Endothelin Enhances and Inhibits Adrenal Catecholamine Release in Deoxycorticosterone Acetate-Salt Hypertensive Rats

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Abstract—Endothelin (ET) and the sympathoadrenal system contribute to the development and maintenance of deoxycorticosterone acetate (DOCA)-salt hypertension. ET can act directly on the adrenal medulla to enhance the release of catecholamines. In addition, the level of ET peptide is increased in the adrenal glands of DOCA-salt hypertensive rats. Therefore, we tested the hypothesis that ET enhances adrenal medullary catecholamine release during DOCA-salt hypertension. The infusion of exogenous ET-1 into an isolated, perfused adrenal gland preparation resulted in an increase in the basal release of norepinephrine (NE) and epinephrine (EPI) in control and DOCA-salt hypertensive rats. Nerve-stimulated (0.3 Hz) release of NE was significantly inhibited during ET-1 infusion in the DOCA-salt hypertensive rats but not in the control rats. The role of endogenous ET on basal and nerve-stimulated NE and EPI release was also examined. An infusion of either BQ-123 (10^{-7} mol/L), an ET_{A} receptor antagonist, or BQ-788 (10^{-7} mol/L), an ET_{B} receptor antagonist, did not alter basal NE or EPI release in either control or DOCA-salt hypertensive rats. BQ-788 did not alter nerve-stimulated release of NE and EPI. In contrast, the nerve-stimulated release of EPI, but not NE, was enhanced during BQ-123 infusion in DOCA-salt hypertensive rats. Nerve-stimulated NE and EPI release was unaffected by BQ-123 in the control rats. These data suggest that ET can stimulate adrenal medullary catecholamine release in normotensive and DOCA-salt hypertensive rats. However, ET also inhibits adrenal medullary catecholamine release in DOCA-salt hypertensive rats. (Hypertension. 2000;35[part 2]:385-390.)

Key Words: endothelin ■ deoxycorticosterone ■ hypertension, sodium-dependent ■ adrenal glands ■ catecholamines
the absence and presence of exogenous ET-1. To determine whether endogenous ET alters adrenal medullary catecholamine release, the splanchnic nerve was electrically stimulated in the presence and absence of ET_A or ET_B receptor antagonists.

### Methods

All experimental protocols were approved by the University of Texas Health Science Center Animal Care and Use Committee. Animals were treated in accordance with the “Guiding Principles for the Use of Animals in Research and Teaching” of the American Physiological Society. The University of Texas Health Science Center laboratory animal facility is fully accredited by AAALAC International.

Male Sprague-Dawley rats (350 to 375 g) were obtained from Harlan Sprague-Dawley Inc. All animals underwent a right nephrectomy while under gaseous anesthesia (Metofane; Mallinkrodt Veterinary). Rats were implanted subcutaneously with either a Silastic pellet containing 200 mg/kg DOCA or a blank Silastic pellet as a control. Animals in the DOCA-salt group were provided a solution of 0.9% NaCl and 0.2% KCl to drink, whereas control rats were provided tap water. All rats were provided normal rat chow (Teklad). Male Sprague-Dawley rats (350 to 375 g) were obtained from Harlan Sprague-Dawley Inc. All animals underwent a right nephrectomy while under gaseous anesthesia (Metofane; Mallinkrodt Veterinary). Rats were implanted subcutaneously with either a Silastic pellet containing 200 mg/kg DOCA or a blank Silastic pellet as a control. Animals in the DOCA-salt group were provided a solution of 0.9% NaCl and 0.2% KCl to drink, whereas control rats were provided tap water. All rats were provided normal rat chow (Teklad).

At 14 days after DOCA-salt initiation, all of the rats were prepared with an arterial catheter while under gaseous anesthesia. The following protocols were performed 3 to 5 days after the implantation of an arterial catheter.

After the measurement of mean arterial pressure and heart rate with the MacLab data acquisition system (AD Instruments) while the animal was conscious, the rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). The adrenal gland was prepared according to Hinson et al21 with some modifications. The inflow catheter was inserted into the abdominal aorta, and the outflow catheter was inserted into the adrenal vein via the renal vein. The gland was continuously perfused with bicarbonate-buffered Krebs’ solution at a rate of 250 to 300 μL/min for the duration of the experiment. The solution contained 3% BSA and 100 mg% glucose and was continuously bubbled with a gaseous mixture of 5% CO_2 and 95% room air.

To determine catecholamine release rate, the concentration of catecholamine in the perfusate sample was multiplied by the rate of perfusate exiting the adrenal gland.

### Perfusion With ET-1

The perfused adrenal gland was allowed 1 hour to equilibrate. All of the following perfusate collections were made into polyethylene tubes on ice that contained 15 μL of an EGTA/glutathione solution. At the end of the 1-hour period, perfusate was collected for 2 minutes to determine the baseline catecholamine release. After the last stimulation, the “pocket” created in the area between the kidney and diaphragm was filled with warm Krebs’ solution. The solution contained continuously recirculated to keep the adrenal gland and surrounding tissues warm and moist for the duration of the experiment. At the end of the experiment, the perfused gland was removed, cleaned of connective tissue and fat, and weighed.

To determine catecholamine release rate, the concentration of catecholamine in the perfusate sample was multiplied by the rate of perfusate exiting the adrenal gland.

### Statistical Analysis

Data are presented as mean±SEM values and were analyzed with the use of multifactor repeated and nonrepeated measures ANOVA. Differences between groups were determined with the use of Student-Newman-Keuls post hoc tests. Differences were considered significant when P<0.05.

### Results

Conscious control rats had normal blood pressure (118±1 mm Hg), and DOCA-salt–treated rats were hypertensive (170±4 mm Hg). The heart rate did not differ statistically between the control and the DOCA-salt hypertensive rats (383±6 versus 366±7 bpm, respectively). After preparation of the adrenal perfusion, the first perfusate sample collected immediately before electrical stimulation was used to determine baseline catecholamine release. The basal release of NE and EPI did not differ between control and DOCA-salt hypertensive rats (383±6 versus 366±7 bpm, respectively). After preparation of the adrenal perfusion, the first perfusate sample collected immediately before electrical stimulation was used to determine baseline catecholamine release. The basal release of NE and EPI did not differ between control and DOCA-salt hypertensive rats (383±6 versus 366±7 bpm, respectively). After preparation of the adrenal perfusion, the first perfusate sample collected immediately before electrical stimulation was used to determine baseline catecholamine release. The basal release of NE and EPI did not differ between control and DOCA-salt hypertensive rats (383±6 versus 366±7 bpm, respectively). After preparation of the adrenal perfusion, the first perfusate sample collected immediately before electrical stimulation was used to determine baseline catecholamine release. The basal release of NE and EPI did not differ between control and DOCA-salt hypertensive rats (383±6 versus 366±7 bpm, respectively).

### Infusion of ET-1

The administration of ET-1 for 1 hour into the adrenal gland resulted in a significant increase in the release of NE and EPI in both control and DOCA-salt hypertensive rats (Figure 1); however, the release rate was not significantly different between the two groups. In vehicle-infused groups, there were no statistically significant increases in NE or EPI release. Three-way repeated measures ANOVA indicated a significant interaction of time and ET-1 or vehicle infusion, suggesting that changes in catecholamine release were different between the rats that received vehicle or ET-1.

Adrenal medullary catecholamine release induced through splanchnic nerve stimulation was compared in control and DOCA-salt hypertensive rats before and after ET or vehicle infusion (Figure 2). In the absence of ET-1 (Figure 2, open columns), the nerve-stimulated release of NE or EPI did not differ between control and DOCA-salt hypertensive rats.
At 1 hour after the start of the ET-1 infusion, the electrical stimulation was repeated (Figure 2, filled columns). In the control rats, the presence of ET-1 did not alter NE or EPI release. In contrast, the release of NE from DOCA-salt hypertensive rats was significantly decreased during nerve stimulation in the presence of ET-1. There was a slight decrease in EPI release, but this effect was not statistically significant. In the vehicle-infused groups, the release of catecholamines during the second electrical stimulation was not different from that during the first.

Infusion of ET Receptor Antagonists

The changes in the release rate of NE and EPI during ET$_B$ receptor (BQ-788) and ET$_A$ receptor (BQ-123) antagonist infusion are shown in Figure 3. A 30-minute infusion of BQ-788 or BQ-123 did not significantly alter basal catecholamine release in either the control or DOCA-salt groups.

To determine whether ET$_B$ or ET$_A$ receptors altered catecholamine release during nerve stimulation, the changes in catecholamine release were compared in the absence and presence of BQ-788 and BQ-123, respectively. In the absence of the antagonists, a frequency-dependent increase in adrenal medullary catecholamine release was observed in both control and DOCA-salt hypertensive rats (Figures 4 and 5, open columns). The change in the release of catecholamines at each frequency was similar in control and DOCA-salt hypertensive rats. The release of catecholamines at the 0.3-Hz stimulation in this protocol was similar to that observed before ET-1 infusion (Figure 2).

The presence of BQ-788 did not alter the release rate of NE or EPI in response to splanchnic nerve stimulation in either the control or DOCA-salt groups (Figure 4, filled columns). The infusion of BQ-123 did not affect the nerve-stimulated release of NE in either the control or DOCA-salt groups or the release of EPI in the control group (Figure 5, filled columns). However, in the DOCA-salt group, the presence of BQ-123 augmented the release of EPI in response to 1-Hz stimulation.

Discussion

The novel finding of the present study is that ET can have two opposing effects in the adrenal medulla of DOCA-salt hypertensive rats: ET alone stimulates the release of catechol-
amines from the adrenal medulla but inhibits adrenal medullary catecholamine release during splanchnic nerve stimulation. In normotensive rats, ET alone causes an increase in catecholamine release, but the nerve-stimulated release of catecholamines is not affected by the presence of ET.

No differences were observed in the basal release of adrenal catecholamines between control and DOCA-salt hypertensive rats. When two different electrical stimuli were applied to the splanchnic nerve, the release rate of catecholamines increased in a frequency-dependent manner. The change in the release rate of catecholamines in response to nerve stimulation was not different between control and DOCA-salt hypertensive rats. Thus, the short (30 seconds) and low-frequency (0.3 and 1 Hz) stimulations used in this study did not reveal any differences in the release of adrenal medullary catecholamines between control and DOCA-salt hypertensive animals.

Frequency-dependent release of adrenal medullary catecholamines has been observed during splanchnic nerve stimulation in other studies. When different electrical stimuli were applied to the splanchnic nerve, the release rate of catecholamines increased in a frequency-dependent manner. The change in the release rate of catecholamines in response to nerve stimulation was not different between control and DOCA-salt hypertensive rats. Thus, the short (30 seconds) and low-frequency (0.3 and 1 Hz) stimulations used in this study did not reveal any differences in the release of adrenal medullary catecholamines between control and DOCA-salt hypertensive animals.

Exogenous ET

Cells in the adrenal gland are capable of synthesizing ET-1, and several studies have shown that ET can stimulate adrenal medullary catecholamine release. The addition of ET-1 to isolated chromaffin cells causes an increase in catecholamine release. When ET-1 was administered to the adrenal gland of normotensive dogs in vivo, catecholamine release was also increased. In the present study, we observed that ET-1 infusion into the isolated, perfused adrenal gland of rats also increased adrenal medullary catecholamine release. The increase in the release rate of NE and EPI in response to ET-1 was not different between sham and DOCA-salt hypertensive rats. These results demonstrate that DOCA-salt hypertension does not alter the effect of ET on adrenal medullary catecholamine release.

The ability of ET to enhance adrenal medullary catecholamine release during adrenal medullary stimulation was also examined in the present study. In normotensive rats, we observed that the nerve-stimulated release of adrenal NE and EPI in the presence of ET was similar to that obtained before ET-1 administration. In contrast, the release of NE was prevented in the presence of ET-1 in the DOCA-salt hypertensive rats. There was a trend for an attenuated EPI release in the presence of ET-1, but this effect was not statistically significant. These data suggest that there is an ET receptor that inhibits adrenal medullary catecholamine release in DOCA-salt hypertensive rats but not in normotensive rats.

Previous studies in normotensive dogs demonstrated that ET augmented nerve-stimulated adrenal medullary catecholamine release. In the present study, it was observed that the nerve-stimulated release of adrenal catecholamines from normotensive rats was not enhanced by the presence of ET. In
Endogenous ET

We also examined the role of endogenous ET in the modulation of adrenal catecholamine release. Because the adrenal gland was perfused with Krebs’ solution, the source of endogenous ET was the adrenal gland and not the plasma. Constant perfusion of the adrenal glands with BQ-123 or BQ-788 (ET$_A$ and ET$_B$ receptor antagonist, respectively) did not significantly alter resting adrenal medullary catecholamine release in either control or DOCA-salt hypertensive rats. Similar results were observed in a study in normotensive dogs in which the administration of BQ-123 or BQ-788 did not alter the basal release rate of adrenal catecholamines. Together, these data suggest that endogenous ET does not participate in the tonic, basal release of adrenal medullary catecholamines.

To determine whether endogenous ET alters the nerve-stimulated release of adrenal catecholamines via the ET$_A$ or ET$_B$ receptors during DOCA-salt hypertension, catecholamine release was measured during splanchnic nerve stimulation in the presence of ET receptor antagonists. In response to 0.3- or 1.0-Hz stimulation, the release rates of NE and EPI were not different in the presence and absence of the ET$_B$ receptor antagonist (BQ-788) in either control or DOCA-salt hypertensive rats. These data suggest that the ET$_B$ receptor does not alter the release of adrenal catecholamines in response to splanchnic nerve stimulation in either normotensive or DOCA-salt hypertensive rats.

The release rates of NE and EPI during splanchnic nerve stimulation were also examined in the presence of the ET$_A$ receptor antagonist BQ-123. In response to 1-Hz stimulation, the release of EPI was greater in DOCA-salt hypertensive rats during the infusion of BQ-123. The catecholamine responses in the control animals were not different in the presence of BQ-123. In contrast to our hypothesis, these data suggest that endogenous ET acting via ET$_A$ receptors inhibited EPI release from DOCA-salt hypertensive rats during nerve-induced stimulation of the adrenal medulla.

Together, the data obtained in the present study suggest two different roles for ET in the adrenal gland of DOCA-salt hypertensive rats. On one hand, the increased vascular level of ET peptide in DOCA-salt hypertension$^{1,2}$ may serve to enhance the basal release of catecholamines. On the other hand, ET endogenous to the adrenal medulla may protect the gland in DOCA-salt hypertension through inhibition of catecholamine release during adrenal activation. In this capacity, ET could prevent the adrenal medulla from quickly depleting its catecholamine content in times of stress during DOCA-salt hypertension.

The mechanism by which ET inhibits catecholamine release in DOCA-salt hypertensive rats is not known. Previous studies have demonstrated that ET$_A$ receptors found on the chromaffin cells of normotensive animals enhance catecholamine release,$^{12-15}$ During DOCA-salt hypertension, however, an ET$_A$ receptor may be expressed on the prejunctional nerve terminal that inhibits acetylcholine release and subsequent catecholamine release from the chromaffin cell. This hypothesis is supported by the observation that ET can inhibit acetylcholine release from peripheral sympathetic nerve endings in normotensive animals.$^{16}$

In summary, the basal and stimulated release rates of adrenal medullary catecholamines did not differ between control and DOCA-salt hypertensive rats. Exogenous ET-1 enhanced the release of catecholamines from both normotensive and DOCA-salt hypertensive rats. When the adrenal gland was stimulated in the presence of ET-1, NE release was inhibited from the DOCA-salt hypertensive rats but not from the normotensive rats. The infusion of BQ-123 or BQ-788 did not alter basal catecholamine release from either control or DOCA-salt hypertensive rats. EPI release in the DOCA-salt hypertensive rats was enhanced in the presence of BQ-123. These data suggest that ET can stimulate and inhibit the release of adrenal medullary catecholamines in DOCA-salt hypertension.

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References
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