline group), $P<0.05$ (ANOVA).

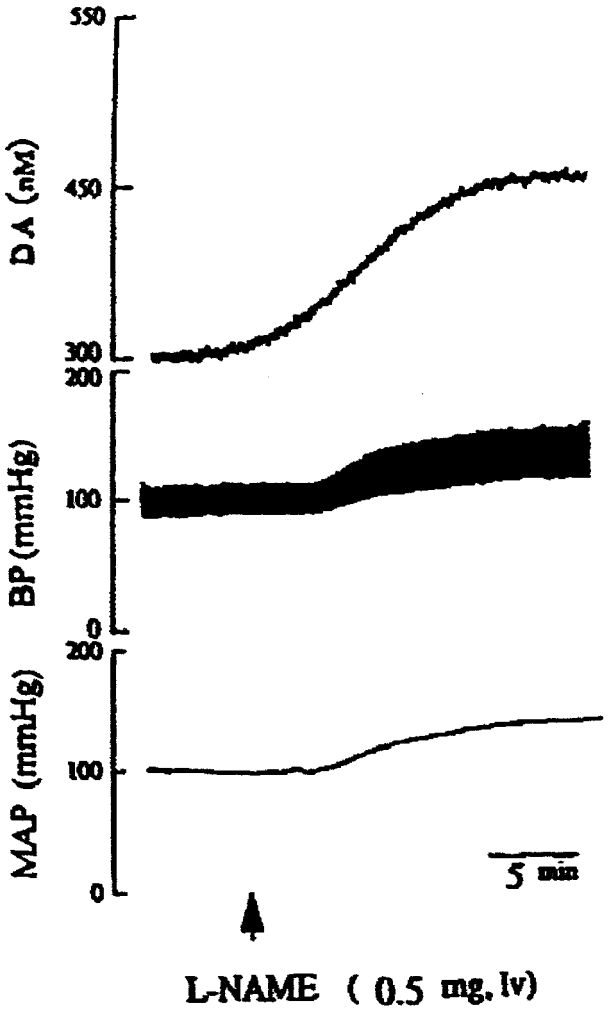

Figure 2 Representative tracings showing the effects of administration of L-NAME into the lateral cerebral ventricle (lv) on DA release recorded in the OVLT area, blood pressure (BP) and mean arterial pressure (MAP) in a rat. The basal level of DA in the OVLT is 302 nM.

injection of a large amount of amphetamine (2 mg kg^{-1}, i.v.), SKF38393 (0.2 mg kg^{-1}, i.v.), apomorphine (0.2 mg kg^{-1}, i.v.) or haloperidol (0.1 mg kg^{-1}, i.v.) caused an insignificant effect on MAP (Table 4).

Discussion

In the present study, *in vivo* voltammetry was used in combination with electrochemically treated nafion coated carbon fibre electrodes for measuring the DA release in the OVLT of rat brain. Direct administration of NO donors such as SNP (0.01–0.1 mg in 5 μl) and hydroxylamine (0.01–0.1 mg in 5 μl) into the cerebroventricular fluid system elicits a decrease in both MAP and DA release in the OVLT of rat brain. In contrast, ICV administration of L-NAME (0.01–0.1 mg in 5 μl; an endothelial NO synthase inhibitor), AG (1.0–2.0 mg in 5 μl; an immunologic NO synthase inhibitor) or 7-NI (0.1–1.0 mg in 5 μl; a neuronal NO synthase inhibitor) elicits an increase in both MAP and OVLT DA release. The BP responses caused by ICV injection of either NO donors or NOS inhibitors could be mimicked by direct administration of DA receptor antagonists or agonists into the OVLT area of rat brain, respectively. In addition, the pressor effects induced by intra-OVLT injection of L-NAME (0.05–0.2 mg in 0.5 μl), AG (0.05–0.2 mg in 0.5 μl) or 7-NI (0.05–0.2 mg in 0.5 μl) could be attenuated by pretreatment with intra-OVLT injection of DA receptor antagonists, while the depressor effects produced by intra-OVLT injection of NO donors could be attenuated by pretreatment with intra-OVLT injection of DA receptor agonists. The data suggest that there exists a NO-DA link pathway within the OVLT of rat brain which exert control over blood pressure. Therefore, NO results in diminished arterial pressure due to inhibition of dopamine release within the OVLT. Within the OVLT, inhibition of NOS enhances DA release and results in pressor effects, while activation of NO pathways within the OVLT inhibits DA release and results in depressor effects in rats.

There are three NOS isoforms that are named after the tissue from which they were first cloned and numbered in the order in which they were cloned (Bredt & Snyder, 1994; Marletta, 1994). nNOS (Type I) and endothelial NOS (eNOS) (type III) are constitutively expressed and are calcium

Table 3 Maximal changes in MAP produced by intra-OVLT injection of NOS inhibitors in saline-, haloperidol-, SCH23390-treated and 6-OHDA-treated rats

Treatment	ΔMAP (mmHg)
Saline-treated rats	
0.9% NaCl	2±1
4.5% NaCl	3±2†
L-NAME 0.05 mg	12±2
L-NAME 0.10 mg	28±3
L-NAME 0.20 mg	47±3
AG 0.05 mg	2±1
AG 0.1 mg	13±2
AG 0.2 mg	33±2
7-NI 0.05 mg	11±2
7-NI 0.1 mg	25±2
7-NI 0.2 mg	44±3
Haloperidol (0.005 mg)-treated rats	
0.9% NaCl	3±2
L-NAME 0.05 mg	5±2*
L-NAME 0.10 mg	11±3*
L-NAME 0.20 mg	18±2*
AG 0.05 mg	3±2
AG 0.1 mg	5±2*
AG 0.2 mg	15±3*
7-NI 0.05 mg	4±2*
7-NI 0.1 mg	10±2*
7-NI 0.2 mg	19±3*
SCH23390 (0.005 mg)-treated rats	
0.9% Saline	2±1
L-NAME 0.05 mg	6±2*
L-NAME 0.10 mg	13±2*
L-NAME 0.20 mg	21±3*
AG 0.05 mg	3±2*
AG 0.1 mg	5±2*
AG 0.2 mg	12±3*
7-NI 0.05 mg	4±2*
7-NI 0.1 mg	11±2*
7-NI 0.2 mg	22±4*
6-OHDA-treated rats	
0.9% Saline	4±2
L-NAME 0.05 mg	3±2*
L-NAME 0.10 mg	7±3*
L-NAME 0.20 mg	18±2*
AG 0.05 mg	3±2
AG 0.1 mg	4±2*
AG 0.2 mg	10±2*
7-NI 0.05 mg	3±1*
7-NI 0.1 mg	6±2*
7-NI 0.2 mg	20±3*

Values are means±s.e.mean for five rats per group. Δ, values that denote the difference between the control values before and 20 min after the start of L-NAME, AG or 7-NI injection; the drug injection was given 1 h after the haloperidol or SCH23390 injection or 1 week after the 6-OHDA injection. *Significantly different from corresponding control values (saline-treated group), $P<0.05$ (ANOVA). †Insignificantly different from control values (0.9% NaCl group), $P>0.05$ (ANOVA).

Table 4 Maximal changes in mean arterial pressure (MAP) produced by injection of amphetamine, SKF38393, apomorphine, SCH23390 or haloperidol in rats

Treatment	ΔMAP (mmHg)
Amphetamine 0.1 mg, intra-OVLT	14±2*
Amphetamine 0.2 mg, intra-OVLT	26±3*
Amphetamine 0.3 mg, intra-OVLT	39±4*
Amphetamine 2 mg kg^{-1}, i.v.	4±2
SKF38393 0.01 mg, intra-OVLT	15±2*
SKF38393 0.02 mg, intra-OVLT	25±3*
SKF38393 0.03 mg, intra-OVLT	36±3*
SKF38393 0.2 mg kg^{-1}, i.v.	3±2
Apomorphine 0.01 mg, intra-OVLT	11±2*
Apomorphine 0.02 mg, intra-OVLT	19±2*
Apomorphine 0.03 mg, intra-OVLT	32±3*
Apomorphine 0.2 mg kg^{-1}, i.v.	3±2
SCH23390 0.005 mg, intra-OVLT	−4±2
SCH23390 0.010 mg, intra-OVLT	−12±3*
SCH23390 0.020 mg, intra-OVLT	−23±3*
SCH23390 0.1 mg kg^{-1}, i.v.	3±2
Haloperidol 0.005 mg, intra-OVLT	−5±3*
Haloperidol 0.010 mg, intra-OVLT	−13±2*
Haloperidol 0.020 mg, intra-OVLT	−33±3*
Haloperidol 0.1 mg kg^{-1}, i.v.	4±2

Values are means±s.e.mean for four rats per group. Δ, values that denote the difference between the control values before and 20 min after the start of drug injection. *Significantly different from corresponding control values (saline group), $P<0.05$ (ANOVA).

dependent. Immunologic NOS (iNOS) (type II) is expressed after immunologic challenge and neuronal injury and is calcium independent under most circumstances (Yun & Dawson, 1996). The concentrations of NO donors and NOS inhibitors used for intracerebral injections in the present results seem quite high. However, systemic administration of these drugs at a 5 fold concentration was shown to produce an insignificant effect on BP. In addition, intracerebral injection of NO donors or NOS inhibitors at an equivalent amount caused a depressor and a pressor effect, respectively. Furthermore, intracerebral injection of a hypertonic solution (e.g., 4.5% NaCl) still failed to elicit a BP response in our rats. Thus it appears that non-specific and peripheral effects can be ruled out in the present results.

The concentration (10 mg kg^{-1}, i.v.) of L-NAME used in the present study had no effect on systemic blood pressure. This concentration of L-NAME has been shown previously to increase systemic blood pressure in anaesthetized rats by over 30 mmHg (Rees *et al*., 1990); indeed, concentration of L-NAME as low as 0.01 mg kg^{-1} elicit increases in systemic blood pressure in anaesthetized rats. The reason for this discrepancy may be due to the difference in analysing the pressure response patterns. In our results, a transient rise for a few minutes in BP produced by L-NAME was not counted. We counted only the concentration of L-NAME which is able to produce a long-lasting BP change for at least 30 min after drug administration. It should be mentioned that the lipophilic nature of NO means that microinjection of an NO-donor will effect a fairly large area of the brain (due to diffusion of free NO) and not be restricted to the site of administration. The possibility has been tested in our previous results (Lin *et al*., 1999). We observed that, compared to those caused by intra-OVLT injection of a NO donor, administration of the same amount of a NO donor into the neighbouring areas of the OVLT caused decreased BP changes. The problem of NO diffusion could also be circumvented by the fact that the same depressor effect is produced by microinjecting a cyclic GMP analogue into the OVLT as shown in the present results.

In the present results, there is a lag period between OVLT/ICV application of NOS inhibitors/NO donors and the change in MAP. In fact, numerous chemical substances pass from the bloodstream into the brain at rates that are far slower than for entry into all other organs in the body. There

are similar slow rates of transport between the cerebrospinal fluid and the brain (Cooper *et al*., 1996). These permeability barriers appear to be the end result of many contributing factors that present diffusional obstacles to chemicals on the basis of molecular size, change, solubility, and specific carrier system.

As suggested in the Introduction, the dopamine D_2 system in the CNS possesses a pressor action in rats (Nagahama *et al*., 1986; Lin & Yang, 1994). The present results further demonstrated that direct injection of DA receptor agonists such as amphetamine, SKF38393 (a DA D_1 receptor agonist) or apomorphine (a DA $D_{2,3}$ receptor agonist) into the OVLT of rat brain caused pressor effects in rats. On the other hand, intra-OVLT injection of DA receptor antagonists such as SCH23390 (a DA D_1 receptor antagonist) or haloperidol (a DA $D_{2,3}$ receptor antagonist) caused depressor effects in rats. The pressor or depressor effects produced by intracerebral injection of NOS inhibitors or NO donors, respectively, was associated with an increase or a decrease in DA release recorded in the OVLT of rat brain.

As shown in our previous results (Lin *et al*., 1999), the depressor effects caused by intra-OVLT administration of NO donors as well as the pressor effects caused by intra-OVLT administration of L-NAME was attenuated by pretreatment with spinal transection. The pressor effects caused by stimulation of substantia nigra pars compacta were also attenuated by spinal transection (Lin & Yang, 1994). These results imply that the decreases of arterial pressure after intra-OVLT administration of NO donors or DA receptor antagonists in sham-operated rats are attributable to inhibition of the sympathetic efferent activity. In contrast, the increases of arterial pressure after intra-OVLT administration of NOS inhibitors or DA receptor agonists in sham-operated rats are attributable to stimulation of the sympathetic efferent activity. In fact, eNOS normally regulates basal vascular tone while nNOS is involved in central and peripheral neurotransmission, including the nonadrenergic noncholinergic nervous system (Moncada *et al*., 1991). Nagashima *et al*. (1994) reported that L-NAME inhibited the norepinephrine-induced increase in blood flow through brown adipose tissue. In addition, nNOS has been isolated in sympathetic preganglionic neurons and in spinal cord neurons, which mediate sympathetic output to various peripheral organs (Vizzard *et al*., 1994). Because the sympathetic nervous system is the principal regulator of blood pressure, activation of NO-DA pathways in the CNS may modulate the BP response by affecting the sympathetic efferent activity. In the present results, as compared with those of an iNOS inhibitor, intracerebral administration of either an eNOS or an nNOS inhibitor had a greater pressor effect and a greater release of OVLT DA. Thus it appears that more than one isoform of NOS may be involved in regulating blood pressure.

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