Effects of Yohimbine and Desipramine on Adrenal Catecholamine Release in Response to Splanchnic Nerve Stimulation in Anesthetized Dogs

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The effects of yohimbine and desipramine on adrenal catecholamine (CA) release in response to splanchnic nerve stimulation (SNS) were examined in anesthetized dogs. SNS at 1 and 3 Hz produced frequency-dependent increases in epinephrine (EPI) and norepinephrine (NE) output determined from adrenal venous blood. Yohimbine (30 and 100 $\mu\text{g/kg}$, i.v.), a selective α_2 -adrenoceptor antagonist, enhanced the SNS-induced increases in both EPI and NE output. Desipramine (100 and 300 $\mu\text{g/kg}$, i.v.), an amine pump inhibitor, enhanced the SNS-induced increases in NE output, whereas no enhancement of EPI output was produced. After desipramine treatment, yohimbine further enhanced the SNS-induced increases in EPI and NE output. After yohimbine treatment, desipramine further enhanced the SNS-induced increase in NE output. These results suggest that the release of adrenal CA in response to SNS is inhibited by α_2 -adrenoceptors, and that released NE, rather than EPI, is predominantly taken up into the dog adrenal medullary cells.

Key words adrenal catecholamine release; yohimbine; desipramine

It is generally accepted that the activation of presynaptic α_2 -adrenoceptors inhibits the subsequent release of norepinephrine (NE) from sympathetic nerve terminals,^{1,2)} and that some of NE released into the synaptic cleft is taken up into the nerves by an amine pump.³⁾ From the viewpoint of functional homology of adrenal medullary cells with sympathetic postganglionic neurons, many studies have examined these two mechanisms in the adrenal medulla. However, the observations obtained remain controversial. The α_2 -adrenoceptor-mediated feedback control of inhibition of adrenal catecholamine (CA) release has been postulated from findings in *in vitro*^{4–6)} and *in vivo* preparations.^{7,8)} In contrast, several investigators have provided evidence suggesting that α_2 -adrenoceptors do not play a functional role in regulating adrenal CA release.^{9–11)} Similar questions remain in the case of the presence^{12,13)} or absence¹⁴⁾ of an uptake system in the adrenal medulla.

In the present study, the effects of yohimbine and desipramine on adrenal CA release in response to splanchnic nerve stimulation (SNS) were examined in anesthetized dogs in order to characterize an α_2 -adrenoceptor-mediated feedback control and uptake system in the adrenal medulla. The effects of the combination of yohimbine and desipramine were also examined, since the neuronal uptake of NE has been reported to affect the α_2 -adrenoceptor-mediated inhibition of NE release in sympathetic nerve terminals.^{15–19)}

MATERIALS AND METHODS

Animal Preparation Mongrel dogs of either sex, weighing 7 to 14 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and a constant level of anesthesia was then maintained by an infusion of sodium pentobarbital (4–6 mg/kg/h, i.v.) with an infusion pump (201B, Atom Co., Tokyo, Japan). After endotracheal intubation, artificial respiration was performed by a respiration pump (Model-607, Harvard Apparatus Co., Inc., Millis, U.S.A.), with room air being administered at

18 strokes/min (20 ml/kg tidal volume). The surgical procedure used in the present study was described previously.²⁰⁾ 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 directly returned to the vena cava. After the diaphragm was incised, the left splanchnic nerves were dissected free from surrounding tissues and cut. A bipolar platinum electrode was placed in contact with the distal end of the splanchnic nerves. Coagulation of blood was prevented by an initial injection of sodium heparin (500 U/kg, i.v.) and hourly i.v. injection of 100 U/kg. Systemic blood pressure in the right brachial artery was measured by a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan). Heart rate was measured by a cardi tachometer (RT-5, Nihon Kohden) triggered by an electrocardiogram. Changes in parameters were recorded on a heat-writing oscillograph (RJG-4128, Nihon Kohden).

The procedure of intraarterial (i.a.) injection of drug into the adrenal gland was described previously.²¹⁾ The left phrenicoabdominal artery was dissected to expose its origin from the abdominal aorta. A 27-gauge needle connected to the polyethylene tube was inserted into the phrenicoabdominal artery at its origin for i.a. injection of tyramine into the adrenal gland. Tyramine in a dose of 30 μg was injected for 3 s by an infusion pump (Model-975E, Harvard Apparatus Co., Ltd.).

SNS The splanchnic nerves were stimulated for 6 min, 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). Stimulus frequency was applied at 1 Hz for

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3 min, and subsequently at 3 Hz for another 3 min during a 6-min stimulus period.

Experimental Protocol The dogs were divided into three groups. In group 1 ($n=8$), after the first SNS trial as a control, yohimbine in doses of 30 and 100 $\mu\text{g}/\text{kg}$ was injected i.v. 5 min before the second and third SNS trials, respectively. Subsequently, desipramine (100 $\mu\text{g}/\text{kg}$, i.v.) was injected 5 min before the fourth SNS trial in order to examine the effect of uptake inhibition on adrenal CA release in the presence of α_2 -adrenoceptor inhibition. In group 2 ($n=6$), the effects of desipramine (100 and 300 $\mu\text{g}/\text{kg}$, i.v.) and subsequently injected yohimbine (100 $\mu\text{g}/\text{kg}$, i.v.) were examined following the same protocol used in group 1. In these groups, SNS was conducted at 30 min intervals. It was confirmed in preliminary experiments that the effects of yohimbine (100 $\mu\text{g}/\text{kg}$) and desipramine (300 $\mu\text{g}/\text{kg}$) on the SNS-induced increases in CA output persisted over 60 min. Previously, we reported that the SNS-induced increases in CA output were reproducible during repetitive SNS periods.²⁰ In group 3 ($n=6$), the effect of tyramine (30 μg) injected into the phrenicoabdominal artery on epinephrine (EPI) and NE output was examined.

Adrenal venous blood (1 or 2 ml) was sampled during the basal state, during SNS and during tyramine injection to determine basal CA output and SNS- and tyramine-induced increases in CA output, respectively. The time required to collect 1 or 2 ml of blood served as an estimate of adrenal venous blood flow rate.

Determination of Adrenal CA Output Adrenal blood samples were centrifuged to obtain plasma samples. CA was extracted from plasma by the alumina adsorption method, and plasma EPI and NE concentration was determined by high-performance liquid chromatography with electrochemical detection (LC-304, Bioanalytical Systems, West Lafayette, U.S.A.), as described previously.²⁰ Adrenal EPI and NE output (ng/min) was calculated by multiplying plasma CA concentration (ng/ml) by adrenal plasma flow rate (ml/min). Adrenal plasma flow rate was determined by the following equation: adrenal venous blood flow rate (ml/min) $\times$ [1 - hematocrit of adrenal venous blood]. The SNS- or tyramine-induced increases in CA output were calculated by subtracting the basal CA output from that obtained during the stimulus state.

Analysis of Data Results were expressed as the mean $\pm$ S.E. throughout the study. The Student's t -test was used for statistical analysis of paired data. Analysis of variance was used for statistical analysis of multiple comparisons of data. When multiple comparisons were made with a single control, Dunnett's test was used to determine significance level. Differences were considered significant at $p < 0.05$.

Drugs The drugs used were yohimbine hydrochloride (Sigma Chemical Co., St. Louis, U.S.A.), desipramine hydrochloride (Nihon Ciba-Geigy, Takarazuka, Japan) and tyramine (Sigma Chemical Co.). All drugs were dissolved in 0.9% saline solution.

RESULTS

Effects of Yohimbine and Desipramine on the SNS-Induced Increases in CA Output SNS at 1 and 3 Hz produced marked increases in both EPI and NE output from the adrenal gland. In group 1, yohimbine (30 and 100 $\mu\text{g}/\text{kg}$, i.v.) significantly enhanced the SNS-induced increases in EPI and NE output. A dose-response relationship in the effect of yohimbine was not observed. This result suggests that 30 $\mu\text{g}/\text{kg}$ of yohimbine is sufficient to produce a maximal effect. Subsequently administered desipramine (100 $\mu\text{g}/\text{kg}$, i.v.) further enhanced the SNS-induced increases in NE output significantly, but it had no effect on EPI output (Fig. 1). The effects of yohimbine and desipramine on the ratio of EPI to NE in CA output during SNS, the basal output of EPI and NE, the mean arterial pressure and heart rate are shown in Table 1. The ratio of EPI to NE in SNS-induced increases in CA output was not changed by yohimbine, but was decreased by desipramine. Yohimbine did not affect the basal EPI or NE output. After the yohimbine treatment, desipramine increased the basal CA output. Yohimbine increased the heart rate slightly at 30 $\mu\text{g}/\text{kg}$ and significantly at 100 $\mu\text{g}/\text{kg}$, but had a little effect on arterial pressure. After the yohimbine treatment, desipramine significantly increased arterial pressure and heart rate.

Figure 2 shows the effects of desipramine and subsequently administered yohimbine on the SNS-induced

Fig. 1. Effects of Yohimbine (Yoh) and Subsequently Injected Desipramine (DMI) on EPI and NE Output from the Adrenal Gland in Response to SNS

Histograms and vertical bars represent the mean $\pm$ S.E.M. obtained from 8 dogs. ** $p < 0.01$: values obtained with yohimbine 30 and 100 $\mu\text{g}/\text{kg}$ were compared with corresponding control values. † $p < 0.05$, †† $p < 0.01$: values obtained with desipramine 100 $\mu\text{g}/\text{kg}$ after the yohimbine treatment were compared with values obtained with yohimbine 100 $\mu\text{g}/\text{kg}$.

Table 1. Effects of Yohimbine (Yoh) and Desipramine (DMI) on Ratio of EPI to NE in Catecholamine Output during SNS, Basal Output of EPI and NE, Mean Arterial Pressure (MAP) and Heart Rate (HR)

Treatment	Ratio of EPI to NE		Basal output (ng/min)		MAP (mmHg)	HR (beats/min)
	1 Hz SNS	3 Hz SNS	EPI	NE		
Group 1 (<i>n</i> =8)						
Control	6.6±0.8	5.5±0.6	1.6±0.3	0.2±0.1	108±5	115±5
Yoh 30 µg/kg	6.1±0.7	5.4±0.5	1.5±0.3	0.2±0.1	113±5	124±11
Yoh 100 µg/kg	5.8±0.7	5.3±0.6	1.7±0.3	0.3±0.1	113±6	134±13**
Yoh + DMI 100 µg/kg	3.5±0.5††	4.4±0.6††	3.5±0.9††	5.8±0.2††	122±7†	157±12††
Group 2 (<i>n</i> =6)						
Control	5.6±0.9	4.8±0.9	2.3±2.0	0.7±0.5	124±6	150±15
DMI 100 µg/kg	3.5±0.7**	3.9±0.7**	2.0±1.0	0.7±0.3	129±6	147±18
DMI 300 µg/kg	3.1±0.7**	3.7±0.7**	2.7±1.0	0.7±0.3	128±6	149±17
DMI + Yoh 100 µg/kg	3.2±0.5	4.2±0.8	4.8±3.3	1.8±1.1††	143±6††	191±23†

** *p* < 0.01; values obtained with Yoh 30 and 100 µg/kg (group 1), and DMI 100 and 300 µg/kg (group 2) were compared with corresponding control values. † *p* < 0.05, †† *p* < 0.01; values obtained with DMI 100 µg/kg after Yoh were compared with values obtained with Yoh 100 µg/kg in group 1, and values with Yoh 100 µg/kg after DMI compared with values with DMI 300 µg/kg in group 2.

Fig. 2. Effects of Desipramine (DMI) and Subsequently Injected Yohimbine (Yoh) on EPI and NE Output from the Adrenal Gland in Response to SNS

Histograms and vertical bars represent the mean ± S.E.M. obtained from 6 dogs. ** *p* < 0.01; values obtained with desipramine 100 and 300 µg/kg were compared with corresponding control values. † *p* < 0.05, †† *p* < 0.01; values obtained with yohimbine 100 µg/kg after the desipramine treatment were compared with values obtained with desipramine 300 µg/kg.

increases in EPI and NE output (group 2). Desipramine (100 and 300 µg/kg, i.v.) significantly enhanced the SNS-induced increases in NA output, but it had no effect on EPI output. No dose-response relationship in the effect of desipramine on NE output was observed. This suggests that 100 µg/kg of desipramine is sufficient to produce a maximal effect. Subsequently administered yohimbine (100 µg/kg, i.v.) significantly enhanced the SNS-induced increases in EPI and NE output when compared with the values obtained with desipramine

Fig. 3. EPI and NE Output in Response to Intraarterial Injection of Tyramine into the Phrenicoabdominal Artery

Histograms and vertical bars represent the mean ± S.E.M. obtained from 6 dogs. * *p* < 0.05; values obtained with tyramine were compared with the corresponding basal values.

alone (300 µg/kg). The ratio of EPI to NE in the SNS-induced increases in CA output was decreased by desipramine (Table 1). Desipramine did not affect the basal EPI or NE output. After the desipramine treatment, yohimbine increased the basal NE output (Table 1). Desipramine did not affect arterial pressure and heart rate. After the desipramine treatment, yohimbine significantly increased arterial pressure and heart rate (Table 1).

CA Output Induced by i.a. Injection of Tyramine In group-3, tyramine (30 µg) injected into the phrenicoabdominal artery significantly increased both EPI and NE output from the adrenal gland (Fig. 3). The ratio of EPI to NE in tyramine-induced increases in CA output was 4.8 ± 0.5 . Arterial pressure (mean pressure: 104 ± 10 mmHg, *n*=6) and heart rate (127 ± 13 beats/min) were not affected by i.a. injections of tyramine.

DISCUSSION

Yohimbine significantly enhanced both EPI and NE output from the adrenal gland in response to SNS without affecting basal CA output. These results confirm and support the presence of α_2 -adrenoceptors subserving the inhibition of CA release in the dog adrenal gland.⁸⁾ CA released from adrenal chromaffin cells would inhibit its

own release by activating α_2 -adrenoceptors, probably located on the adrenal chromaffin cells. Blockade by yohimbine of α_2 -adrenoceptors would result in the cancellation of this negative feedback control. It is well known that EPI and NE are present in separate adrenal chromaffin cells.²²⁾ In the present study, the ratio of EPI to NE in SNS-induced increases in CA output was not changed by yohimbine, suggesting that EPI-containing cells and NE-containing cells are equally influenced by α_2 -adrenoceptor-mediated feedback control.

Desipramine significantly enhanced the SNS-induced increases in NE output without significant enhancement of EPI output; consequently, the ratio of EPI to NE decreased. These results suggest that NE, rather than EPI, is predominantly taken up into the adrenal chromaffin cells during SNS. It has been demonstrated that the uptake system in guinea-pig adrenal chromaffin cells recognized CA in the affinity sequence of dopamine > NE > EPI, and also that CA uptake was inhibited by acetylcholine and veratridine, both of which depolarize the adrenal chromaffin cell membrane and release CA.¹³⁾ From these results, it is suggested that the transport system is inactivated by released acetylcholine during splanchnic nerve excitation. In the present study, however, the enhancement by desipramine of NE output was observed during SNS, but not during the unstimulated state. Therefore, it seems likely that the uptake system in adrenal chromaffin cells maintains its activity for NE during splanchnic nerve excitation, even if a depolarization-induced depression is produced. The failure of desipramine to enhance basal CA output during a resting state may be due to insufficient CA levels for operation of the uptake system. The functioning of the uptake system during a resting state was confirmed by the finding that i.a. injection of tyramine into the adrenal gland through the phrenicoabdominal artery produced marked increases in CA output.

The neuronal uptake of NE has been reported to affect the α_2 -adrenoceptor-mediated inhibition of NE release in sympathetic nerve terminals,¹⁵⁻¹⁸⁾ and, thus, an interaction between presynaptic α_2 -adrenoceptors and neuronal uptake mechanism has been postulated.^{19,23,24)} In the present study, yohimbine enhanced the SNS-induced increases in EPI and NE output after uptake blockade by desipramine. This effect of yohimbine was qualitatively similar to that obtained when neural uptake is left intact. The same applied in the case of the enhancement by desipramine of SNS-induced increases in NE output after α_2 -adrenoceptor blockade by yohimbine. Thus, we could not obtain data suggesting the interaction between α_2 -

adrenoceptors and an uptake mechanism in the SNS-evoked release of adrenal CA. However, the synergistic effects of α_2 -adrenoceptor blockade and uptake inhibition in the sympatho-adrenal system were observed in basal CA output and postsynaptic responses. Basal CA output was not affected by yohimbine or desipramine alone, but was significantly increased by their combination. Arterial pressure and heart rate were also increased by their combination.

In conclusion, the present study demonstrated that yohimbine enhanced SNS-induced increases in EPI and NE output from the dog adrenal gland *in vivo* and desipramine selectively enhanced NE output. These results suggest that the release of adrenal CA in response to SNS is inhibited by α_2 -adrenoceptors probably located in adrenal medullary cells, and released NE, rather than EPI, is predominantly taken up into the cells.

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