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 (10⁻²⁷ mol/L), an ETₐ receptor antagonist, or BQ-788 (10⁻²⁷ mol/L), an ETₐ 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

Endothelin (ET) is important in the development and maintenance of deoxycorticosterone acetate (DOCA)-salt hypertension. Lariviere et al.¹² demonstrated that ET mRNA and peptide levels are greater in the vasculature of DOCA-salt hypertensive animals than in that of normotensive animals. The development of DOCA-salt hypertension is attenuated during the long-term administration of ETₐ receptor antagonists given throughout DOCA-salt treatment³⁴ and is associated with attenuated production of ET mRNA and peptide.⁵ ET is also involved in the maintenance of hypertension, because the administration of ETₐ antagonists to animals with established DOCA-salt hypertension leads to a significant reduction in blood pressure.⁶–⁸

Recent data have shown that ET is found in many different tissues,⁹ including the medulla of the adrenal gland. ET mRNA and ET-converting enzyme are found in the adrenal gland and can produce mature ET peptide.⁹–¹¹ In addition, several studies have shown the presence of binding sites for ET in the adrenal cortex and medulla.¹²,¹³ Functionally, ET can elicit catecholamine release from adrenal medullary cells in culture¹⁴,¹⁵ and from the whole adrenal gland in situ independent of cholinergic stimulation.¹⁶,¹⁷ These studies further demonstrate that the binding sites consist of both ETₐ and ETₐ receptors; however, it is the ETₐ receptor that predominantly mediates adrenal medullary catecholamine release. Catecholamine release from the adrenal gland may contribute to the development and maintenance of DOCA-salt hypertension. DOCA-salt hypertension does not develop in the absence of the adrenal glands,¹⁸ and removal of the adrenal medullae in animals with established DOCA-salt hypertension causes a significant decrease in blood pressure.¹⁹ Interestingly, recent data suggest that the level of ET peptide increases in the adrenal gland of DOCA-salt hypertensive rats compared with that of normotensive control animals,²⁰ suggesting that ET may play a role in catecholamine release during DOCA-salt hypertension. Therefore, this study tested the hypothesis that ET enhances catecholamine release from the adrenal medulla of DOCA-salt hypertensive rats. To test this hypothesis, the left adrenal gland of normotensive and DOCA-salt hypertensive rats was isolated and perfused with Krebs’ solution. The effect of ET on catecholamine release from the adrenal medulla in normotensive and DOCA-salt hypertensive rats was assessed through electrical stimulation of the splanchnic nerve and measurement of norepinephrine (NE) and epinephrine (EPI) release in normotensive and DOCA-salt hypertensive rats.
the absence and presence of exogenous ET-1. To determine whether endogenous ET alters adrenal medullary catecholamine release, the splanchic 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). At 14 days after DOCA-salt initiation, all 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 animals were conscious, the rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). The adrenal gland was prepared according to Hinson et al$^{21}$ 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 splanchnic nerve was isolated near the diaphragm. A stimulating electrode was placed around the nerve and covered with 0.2 mL of a fast curing silicone elastomer (Kwik-Sil; World Precision Instruments). After the elastomer had cured, the “pocket” created in the area between the kidney and diaphragm was filled with warm Krebs’ solution. This solution was continuously recirculated to keep the area between the kidney and diaphragm warm and moist for the duration of the experiment.

In a separate group animals, the role of endogenous ET was examined. After the 60-minute equilibration period, the splanchic nerve was stimulated for 30 seconds at either 0.3 or 1.0 Hz. Voltage (10 V), delay (0.1 ms), and duration (1.0 ms) were kept constant. A 30-second sample of perfusate was collected to determine catecholamine release during electrical stimulation. At 30 minutes after the first stimulation, the other stimulation frequency was performed, and the perfusate was collected.

The antagonists were dissolved in Krebs’ solution. At 30 minutes after the second electrical stimulation, a constant infusion of the ET$_A$ or ET$_B$ receptor antagonist BQ-123 (10$^{-7}$ mol/L) or BQ788 (10$^{-7}$ mol/L), respectively, was started. These concentrations of BQ-123 and BQ-788 have previously been shown to inhibit the vasoconstrictor and vasodilator effects of ET-1 on the adrenal vasculature.$^{22}$ Perfusate samples were collected 5, 10, 15, and 30 minutes after the start of the infusion of the antagonists. At the end of 30 minutes, electrical stimulations (0.3 and 1.0 Hz) were repeated at 30-minute intervals. Four groups of rats were used in this experiment: control, vehicle DOCA-salt hypertensive rats infused with BQ-788 and control and DOCA-salt hypertensive rats infused with BQ-123.

**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±2 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 initial basal 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 initial basal 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 initial basal 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 initial basal 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 splanchic 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.

In order to determine the baseline release rate of NE and EPI, the adrenal gland was dissected and removed. The adrenal gland and surrounding tissues were weighed and placed in a 4 mm Hg incubator. The heart rate did not differ statistically between the control and the DOCA-salt hypertensive rats (118±2 versus 118±2 bpm, respectively). After the measurement of mean arterial pressure and heart rate with the MacLab data acquisition system (AD Instruments) while the animals were conscious, the rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). The adrenal gland was prepared according to Hinson et al$^{21}$ 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 splanchnic nerve was isolated near the diaphragm. A stimulating electrode was placed around the nerve and covered with 0.2 mL of a fast curing silicone elastomer (Kwik-Sil; World Precision Instruments). After the elastomer had cured, the “pocket” created in the area between the kidney and diaphragm was filled with warm Krebs’ solution. This solution was continuously recirculated to keep the area between the kidney and diaphragm 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.

**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. The splanchic nerve was stimulated for 30 seconds at 0.3 Hz, Voltage (10 V), delay (0.1 ms), and duration (1.0 ms) were kept constant. A 30-second sample of perfusate was collected to determine catecholamine release during electrical stimulation.

ET-1 was dissolved in Krebs’ solution. At 60 minutes after the initial stimulation, a constant infusion of the ET-1 solution (1 ng/mL) was started. Perfusion samples were collected 5, 15, 30, 45, and 60 minutes after the infusion of ET-1. At this time, the electrical stimulation (0.3 Hz) of the splanchic nerve was repeated in the presence of ET-1. Separate groups of control and DOCA-salt hypertensive rats followed the same protocol except that the vehicle (Krebs’ solution) was infused instead of ET-1. Four groups of rats were used in this experiment: vehicle-infused control rats, vehicle-infused DOCA-salt hypertensive rats, ET-1–infused control rats, and ET-1–infused 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.23,24 Although these studies did not compare the adrenal catecholamine release in normotensive and hypertensive animals, the electrical stimuli used to induce adrenal medullary catecholamine release were of longer duration (several minutes) and of higher frequency (≥1 Hz) than those used in the present study.

Exogenous ET

Cells in the adrenal gland are capable of synthesizing ET-1,9–11 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.14,15 When ET-1 was administered to the adrenal gland of normotensive dogs in vivo, catecholamine release was also increased.16,17 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.25 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
were not different in the presence and absence of the ETB receptor during DOCA-salt hypertension, catecholamine release in either control or DOCA-salt hypertensive rats. Group size is indicated in legend to Figure 3.

Responses for control rats. Right, Responses for DOCA-salt hypertensive rats. *Significant difference from 0.3-Hz stimulation. †Significant difference from absence of BQ-123.

Figure 5. Change in release rate of NE and EPI during splanchnic nerve stimulation in absence (open columns) and presence (filled columns) of ET alpha receptor antagonist BQ-123. Left, Responses for control rats. Right, Responses for DOCA-salt hypertensive rats. Group size is indicated in legend to Figure 3. *Significant difference from 0.3-Hz stimulation. †Significant difference from absence of BQ-123.

the DOCA-salt hypertensive rats, ET inhibited the nerve-stimulated release of NE. Thus, the role of ET in altering adrenal medullary catecholamine release may be species or blood pressure dependent, or both.

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 alpha and ET beta 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 alpha or ET beta 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 beta receptor antagonist (BQ-788) in either control or DOCA-salt hypertensive rats. These data suggest that the ET beta 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 alpha 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 alpha 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 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 alpha receptors found on the chromaffin cells of normotensive animals enhance catecholamine release. During DOCA-salt hypertension, however, an ET alpha 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.

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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