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The role of BK_{Ca} channels in the nitric oxide-mediated regulation of adrenal catecholamine secretion

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Abstract

We examined whether high conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels are involved in the modulatory action of nitric oxide (NO) on the secretion of adrenal catecholamines in response to splanchnic nerve stimulation and acetylcholine in anesthetized dogs. The NO donor 3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-*N*-methyl-1-propanamine (NOC 7), the BK_{Ca} channel blocker charybdotoxin and acetylcholine were administered intraarterially (i.a.) into the adrenal gland. NOC 7 infusion (2 μg min^{-1}) inhibited increases in catecholamine output induced by splanchnic nerve stimulation (1–3 Hz) and acetylcholine (0.75–3 μg). Charybdotoxin infusion (100 ng min^{-1}) did not affect increases in catecholamine output induced by splanchnic nerve stimulation and acetylcholine. Charybdotoxin blocked the NOC 7-induced inhibition of increases in catecholamine output induced by splanchnic nerve stimulation but not by acetylcholine. These results suggest that NO may inhibit the secretion of adrenal catecholamines induced by splanchnic nerve stimulation through activation of BK_{Ca} channels.

Keywords: Catecholamine; adrenal; Nitric oxide (NO); K^+ channel, BK_{Ca}; NOC 7 (3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-*N*-methyl-1-propanamine); Charybdotoxin; Splanchnic nerve stimulation

1. Introduction

Catecholamine secretion from the adrenal medulla is controlled by acetylcholine released from splanchnic nerve terminals. Acetylcholine activates nicotinic receptor-associated cation channels, which in turn leads to membrane depolarization and opening of voltage-dependent Ca^{2+} channels (Cena et al., 1983; Corcoran and Kirshner, 1983). The increase in Ca^{2+} influx through these channels triggers the exocytotic secretion of adrenal catecholamines (Garcia et al., 1984). The nitric oxide (NO) donor, sodium nitroprusside, was reported to reduce the depolarization-induced Ca^{2+} influx by a cyclic GMP-mediated mechanism in PC12 cells (Desole et al., 1994) and in bovine chromaffin cells (Rodriguez-Pascual et al., 1996). Moreover, in vitro studies using NO synthase inhibitors and NO donors have shown that NO may play an inhibitory role in the control of catecholamine secretion (Oset-Gasque et al., 1994; Torres et al., 1994; Macarthur et al., 1995; Rodriguez-Pascual et al., 1996). Taken together with these observations, it seems likely that NO inhibits the secretion of catecholamines by reducing Ca^{2+} influx.

Recently, it has been reported that high conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel blockers counteract the NO-mediated relaxation in tracheal (Bialecki and Stinson-Fisher, 1995) and vascular smooth muscles (Khan et al., 1993; Taniguchi et al., 1993; Bolotina et al., 1994; Bialecki and Stinson-Fisher, 1995). More direct patch-clamp studies have shown that NO causes cyclic GMP-mediated or direct activation of BK_{Ca} channels in ciliary ganglia (Cetiner and Bennett, 1993) and vascular smooth muscles (Taniguchi et al., 1993; Robertson et al., 1993; Bolotina et al., 1994; Miyoshi and Nakaya, 1994). These findings suggest the possibility that NO relaxes vascular smooth muscles through activation of BK_{Ca} channels. The activation of BK_{Ca} channels may facilitate the efflux of K^+ from the cell, and the resulting hyperpolarization of membrane potential leads to inhibition of Ca^{2+} influx. However, it is not known whether activation of BK_{Ca} channels is in-

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volved in the modulatory effect of NO on the secretion of adrenal catecholamines.

In the present study, we investigated the effects of the NO donor 3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-*N*-methyl-1-propanamine (NOC 7), the BK_{Ca} channel blocker charybdotoxin and the combination of NOC 7 and charybdotoxin on the secretion of catecholamines in response to splanchnic nerve stimulation and acetylcholine in anesthetized dogs. This was done in attempt to elucidate the role of BK_{Ca} channels in the NO-mediated regulation of adrenal catecholamine secretion. NOC 7, charybdotoxin and acetylcholine were administered intraarterially (i.a.) into the adrenal gland to eliminate their hemodynamic influences on adrenal catecholamine secretion.

2. Materials and methods

2.1. Animal preparation

All animal protocols were reviewed and approved by the Animal Subjects Committee of Pharmaceutical Institute, Tohoku University. Mongrel dogs of either sex, weighing 9–13 kg, were anesthetized with 30 mg kg^{-1} i.v. of sodium pentobarbital, and a constant level of anesthesia was then maintained by an i.v. infusion of sodium pentobarbital at a rate of 6 mg kg^{-1} h^{-1} with an infusion pump (201B, Atom, Tokyo, Japan). Artificial respiration was performed by means of a respiration pump (Model-607, Harvard Apparatus, Millis, USA), with room air being administered at 18 strokes min^{-1} (20 ml kg^{-1} tidal volume). The surgical procedure used in the present study was described previously (Kimura et al., 1988). 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 thread was placed around the juncture of the adrenolumbar vein with the abdominal vena cava. Adrenal blood samples were obtained by pulling the thread, thus occluding the adrenolumbar vein and causing a retrograde flow of blood to ensue. The 1- or 2-ml blood samples 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 i.v. injection of sodium heparin (500 U kg^{-1}) and hourly i.v. injections of 100 U kg^{-1}. Systemic blood pressure and heart rate were measured with a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan) and a cardiotachometer (RT-5, Nihon Kohden), respectively, and recorded on a heat-writing oscillograph (RJG-4128, Nihon Kohden).

2.2. Administration of drugs into the adrenal gland

The procedure for i.a. administration of drugs into the adrenal gland was reported previously (Kimura et al., 1992). The left phrenicoabdominal artery was dissected to expose its origin from the abdominal aorta. A needle connected to a Y-shaped polyethylene catheter was inserted into the phrenicoabdominal artery at its origin for i.a. infusion of 0.9% saline solution (as a vehicle), NOC 7, charybdotoxin and the combination of NOC 7 and charybdotoxin. These drugs were infused into the adrenal gland by using an infusion pump (1140-001, Harvard Apparatus). Acetylcholine was injected i.a. for 3 s during infusion of saline, NOC 7, charybdotoxin and the combination of NOC 7 and charybdotoxin.

2.3. Splanchnic nerve stimulation

The left splanchnic nerves were dissected free from surrounding tissue and cut. A bipolar platinum electrode was placed in contact with the distal end of the splanchnic nerves. The splanchnic nerves were stimulated with rectangular 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). Stimuli were applied at 1 Hz for 2 min, subsequently 2 Hz for 2 min, and 3 Hz for 2 min during a 6-min stimulus period.

2.4. Experimental protocol

The dogs were divided into six groups. In group 1 ($n = 7$), the effect of NOC 7 on the splanchnic nerve stimulation-induced increases in catecholamine output was examined. Splanchnic nerve stimulation was repeated two times at 30-min interval. The first splanchnic nerve stimulation trial during the infusion of 0.9% saline solution into the adrenal gland was regarded as a control. NOC 7 infusion (2 μg min^{-1}) was started 20 min before the start of the second splanchnic nerve stimulation trial. In group 2 ($n = 6$), the effect of NOC 7 on the acetylcholine-induced increases in catecholamine output was examined. A set of acetylcholine injections (0.75, 1.5 and 3 μg) into the adrenal gland was repeated two times at 40-min interval. The interval between each dose of acetylcholine was 5 min. The first set of acetylcholine injections during the infusion of 0.9% saline solution was regarded as a control. NOC 7 infusion was started 20 min before the second set of acetylcholine injections. The effects of charybdotoxin (100 ng min^{-1}) on the increases in catecholamine output induced by splanchnic nerve stimulation (group 3; $n = 6$) and acetylcholine (group 4; $n = 6$) were examined with the same protocol as used in the NOC 7 experiments. In groups 5 ($n = 6$) and 6 ($n = 6$), the effects of the combination of NOC 7 (2 μg min^{-1}) and charybdotoxin (100 ng min^{-1}) on the increases in catecholamine output induced by splanchnic nerve stimulation and acetylcholine were examined, respectively. Previously, we demonstrated that the splanchnic nerve stimulation-induced increases in cate-

cholamine output were well reproducible during repetitive splanchnic nerve stimulation periods (Kimura et al., 1988). We also demonstrated that repeated acetylcholine injections produced increases in catecholamine output to almost the same extent as in the first trial (Kimura et al., 1992).

2.5. Blood sampling and determination of adrenal catecholamine output

Adrenal venous blood was sampled before and during splanchnic nerve stimulation and acetylcholine injections to determine basal catecholamine output and stimuli-induced increases in catecholamine output, respectively. The sampling during the basal state (during saline, NOC 7, or charybdotoxin infusion or the combined infusion of NOC 7 and charybdotoxin) was performed 2 min before splanchnic nerve stimulation or sets of acetylcholine injections. The time required to collect 1 ml (during basal state or splanchnic nerve stimulation) or 2 ml (during acetylcholine injections) of blood served to estimate adrenal venous flow rate.

Adrenal blood samples were centrifuged to obtain plasma samples. Catecholamines were extracted from plasma by the alumina adsorption method, and plasma epinephrine and norepinephrine concentrations were determined by high-performance liquid chromatography with electrochemical detection (LC-4B, Bioanalytical Systems, West Lafayette, IN, USA), as described previously (Kimura et al., 1988). Epinephrine and norepinephrine output (ng min^{-1}) was calculated by multiplying plasma catecholamine concentration (ng ml^{-1}) by adrenal plasma flow rate (ml min^{-1}), and the total output of epinephrine and norepinephrine was expressed as catecholamine output. Adrenal plasma flow rate was calculated by multiplying adrenal venous blood flow by 1-hematocrit of adrenal venous blood. The basal catecholamine output was determined from samples collected before splanchnic nerve stimulation or acetylcholine injections. The splanchnic nerve stimulation- or acetylcholine-induced changes in catecholamine output were calculated by subtracting the basal catecholamine output from that obtained during the stimulus state.

2.6. Analysis of data

The results were expressed as means ± S.E.M. throughout the study. Student's paired *t*-test was used for statistical analysis of data. *P* values smaller than 0.05 were considered to be statistically significant.

2.7. Drugs

The drugs used were NOC 7 (Dojindo, Kumamoto, Japan), charybdotoxin (Peptide Institute, Osaka, Japan) and acetylcholine chloride (Daiichi Seiyaku, Tokyo, Japan). NOC 7 was dissolved in 0.01 N NaOH. Other drugs were dissolved in 0.9% saline solution.

3. Results

3.1. Increases in catecholamine output in response to splanchnic nerve stimulation and acetylcholine

Splanchnic nerve stimulation (1, 2 and 3 Hz) or i.a. injections of acetylcholine (0.75, 1.5 and 3 μg) into the

Table 1
Effects of NOC 7, charybdotoxin (ChTX) and the combination of NOC 7 and charybdotoxin on adrenal plasma flow during the basal state and during splanchnic nerve stimulation (SNS) and injection of acetylcholine (ACh)

Experiment	Adrenal plasma flow rate (ml min^{-1})	
	Control	NOC 7 infusion (2 μg min^{-1})
Group 1 ($n=7$)		
Basal state	1.4±0.3	1.4±0.3
SNS 1 Hz	1.3±0.2	1.4±0.2
SNS 2 Hz	1.3±0.1	1.4±0.2
SNS 3 Hz	1.5±0.2	1.6±0.3
Group 2 ($n=6$)		
Basal state	1.2±0.3	1.0±0.2
ACh 0.75 μg	1.9±0.5[c]	1.5±0.3
ACh 1.5 μg	1.9±0.5[c]	1.6±0.3
ACh 3 μg	2.0±0.5[c]	1.7±0.3
	Control	ChTX infusion (100 ng min^{-1})
Group 3 ($n=6$)		
Basal state	2.3±0.5	1.9±0.4
SNS 1 Hz	2.1±0.5	1.8±0.4
SNS 2 Hz	2.3±0.5	2.0±0.5
SNS 3 Hz	2.4±0.5	2.2±0.4
Group 4 ($n=6$)		
Basal state	2.1±0.3	1.7±0.2
ACh 0.75 μg	3.2±0.3[c]	2.8±0.4[b]
ACh 1.5 μg	3.3±0.4[c]	2.9±0.3[b]
ACh 3 μg	3.5±0.4[c]	3.0±0.4[b]
	Control	NOC 7 (2 μg min^{-1}) and ChTX (100 ng min^{-1}) infusion
Group 5 ($n=6$)		
Basal state	1.5±0.3	1.8±0.4
SNS 1 Hz	1.4±0.2	1.9±0.4
SNS 2 Hz	1.5±0.1	1.9±0.4
SNS 3 Hz	1.7±0.2	2.1±0.4
Group 6 ($n=6$)		
Basal state	1.4±0.3	1.1±0.2
ACh 0.75 μg	2.3±0.6[c]	1.7±0.3[a]
ACh 1.5 μg	2.3±0.5[c]	1.8±0.3[a]
ACh 3 μg	2.5±0.5[c]	1.8±0.3[a]

[a] $P < 0.05$ as compared with the corresponding control values.
[b] $P < 0.01$ as compared with the corresponding control values.
[c] $P < 0.01$ as compared with the values during the basal state under control conditions.
Values represent means ± S.E.M.

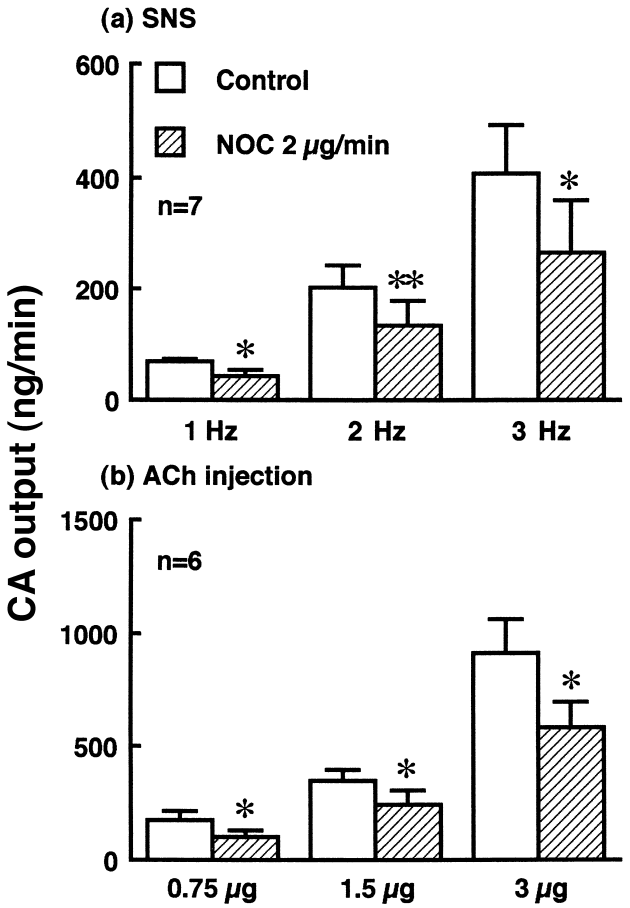

Fig. 1. Effects of NOC 7 (NOC) on catecholamine (CA) output from the adrenal gland in response to splanchnic nerve stimulation (SNS: a) and acetylcholine (ACh: b) injected into the phrenicoabdominal artery. NOC 7 was infused into the same artery. Histograms and vertical bars represent means ± S.E.M. $*P < 0.05$, $**P < 0.01$, compared with corresponding control values obtained before the NOC 7 infusion.

adrenal gland produced frequency- or dose-dependent increases in adrenal venous plasma catecholamine concentration (data not shown). The acetylcholine-induced increases in catecholamine concentration were accompanied by increases in adrenal plasma flow rate (Table 1). Splanchnic nerve stimulation had no effect on adrenal plasma flow rate. Catecholamine output, calculated from catecholamine concentration and the adrenal plasma flow rate, was increased by splanchnic nerve stimulation and acetylcholine injections. The increases in catecholamine output induced by splanchnic nerve stimulation (3 Hz) and acetylcholine (3 μg) during the control stimulation periods were 428 ± 43 ($n = 19$) and 948 ± 108 ($n = 18$), respectively, in groups 1–6, in which basal catecholamine output during the resting state was 4.5 ± 1.1 ng min^{-1} ($n = 37$).

Splanchnic nerve stimulation produced small pressor and bradycardic responses. The increase in blood pressure produced by 3-Hz splanchnic nerve stimulation was 10 ± 2 mm Hg ($n = 19$), and the decrease in heart rate was 14 ± 3 beats min^{-1} ($n = 19$). Injections of acetylcholine decreased blood pressure slightly, but they did not modify heart rate. The decrease in blood pressure produced by 3-μg acetylcholine was 8 ± 2 mmHg ($n = 18$). It is unlikely that baroreflex-mediated catecholamine secretion is involved in the catecholamine response to splanchnic nerve stimulation and acetylcholine, as described previously (Nagayama et al., 1997).

3.2. Effects of NOC 7 on the splanchnic nerve stimulation- and acetylcholine-induced increases in catecholamine output

Intraarterial infusion of NOC 7 (2 μg min^{-1}) into the adrenal gland significantly inhibited increases in catecholamine output induced by splanchnic nerve stimulation and acetylcholine (Fig. 1). Basal catecholamine output was not affected by NOC 7. In groups 1 and 2 ($n = 13$), basal catecholamine output before and during 2-μg min^{-1} NOC 7 infusion were 4.2 ± 1.7 and 7.2 ± 3.6 ng min^{-1}, respectively. Adrenal plasma flow rate was not affected by NOC 7 (Table 1). NOC 7 produced small depressor response, but did not modify heart rate (Table 2).

Table 2
Effects of NOC 7, charybdotoxin (ChTX) and the combination of NOC 7 and charybdotoxin on mean blood pressure and heart rate

Experiment	Mean blood pressure (mmHg)		Heart rate (beats min^{-1})	
	Before	Change	Before	Change
Groups 1 and 2 ($n = 13$)				
Control	128 ± 4	−1 ± 0	130 ± 8	0 ± 0
NOC 7 (2 μg min^{-1})	127 ± 4	−6 ± 1[a]	129 ± 8	3 ± 2
Groups 3 and 4 ($n = 12$)				
Control	121 ± 3	1 ± 1	125 ± 8	−1 ± 1
ChTX (100 ng min^{-1})	120 ± 4	0 ± 1	123 ± 7	−1 ± 1
Groups 5 and 6 ($n = 12$)				
Control	125 ± 5	0 ± 0	132 ± 8	−1 ± 1
NOC 7 (2 μg min^{-1}) and ChTX (100 ng min^{-1})	125 ± 6	−7 ± 2[a]	129 ± 9	2 ± 1

[a] $P < 0.01$ as compared with the values before administration of the drug.
Values represent means ± S.E.M.

3.3. Effects of charybdotoxin

Intraarterial infusion of charybdotoxin (100 ng min^{-1}) into the adrenal gland did not affect increases in catecholamine output induced by splanchnic nerve stimulation and acetylcholine (Fig. 2). Basal catecholamine output was not affected by charybdotoxin. In groups 3 and 4 ($n = 12$), basal catecholamine output before and during 100-ng min^{-1} charybdotoxin infusion were 1.7 ± 0.6 and 1.5 ± 0.4 ng min^{-1}, respectively. Charybdotoxin decreased adrenal plasma flow rate during acetylcholine injections, but did not affect it during the basal state and splanchnic nerve stimulation (Table 1). Charybdotoxin did not affect mean blood pressure and heart rate (Table 2).

3.4. Effects of the combination of NOC 7 and charybdotoxin

The i.a. combined infusion of NOC 7 (2 μg min^{-1}) and charybdotoxin (100 ng min^{-1}) into the adrenal gland did not affect increases in catecholamine output induced by splanchnic nerve stimulation (Fig. 3a). On the other hand, the increases in catecholamine output induced by acetylcholine were significantly inhibited by the combination of NOC 7 and charybdotoxin (Fig. 3b). Basal catecholamine output was not affected by the combination of NOC 7 and charybdotoxin. In groups 5 and 6 ($n = 12$), basal catecholamine output before and during the combined infusion of NOC 7 and charybdotoxin were 7.4 ± 2.3 and 12.0 ± 5.2 ng min^{-1}, respectively. The combination of NOC 7 and charybdotoxin decreases adrenal plasma flow rate during acetylcholine injections, but did not affect it during the basal state and splanchnic nerve stimulation (Table 1). The combination of NOC 7 and charybdotoxin produced small depressor response, but did not modify heart rate (Table 2).

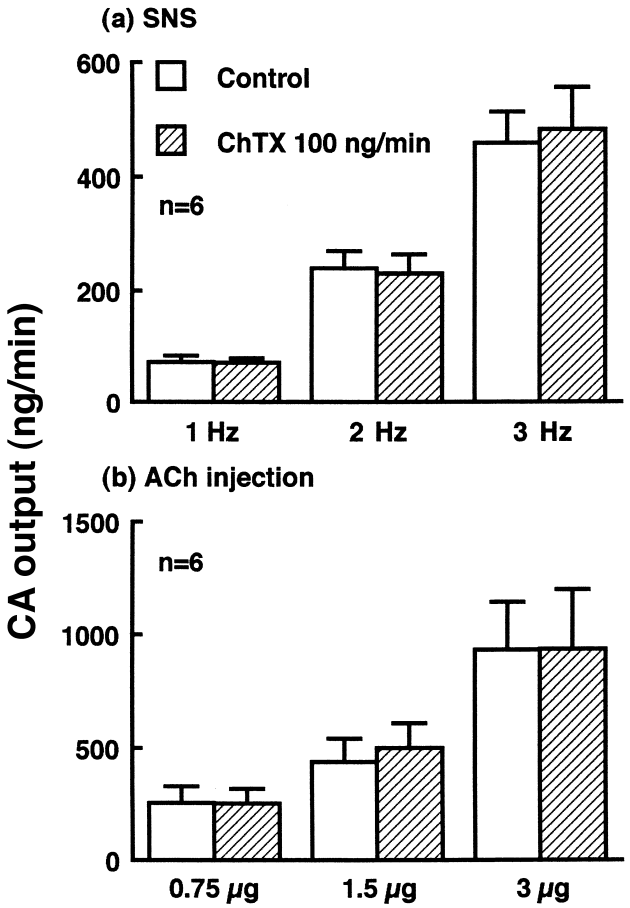

Fig. 2. Effects of charybdotoxin (ChTX) on catecholamine (CA) output from the adrenal gland in response to splanchnic nerve stimulation (SNS: a) and acetylcholine (ACh: b) injected into the phrenicoabdominal artery. Charybdotoxin was infused into the same artery. Histograms and vertical bars represent means ± S.E.M. There were no significant differences ($P > 0.05$) in the splanchnic nerve stimulation- and acetylcholine-induced increases in catecholamine output before (control) and during the charybdotoxin infusion.

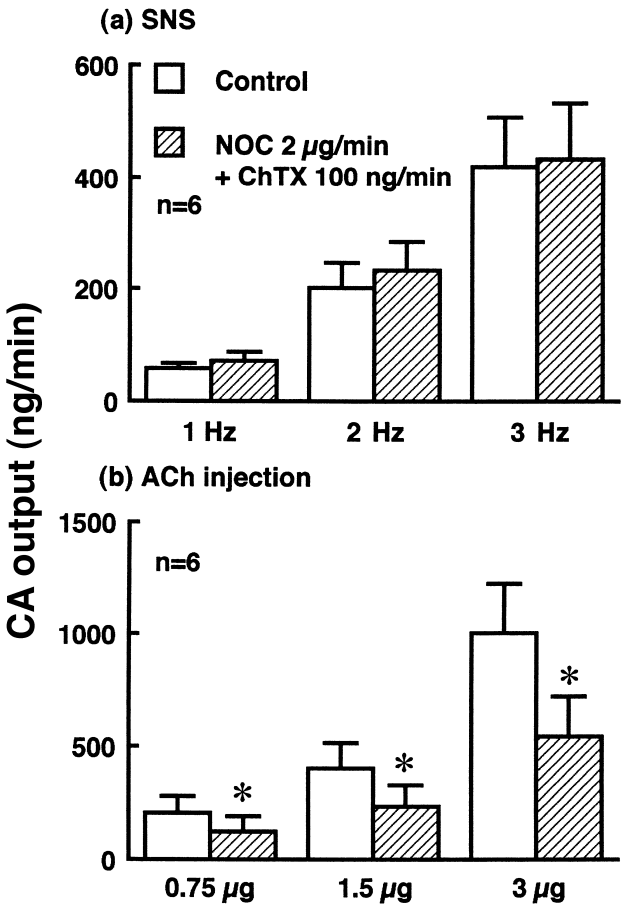

Fig. 3. Effects of the combination of NOC 7 (NOC) and charybdotoxin (ChTX) on catecholamine (CA) output from the adrenal gland in response to splanchnic nerve stimulation (SNS: a) and acetylcholine (ACh: b) injected into the phrenicoabdominal artery. NOC 7 and charybdotoxin were infused into the same artery. Histograms and vertical bars represent means ± S.E.M. $*\ P < 0.05$, compared with corresponding control values obtained before the combined infusion of NOC 7 and charybdotoxin. There were no significant differences ($P > 0.05$) in the splanchnic nerve stimulation-induced increases in catecholamine output before (control) and during the combined infusion.

Finally, it should be noted that the separate analysis of epinephrine and norepinephrine responses showed similar effects of NOC 7 or charybdotoxin on the two catecholamines in all groups.

4. Discussion

NOC 7 is a novel spontaneous NO donor developed by Hrabie et al. (1993) and it is distinct from other NO donors, such as sodium nitroprusside and nitroglycerin. Thus, NOC 7 was thought to be available to clarify a direct action of NO on adrenal catecholamine secretion in response to splanchnic nerve stimulation and exogenous acetylcholine under physiological condition. NOC 7 infused into the adrenal gland significantly inhibited the splanchnic nerve stimulation- and acetylcholine-induced increases in catecholamine output. Previously, we demonstrated that the splanchnic nerve stimulation-induced catecholamine secretion in the dog adrenal gland is mainly mediated by nicotinic receptors and that acetylcholine stimulates the secretion by activating both nicotinic and muscarinic receptors (Shimamura et al., 1991; Kimura et al., 1992). Therefore, the results of the present study suggest that NO derived from NOC 7 acts on the adrenal medullary cells and inhibits the nicotinic receptor-mediated catecholamine secretion in response to splanchnic nerve stimulation and acetylcholine. These results are consistent with the observations that pure NO produces an inhibition of the catecholamine secretion induced by high doses of nicotine in cultured bovine chromaffin cells (Oset-Gasque et al., 1994) and that sodium nitroprusside inhibits the K^+-evoked secretion of dopamine in PC 12 cells (Macarthur et al., 1995) and the acetylcholine-induced secretion of catecholamines in bovine chromaffin cells (Rodriguez-Pascual et al., 1996). From these results, it is suggested that NO plays an inhibitory role in the catecholamine secretion from adrenal medullary cells.

Charybdotoxin did not affect increases in catecholamine output in response to splanchnic nerve stimulation and acetylcholine under in vivo condition in dogs, as reported previously (Nagayama et al., 1997). These results are consistent with the observations that charybdotoxin has no effect on the transmural electrical stimulation-induced secretion of catecholamines in the perfused cat adrenal gland (Montiel et al., 1995) and that another BK_{Ca} channel blocker tetraethylammonium does not affect the catecholamine secretion induced by methacholine in cat chromaffin cells (Uceda et al., 1992). Therefore, it is suggested that BK_{Ca} channels have no role in the secretion of catecholamines from adrenal medullary cells.

NO is suggested to produce relaxation through activation of BK_{Ca} channels in vascular smooth muscle (see Section 1). In order to evaluate whether blockade of BK_{Ca} channels counteracts the NO-induced inhibition of catecholamine secretion, the effects of the combination of NOC 7 and charybdotoxin were examined. The combined infusion of NOC 7 and charybdotoxin into the adrenal gland did not affect the splanchnic nerve stimulation-induced increases in catecholamine output. This result indicates that charybdotoxin blocks the inhibitory action of NOC 7, and suggests that BK_{Ca} channels are involved in the NO-induced inhibition of catecholamine secretion in response to splanchnic nerve stimulation. It is reported that the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester, does not affect the splanchnic nerve stimulation-induced secretion of catecholamines in the dog adrenal gland in vivo (Breslow et al., 1992, 1993). In the present study, charybdotoxin by itself did not affect the splanchnic nerve stimulation-induced increases in catecholamine output. Thus, endogenous NO may not be inhibiting the splanchnic nerve stimulation-induced secretion of catecholamines through activation of BK_{Ca} channels under in vivo condition, probably because an amount of spontaneously released NO is not sufficient to cause the effect.

NO was reported to cause cyclic GMP-mediated or direct activation of BK_{Ca} channels in ciliary ganglia (Cetiner and Bennett, 1993) and vascular smooth muscles (Taniguchi et al., 1993; Robertson et al., 1993; Bolotina et al., 1994; Miyoshi and Nakaya, 1994). The activation of BK_{Ca} channels increases K^+ efflux from the cell, and subsequently hyperpolarization leads to inhibition of Ca^{2+} influx (Wada et al., 1995). Thus, it is likely that NO derived from NOC 7 causes activation of BK_{Ca} channels and subsequently inhibition of Ca^{2+} influx. On the other hand, the combination of NOC 7 and charybdotoxin significantly inhibited the acetylcholine-induced increases in catecholamine output in the same manner as observed in the experiment in which NOC 7 was infused alone. This indicates that BK_{Ca} channels are not involved in the NO-induced inhibition of the secretion of catecholamines induced by acetylcholine. Thus, the mechanism of the NO-induced inhibition of catecholamine secretion in response to acetylcholine remains to be resolved.

The different contribution of BK_{Ca} channels to the NO-induced inhibition of catecholamine secretion elicited by endogenous and exogenous acetylcholine is surprising. Here the question arises as to why BK_{Ca} channels act on the NO-induced inhibition of catecholamine secretion differently; an inhibition in the case of splanchnic nerve stimulation, and no effect in the case of acetylcholine injection. As a possible explanation for this, the different distribution of BK_{Ca} channels on the medullary cell membrane in synaptic and extrasynaptic regions can be considered. Endogenous acetylcholine released from the splanchnic nerve terminals would predominantly activate intrasynaptic nicotinic receptors. Exogenous acetylcholine delivered through the arterial supply could diffuse into extrasynaptic regions and would predominantly activate extrasynaptic nicotinic receptors. If BK_{Ca} channels are primarily concentrated in synaptic zones but not in extrasynaptic regions, they could affect the depolarization due to activa-

tion of intrasynaptic nicotinic receptors but not affect the depolarization due to activation of extrasynaptic nicotinic receptors. Thus, it might be possible to suppose that NO inhibits catecholamine secretion through activation of BK_{Ca} channels located in synaptic zones under physiological conditions.

Basal catecholamine output was not affected by NOC 7, suggesting that NO plays no role in basal secretion of catecholamines. This result is not consistent with the observation that basal efflux of catecholamines is increased by the NO synthase inhibitor, N^G-monomethyl-L-arginine, and decreased by the NO donor, sodium nitroprusside, in perfused dog adrenal glands (Ward et al., 1996). The different result may be due to different experimental conditions, such as in vivo and in vitro.

Adrenal plasma flow rate was not affected by NOC 7 under any conditions, during the basal state, splanchnic nerve stimulation and acetylcholine injection. Charybdotoxin in the presence or absence of NOC 7 had no effect on adrenal plasma flow rate during the basal state and splanchnic nerve stimulation, but attenuated the acetylcholine-induced increases in the flow rate. These results suggest that the acetylcholine-induced vasodilation may be partially mediated by activation of BK_{Ca} channels, as evidenced in the isolated rabbit mesenteric artery (Khan et al., 1993).

In conclusion, this study demonstrates that NOC 7 inhibits adrenal catecholamine secretion in response to splanchnic nerve stimulation and acetylcholine, and that charybdotoxin has no effect on catecholamine secretion in anesthetized dogs. Charybdotoxin blocked the NOC 7-induced inhibition of the secretion induced by splanchnic nerve stimulation but not by acetylcholine. These results suggest that NO may play an inhibitory role in the secretion of catecholamines through activation of BK_{Ca} channels located in synaptic zones.

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