Role of nitric oxide in adrenal catecholamine secretion in anesthetized dogs

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Role of nitric oxide in adrenal catecholamine secretion in anesthetized dogs. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1075–R1081, 1998.—We examined the role of nitric oxide (NO) in adrenal catecholamine secretion in response to splanchnic nerve stimulation (SNS) and exogenous acetylcholine (ACH) in anesthetized dogs. The NO synthase inhibitor N\textsuperscript{\textsubscript{\textsuperscript{-}}}-nitro-L-arginine methyl ester (L-NAME), NO donor 3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-1-propanamine (NOC 7), and ACH were administered intra-arterially into the adrenal gland. The increases in catecholamine output induced by ACH (0.75–3 µg) were enhanced by L-NAME (0.1–1 mg/min) and inhibited by NOC 7 (0.2–2 µg/min). Inhibition by NOC 7 (2 µg/min) was observed during treatment with L-NAME (1 mg/min). The increases in catecholamine output induced by SNS (1–2 Hz) were inhibited by L-NAME and by NOC 7. No inhibitory effect of NOC 7 was observed during treatment with L-NAME. These results suggest that NO may play an inhibitory role in the regulation of adrenal catecholamine secretion in response to exogenous ACH.

adrenal gland; N\textsuperscript{\textsuperscript{-}}\textsuperscript{-}nitro-L-arginine methyl ester; 3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-1-propanamine; splanchnic nerve stimulation; acetylcholine

NITRIC OXIDE (NO) is produced enzymatically from the terminal guanidino nitrogen of L-arginine by the action of NO synthase (NOS) (12, 14). There are at least three isoforms of NOS: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS. The adrenal medulla possesses characteristic postganglionic sympathetic neurons, and the presence of nNOS has been demonstrated (7, 9, 11, 15). In vitro studies using NOS inhibitors and NO donors were performed to examine the role of NO in modulating catecholamine secretion from the adrenal medulla but the results remain controversial. It has been reported that the NOS inhibitor N\textsuperscript{\textsuperscript{-}}\textsuperscript{-}nitro-L-arginine methyl ester (L-NAME) inhibits acetylcholine (ACH)-induced catecholamine secretion in bovine chromaffin cells (17) and that the NO donor sodium nitroprusside (SNP) enhances nicotine-induced catecholamine secretion in cultured bovine chromaffin cells (10). These findings suggest that NO may facilitate cholinergic agonist-induced catecholamine secretion. In contrast, it has been reported that the NOS inhibitor L-NAME enhances K\textsuperscript{\textsuperscript{\textsuperscript{-}}}-stimulated catecholamine secretion in cultured bovine chromaffin cells (16) and that SNP inhibits ACh-induced catecholamine secretion in bovine chromaffin cells (13). These studies suggest that NO may play an inhibitory role in the control of catecholamine secretion. Moreover, the presence of endothelial cells has been reported to inhibit the K\textsuperscript{\textsuperscript{-}}-induced or the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium-induced catecholamine secretion in cultured bovine chromaffin cells (16), suggesting that not only nNOS but also eNOS may play roles in modulating adrenal catecholamine secretion. On the other hand, a few in vivo studies have suggested that NO does not play a role in regulation of adrenal catecholamine secretion (1, 2).

In the present study, we investigated the effects of L-NAME and the NO donor 3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-1-propanamine (NOC 7) on the secretion of catecholamine induced by splanchnic nerve stimulation (SNS) and ACH in anesthetized dogs to elucidate the functional role of NO in the control of adrenal catecholamine secretion. The effects of NOC 7 during treatment with L-NAME were also examined. L-NAME, NOC 7, and ACH were administered intra-arterially into the adrenal gland to eliminate hemodynamic effects on adrenal catecholamine secretion.

MATERIALS AND METHODS

Animal preparation. Mongrel dogs of either sex, weighing 10–14 kg, were anesthetized with 30 mg/kg iv of pentobarbital sodium, and a constant level of anesthesia was then maintained by intravenous infusion of pentobarbital sodium at a rate of 6 mg·kg\textsuperscript{-1}·h\textsuperscript{-1} with an infusion pump (201B, Atom, Tokyo, J apan). Artificial respiration was performed using a respiration pump (model-607, Harvard Apparatus, Millis, MA), with room air administered at 18 strokes/min (20 ml/kg tidal volume). The surgical procedure used in the present study was described previously (4). The left adrenal gland was exposed by a retroperitoneal flank incision, and a polyethylene cannula was inserted into the left adrenolumbar vein for collection of the venous effluent blood from the adrenal gland. A ligature was placed around the juncture of the adrenolumbar vein and causing retrograde flow of blood. Blood samples of 1 or 2 ml were collected in chilled test tubes containing disodium EDTA. When not being sampled, adrenal venous blood was returned directly to the vena cava. Coagulation of blood was prevented by an initial intravenous injection of sodium heparin (500 U/kg) and hourly intravenous injections of 100 U/kg. Systemic blood pressure and heart rate were measured with a pressure...
transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan) and a
heart-rate monitor (RT-5, Nihon Kohden, respectively, and
recorded on a heat-writing oscillograph (RJ-4128, Nihon
Kohden).

Administration of drugs into the adrenal gland. The proce-
dure for intra-arterial administration of drugs into the adre-
nal gland was reported previously (5). The left phrenicoba-
dominal artery was dissected to expose its origin from the
abdominal aorta. A needle connected to a Y-shaped polyeth-
ylene catheter was inserted into the phrenicobdominal artery
at its origin for intra-arterial infusion of 0.9% saline solution
(as a vehicle), l-NNAME, NOC 7, and the combination of NOC
7 with l-NNAME. These drugs were infused into the adrenal
gland using an infusion pump (1140–001, Harvard Appara-
tus). AC was injected intra-arterially for 3 s during infusion
of saline, l-NNAME, NOC 7, and the combination of NOC
7 with l-NNAME.

SNS. The left splanchnic nerves were dissected free from
surrounding tissue and cut. A bipolar platinum electrode was
placed in contact with the distal ends of the splanchnic
nerves. The splanchnic nerves were stimulated with rectangu-
lar pulses of 1 ms and 10 V (supramaximal voltage) delivered
by an electronic stimulator (SEN-1101, Nihon Kohden) and
an isolation unit (SS-101J, Nihon Kohden). Stimulation were
applied at 1 Hz for 2 min, subsequently at 2 Hz for 2 min and
3 Hz for 2 min during a 6-min stimulus period.

Experimental protocol. The dogs were divided into eight
groups. In group 1 (n = 6), the effects of repeated SNS on
increases in catecholamine output were examined without
drug treatment. SNS was repeated four times at 30-min
intervals. Infusion of 0.9% saline was started 20 min before
the start of the first, second, third, and fourth SNS. In group 2
(n = 6), the effects of repeated injection of AC on increases
in catecholamine output were examined. A set of AC injections
(0.75, 1.5, and 3 µg) into the adrenal gland was repeated four
times at 35-min intervals. The interval between doses of AC
was 5 min. Infusion of 0.9% saline was started 20 min before
the first, second, third, and fourth set of AC injection. In
group 3 (n = 8), the effects of l-NNAME on the SNS-induced
increases in catecholamine output were examined by the
same protocol as used in group 1. The first SNS trial during
saline infusion was regarded as a control. l-NNAME infusion
(0.1, 0.3, and 1 mg/min) was started 20 min before the start of
the second, third, and fourth SNS, respectively. In group 4
(n = 6), the effects of NOC 7 on the AC-induced increases
in catecholamine output were examined with the same proto-
col as used in group 2. The first set of AC injection during
saline infusion was regarded as a control. l-NNAME infusion
was started 20 min before the start of the second, third,
and fourth set of AC injection. The effects of NOC 7 (0.2, 0.6,
and 2 µg/min) on increases in catecholamine output induced
by SNS (group 5; n = 8) and AC (group 6; n = 6) were examined
by the same protocol as used in groups 3 and 4, respectively.
In group 7 (n = 8), after the first SNS trial as a control,
l-NNAME infusion (1 mg/min) was started 20 min before the
second SNS trial. Subsequently, the combined infusion of
NOC 7 (2 µg/min) and l-NNAME was started 20 min before the
third SNS trial. In group 8 (n = 8), the effects of NOC 7 during
treatment with l-NNAME on increases in catecholamine output
induced by AC were examined with the same protocol as
used in group 7.

Blood sampling and determination of adrenal catechol-
amine output. Adrenal venous blood was sampled before and
during SNS and AC injections to determine basal catechol-
amine output and stimuli-induced increases in catecholamine
output, respectively. Sampling during the basal state (during
saline, l-NNAME, or NOC 7 infusion or the combined infusion
of NOC 7 and l-NNAME) was performed 2 min before SNS or
sets of AC injections. The time required to collect 1 (during
basal state or SNS) or 2 ml (during AC injections) of blood
served to estimate adrenal venous flow rate.

Adrenal blood samples were centrifuged to obtain plasma
samples. Catecholamine was extracted from plasma by the
alumina adsorption method, and plasma epinephrine (Epi)
and norepinephrine (NE) concentrations were determined by
high-performance liquid chromatography with electrochemi-
detection (LC-4B, Bioanalytical Systems, West Lafayette,
IN), as described previously (4). Epi and NE output (ng/min)
were calculated by multiplying plasma catecholamine concen-
tration (ng/ml) by adrenal plasma flow rate (ml/min), and the
total output of Epi and NE was expressed as catecholamine
output. Adrenal plasma flow rate was calculated by multiply-
ing adrenal venous blood flow by 1 – hematocrit of adrenal
venous blood. The basal catecholamine output was deter-
mained from samples collected before SNS or AC injections.
The SNS- or AC-induced changes in catecholamine output were
calculated by subtracting the basal catecholamine output
from that obtained during the stimulus state.

Analysis of data. The results are expressed as means ± SE
throughout the study. Single-factor ANOVA was used for
statistical analysis of data. When ANOVA showed a signifi-
cant difference, Dunnett’s test or Scheffe’s test was used to
determine significance level. P values <0.05 were considered
to be statistically significant.

Drugs. The drugs used were l-NNAME (Sigma Chemical),
NOC 7 (Daiichi Seiyaku, Tokyo, Japan), and AC chloride (Daii-
chi Seiyaku, Tokyo, Japan). NOC 7 was dissolved in 0.01 N
NaOH. Other drugs were dissolved in 0.9% saline solution.

RESULTS

Increases in catecholamine output in response to SNS and
AC. SNS (1, 2, and 3 Hz) or intra-arterial injection of AC (0.75, 1.5,
and 3 µg) into the adrenal gland produced frequency- or dose-dependent increases
in adrenal venous plasma catecholamine concentration (data not shown). The 3-Hz SNS- and AC-induced
increases in catecholamine concentration were accompa-
nied by increases in adrenal plasma flow rate (Tables 1
and 2). SNS at 1 and 2 Hz had no effect on adrenal plasma flow rate. Catecholamine output, calculated
from catecholamine concentration and adrenal plasma
flow rate, was increased by SNS and AC injection
(Table 1). The increases in catecholamine output ind-
uced by SNS and AC during the four stimulation
periods are shown in Table 1. Respective increases
in catecholamine output induced by SNS and AC did not
vary during the time course of the experiment. The
increases in catecholamine output induced by SNS (3
Hz) and AC (3 µg) during the control stimulation
periods were 438 ± 33 (n = 30) and 570 ± 85 ng/min
(n = 26), respectively, in groups 1–8, in which basal
catecholamine output during the resting state was
3.0 ± 0.5 ng/min (n = 56).

SNS produced small pressor and bradycardic re-
responses. The increase in blood pressure produced by
3-Hz SNS was 11 ± 3 mmHg (n = 30), and the decrease
in heart rate was 16 ± 3 beats/min (n = 30). Injection of
AC decreased blood pressure slightly but did not
modify heart rate. The decrease in blood pressure
produced by 3 µg of AC was 8 ± 2 mmHg (n = 26). It is
unlikely that baroreflex-mediated catecholamine secre-
tion is involved in the catecholamine response to SNS and ACh, as described previously (8).

Effects of l-NAME on the SNS- and ACh-induced increases in catecholamine output. Intra-arterial infusion of l-NAME (0.1, 0.3, and 1 mg/min) into the adrenal gland inhibited increases in catecholamine output induced by SNS; statistically significant effects were observed with 0.3 or 1 mg/min at 1 Hz and 1 mg/min at 2 Hz (Fig. 1A). On the other hand, l-NAME enhanced increases in catecholamine output induced by ACh, but the degree of enhancement decreased in proportion to increases in the dose of l-NAME (Fig. 1B). Basal catecholamine output was not affected by l-NAME. In groups 3 and 4 (n = 14), basal catecholamine output before and during 0.1, 0.3, and 1 mg/min l-NAME infusion were 2.9 ± 0.8, 2.1 ± 0.5, 1.9 ± 0.5, and 1.8 ± 0.4 ng/min, respectively. Adrenal plasma flow rate was decreased by l-NAME (Table 2). l-NAME produced a small bradycardic response but did not affect blood pressure (Table 3).

Effects of NOC 7. Intra-arterial infusion of NOC 7 (0.2, 0.6, and 2 µg/min) into the adrenal gland inhibited increases in catecholamine output induced by SNS and ACh (Fig. 2, A and B). Basal catecholamine output was not affected by NOC 7. In groups 3 and 4 (n = 14), basal catecholamine output before and during 0.2, 0.6, and 2 µg/min NOC 7 infusion were 3.4 ± 0.8, 3.4 ± 0.7, and 3.0 ± 0.5, respectively. Adrenal plasma flow rate was decreased by NOC 7 (Table 2). NOC 7 produced a small depressor response but did not affect heart rate (Table 3).

Effects of NOC 7 during treatment with l-NAME. Intra-arterial infusion of l-NAME (1 mg/min) into the adrenal gland inhibited increases in catecholamine output induced by SNS at 1 and 2 Hz but did not affect 3-Hz-induced catecholamine response in the same manner as observed in group 1. During treatment with l-NAME, intra-arterial infusion of NOC 7 (2 µg/min) did not affect the SNS-induced increases in catecholamine output compared with the values obtained with l-NAME (Fig. 3A). On the other hand, l-NAME enhanced increases in catecholamine output induced by ACh in the same manner as observed in group 2. During treatment with l-NAME, NOC 7 inhibited the ACh-induced increases in catecholamine output compared with the values obtained with l-NAME (Fig. 3B).

Basal catecholamine output was not affected by l-NAME or NOC 7 during treatment with l-NAME. In groups 7 and 8 (n = 16), basal catecholamine output before, during l-NAME infusion, and during combined infusion of NOC 7 with l-NAME was 3.6 ± 0.6, 2.4 ± 0.7, and 2.5 ± 0.6 ng/min, respectively. Adrenal plasma flow rate was decreased by l-NAME. During treatment with l-NAME, NOC 7 increased adrenal plasma flow rate under basal conditions and SNS but had no effect after ACh injection (Table 2). l-NAME produced a small bradycardic response but did not affect blood pressure. NOC 7 produced a depressor response and slightly increased heart rate during treatment with l-NAME (Table 3).

DISCUSSION

The present study was performed to elucidate the role of NO in regulation of adrenal catecholamine secretion. We assessed the effects of l-NAME, NOC 7...
It has been suggested that NO plays an inhibitory role in controlling catecholamine secretion induced by various stimuli in bovine chromaffin cells (9, 13, 16) and PC12 cells (6). However, a few in vivo studies using the dog adrenal gland provided evidence suggesting that NO plays no role in catecholamine secretion in response to SNS (1, 2). In this study, L-NAME infused into the adrenal gland significantly enhanced the secretion of adrenal catecholamine in response to ACh. It can be assumed that ACh acts on endothelial cells and/or chromaffin cells and produces NO through activation of eNOS and/or nNOS, respectively. Therefore, this result suggests that endogenously released NO (basal NO plus NO produced by the ACh-induced activation of NOS) inhibits the ACh-induced secretion of catecholamine. However, no dose-response effect of L-NAME on ACh-induced catecholamine secretion was observed; the enhancement declined at the highest dose of L-NAME examined. Previously, we demonstrated under the same experimental conditions that ACh stimulated the secretion of catecholamine by activating both nicotinic and muscarinic receptors (5). It has been reported that L-NAME, in addition to inhibiting NOS, also functions as an antagonist at muscarinic receptors (3). This may explain the lack of a dose-response effect of L-NAME: the muscarinic component of catecholamine secretion was inhibited by increasing doses of L-NAME. NOC 7 inhibited the secretion of catecholamine in response to ACh, suggesting that further inhibition of catecholamine secretion is produced by NO derived from NOC 7 in addition to the inhibitory

alone, and NOC 7 during L-NAME treatment on catecholamine secretion induced by SNS and exogenous ACh. The changes in catecholamine response to stimuli during treatment with L-NAME suggest that the effect of endogenously released NO on catecholamine secretion is the opposite of that of L-NAME. The changes in catecholamine response during treatment with NOC 7 in the absence or presence of L-NAME suggest that the action of NO on catecholamine secretion is due to NOC 7 under conditions in which endogenously released NO is or is not functional, respectively.

Table 2. Effects of L-NAME, NOC 7, and the combination of NOC 7 with L-NAME on adrenal plasma flow under basal conditions and during SNS and ACh injection

<table>
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<tr>
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<th>Adrenal Plasma Flow Rate, ml/min</th>
<th>L-NAME infusion, mg/min</th>
<th>NOC 7 Infusion, µg/min</th>
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<tr>
<td></td>
<td>Control</td>
<td>0.1</td>
<td>0.3</td>
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Values are means ± SE; n = number of dogs per group. L-NAME, N-nitro-L-arginine methyl ester; NOC 7, 3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-1-propanamine. *P < 0.05, †P < 0.01 compared with the corresponding control values. ‡P < 0.01 compared with the values under basal conditions. §P < 0.01 compared with the corresponding values obtained with L-NAME alone in groups 5 and 6.

![Fig. 1. Effects of N-nitro-L-arginine methyl ester (L-NAME) on adrenal catecholamine output in response to splanchnic nerve stimulation (SNS) (A) and ACh (B). L-NAME and ACh were infused and injected intra-arterially into the adrenal gland. Histograms and vertical bars indicate means ± SE from 8 and 6 dogs (n) in SNS and ACh experiments, respectively. *P < 0.05; **P < 0.01, compared with corresponding control values obtained before L-NAME infusion.](http://ajpregu.physiology.org/)

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Effects of L-NAME and NOC 7 on the SNS-induced secretion of catecholamine was observed when NOC 7 was administered concomitantly with L-NAME. These results suggest that NO has an inhibitory effect on the secretion of catecholamine from the dog adrenal gland in response to exogenous ACh in vivo.

Similar to the ACh-induced secretion of catecholamine, that induced by SNS was inhibited by NOC 7. This could be explained by the inhibitory action of NO on catecholamine secretion. However, the secretion of catecholamine in response to SNS at 1 and 2 Hz was inhibited by L-NAME. Furthermore, no inhibition by NOC 7 of SNS-induced secretion of catecholamine was observed when NOC 7 was administered concomitantly with L-NAME. These results are not consistent with the observations in the ACh experiments suggesting that NO inhibits the secretion of catecholamine. SNS causes the release of ACh from splanchnic nerve terminals, and this released ACh stimulates medullary cells. Exogenously administered ACh stimulates medullary cells directly. Therefore, the discrepant results of the effects of L-NAME and NOC 7 on the SNS-induced secretion of catecholamine might be related to a presynaptic action of NO on ACh release from splanchnic nerve terminals. If NO facilitates the release of ACh presynaptically, ACh release would increase and subse-

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<th>Table 3. Effects of vehicle, L-NAME, NOC 7, and the combination of NOC 7 with L-NAME on mean blood pressure and heart rate</th>
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<tr>
<td>Mean Blood Pressure, mmHg</td>
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<td>Groups 1 and 2 (n = 12)</td>
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<tr>
<td>1st</td>
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| Groups 3 and 4 (n = 14) | Control L-NAME, mg/min | 0.1 | 128 ± 5 | -2 ± 1 | 153 ± 8 | -5 ± 2 
| | | 0.3 | 125 ± 5 | 2 ± 1 | 147 ± 8 | -9 ± 2 |
| | | 1 | 127 ± 5 | 3 ± 1 | 137 ± 8 | -14 ± 2 |
| Groups 5 and 6 (n = 14) | Control NOC 7, µg/min | 0.2 | 131 ± 5 | 1 ± 1 | 133 ± 11 | -1 ± 1 |
| | | 0.6 | 126 ± 5 | -7 ± 1 † | 130 ± 10 | -2 ± 2 |
| | | 2 | 118 ± 5 | -7 ± 1 † | 129 ± 10 | 3 ± 1 |
| Groups 7 and 8 (n = 16) | Control L-NAME, mg/min | 125 ± 6 | 0 ± 0 | 149 ± 8 | 0 ± 0 |
| | + NOC 7 (1 µg/min) | 126 ± 6 | 2 ± 2 | 149 ± 8 | -28 ± 5 † |
| | + NOC 7 (2 µg/min) | 129 ± 7 | -12 ± 4 † | 118 ± 7 | 9 ± 4 |

Values are means ± SE; n = number of drugs. *P < 0.05, †P < 0.01 compared with values before administration of the drug.

effect of endogenously released NO. Furthermore, NOC 7-induced inhibition of ACh-induced secretion of catecholamine was observed when NOC 7 was administered concomitantly with L-NAME. These results suggest that NO has an inhibitory effect on the secretion of catecholamine from the dog adrenal gland in response to exogenous ACh in vivo.
and by NOC 7. During treatment with L-NAME, no
from adrenal medullary cells but also a facilitatory role
not only an inhibitory role in catecholamine secretion
inhibition of SNS-induced secretion by NOC 7 was
in the absence of L-NAME because the vasodilatory
SNS, suggesting that NO derived from NOC 7 acts on
in the absence of L-NAME. Therefore, it seems likely that NO
by canceling the presynaptic facilitatory action of NO.
perspectives
These results suggest that NO may play an
in ACh release from splanchnic nerve terminals. For
of NO on ACh release from splanchnic nerve terminals.
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REFERENCES

