Alterations in Cardiovascular Responsiveness and Adrenoceptor Binding during Catecholamine Infusion Hypertension in Rats (43226)

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Abstract. **Chronic continuous infusion of norepinephrine in rats causes alterations in biochemical and physiologic responses of the cardiovascular system and in cardiovascular adrenoceptor number. The response of cardiac and aortic ornithine decarboxylase (ODC) activity to stimulation by norepinephrine was decreased in rats receiving norepinephrine infusion. These responses are due to stimulation of β- and α-adrenergic receptors, respectively. Additionally, there was reduced stimulation of aortic ODC activity by angiotensin II and vasopressin. The cardiac ODC response to angiotensin II was decreased, but the response to vasopressin was not affected.**

The decreased ODC response is accompanied by decreased pressor responses to the α-adrenergic agonist phenylephrine. Decreased numbers of α- and β-adrenoceptor binding sites (as measured by the binding of [^{3}H]prazosin and [^{125}I]pindolol) might mediate, in part, the altered responses to adrenergic agonists. The decreased cardiovascular responsiveness measured in these animals after several days of norepinephrine infusion hypertension contrasts with the increased responses found in most other forms of hypertension. This provides a useful model in which to examine the consequences of prolonged adrenergic receptor stimulation. [P.S.E.B.M. 1991, Vol 197]

Despite evidence that sympathetic nervous system stimulation can acutely elevate blood pressure and that suppression of sympathetic activity is efficacious in controlling high blood pressure, there is considerable controversy about the role of sympathetic function in the sustained phase of the disease. One reason for the controversy is that cardiovascular responses to sympathetic agonists decrease after repeated stimulation, whereas cardiovascular responses are increased in most forms of hypertension. Decreased cardiac response to β-receptor stimulation occurs during chronic exposure to isoproterenol both *in vivo* (1–4) and *in vitro* (5). Similarly, decreased arterial response to α-receptor stimulation results from chronic stimulation by α agonists both *in vivo* (6) and *in vitro* (7–9). Therefore, increased adrenergic activity may not be directly responsible for the increased cardiovascular responsiveness that occurs during hypertension, and alterations in the structure of vascular smooth muscle may help to account for increased cardiovascular responsiveness (10).

The studies presented here were designed to investigate alterations in biochemical and physiologic indices of cardiovascular responsiveness in an animal model of hypertension in which elevated blood pressure is due to chronic pharmacologic stimulation of adrenergic receptors. Hypertension was produced by long-term continuous infusion of norepinephrine (NE) subcutaneously with Alzet osmotic minipumps. This model of hypertension was utilized to investigate the effects of a chronic hyperadrenergic state on pressor responses and on the responses of cardiovascular ornithine decarboxylase (ODC) activity.

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The activity of ODC is a convenient and reliable index of tissue response to stimulation by a hormone or neurotransmitter (11, 12). After stimulation, ODC activity reaches a maximum in 3–5 hr. The primary advantage of ODC as an index is that it monitors events *in vivo* in freely moving animals, thus avoiding the problems inherent in many physiologic measurements. This provides a useful complement to evaluation of pressor responses that require the use of anesthesia and reflect actions at several sites. The present studies utilize the enzyme to examine alterations in cardiac and vascular responses during hypertension produced by chronic adrenoceptor stimulation. In a previous report, we demonstrated that NE stimulates cardiac and aortic ODC activity by activating β- and α-adrenergic receptors, respectively (13). Thus, stimulation of enzyme activity by NE provides a convenient index of adrenergic receptor responsiveness in the heart and vasculature. ODC has been used in this way to assess tissue responsiveness in studies of central and peripheral neuronal development (14, 15), kidney development (16), maternal-infant interactions (17), and stress (18). The results of this study demonstrate marked reductions in cardiovascular responsiveness during hypertension secondary to chronic adrenoceptor stimulation.

Materials and Methods

Animals—General Care. Male Sprague-Dawley rats weighing 175–200 g were purchased from Zivic Miller Laboratories (Allison Park, PA). Purina Rodent Chow and tap water were provided *ad libitum*. Ambient temperature was maintained at 22°C, as was a 12-hr light:dark cycle.

ODC Activity. Rats were killed by decapitation and hearts and aortas were rapidly excised. Hearts were blotted dry, weighed, and homogenized (Polytron, setting 8, 10 sec) in 19 volumes of ice-cold 10 m*M* Tris-HCl buffer (pH 7.2). Aortas were blotted dry, weighed, and homogenized (glass-to-glass) in 9 volumes of the same buffer. The homogenates were centrifuged at 26,000*g* for 20 min and an aliquot of the supernatant was assayed for ODC activity, as described previously (14). The incubation medium contained final concentrations of 5 μM pyridoxal phosphate, 0.5 m*M* dithiothreitol, and 12 μM (heart) or 60 μM (aorta) [^{14}C] ornithine as substrate. The reaction was allowed to proceed in a closed vial for 40 min at 37°C. It was stopped by addition of 1 ml of citric acid (1 *M*), and the $^{14}CO_2$ evolved was trapped by hyamine hydroxide and counted by liquid scintillation spectrometry (Aquasol) at an efficiency of 85%.

Blood Pressure Determination. Rats were anesthetized with sodium pentobarbital (35 mg/kg ip), and a ventral midline incision 2 cm in length was made in the neck. The trachea was intubated with polyethylene tubing (PE-240; Becton-Dickinson, Parsippany, NJ). The left common carotid artery was exposed and freed from its surrounding neurovascular sheath by blunt dissection. The artery was catheterized with polyethylene tubing (PE-50) containing heparinized saline (1000 IU/ml 0.9% saline) and advanced to the level of the aortic arch. The catheter was connected to a pressure transducer (model P23 ID; Gould Statham, Oxnard, CA) and blood pressure was recorded with a polygraph (Grass model 5D). The jugular vein was exposed by blunt dissection and catheterized with PE-50 tubing for intravenous drug administration.

Catecholamine Infusion. Rats were anesthetized with ether. A small incision (1 cm) was made in the intrascapular region and a pocket prepared by blunt dissection. An Alzet osmotic minipump (model 2001) containing (−)NE in 0.2% ascorbic acid as an antioxidant was inserted and the incision was closed with wound clips. The concentration of catecholamine in the minipumps was such that animals received a dose of 0.1 mg/kg/hr. Control animals received vehicle infusions.

Radioligand Binding. Hearts were homogenized with a Polytron (setting 5.5) for 20 sec in 40 vol of ice-cold buffer (20 m*M* Tris-HCl, 2 m*M* $MgCl_2$, and 140 m*M* NaCl, pH 7.5). Homogenates were filtered through two layers of gauze and centrifuged at 40,000*g* for 15 min at 4°C. The supernatant was discarded and the pellet resuspended and recentrifuged twice in the original volume of buffer. After the final wash, the pellet was resuspended in 50 m*M* Tris-HCl, 2 m*M* $MgCl_2$, and 0.25 *M* sucrose (pH 7.5) by Teflon to glass homogenization (10 strokes by hand) at a dilution of 3 ml of buffer/g initial tissue weight. For analysis of α-receptors, an aliquot of the membrane suspension was diluted 1:1 in ice-cold 50 m*M* Tris-HCl and 10 m*M* $MgCl_2$ (pH 7.5), and 100-μl aliquots were incubated with [^{3}H]prazosin (0.25–3.0 n*M*) in a total volume of 400 μl. Phentolamine (10^{-5} *M*) was used to determine nonspecific binding. Triplicate samples were incubated at 4°C for 50 min. The reaction was terminated by the addition of 7.5 ml of ice-cold incubation buffer and filtered under vaccum through Whatman GF/C filters. Filters were rapidly washed with an additional 7.5 ml of buffer and then counted by liquid scintillation spectrometry. Nonspecific binding was typically 30–50% of total binding. For analysis of β-receptor binding, membranes were diluted 1:5 with 20 m*M* Tris-HCl, 2 m*M* $MgCl_2$, 145 m*M* NaCl, and 1 m*M* sodium ascorbate (pH 7.5), and 100-μl aliquots were incubated with [^{125}I] pindolol (0.01–0.2 n*M*) in a total volume of 400 μl. Isoproterenol (10^{-5} *M*) was used to determine nonspecific binding. Triplicate samples were incubated at 22°C for 20 min. The reaction was terminated by the addition of 8 ml of ice-cold 10 m*M* Tris-HCl and 145 m*M* NaCl (pH 7.5) and filtered under vacuum through Whatman GF/C filters. The filters were rapidly washed twice with

8 ml of buffer and then counted in a gamma counter (Packard A-500C). Nonspecific binding was typically 5–15% of total binding. (−)Pindolol was iodinated to a sp act of 2.2 Ci/μmol according to the method of Witkin and Harden (18).

Protein Measurement. Protein was measured according to the method of Lowry *et al.* (19) using bovine serum albumin as the standard.

Statistics. Results are expressed as the mean ± SE. B_{max} and K_d values were calculated by Scatchard analysis of binding data using linear regression analysis. Dose-response curves for blood pressure and ODC activity were analyzed by repeated measures analysis of variance followed by post hoc tests (Games and Howell variant of the Tukey test and *t* test) to compare the two groups at each dose separately. For blood pressure, analysis of variance revealed a significant group main effect, dose main effect and group by dose interaction. The latter effect indicates that group differences varied as a function of dose; therefore, a series of unpaired *t* tests was performed to compare groups at each dose.

Materials. L-[1-^{14}C]Ornithine hydrochloride (40–60 mCi/mmol), [^{3}H]prazosin (10–30 Ci/mmol), and ^{125}I (carrier-free) were purchased from the Amersham Corp. (Arlington Heights, IL). (−)Norepinephrine hydrochloride, pindolol, angiotensin II, and arginine vasopressin were purchased from Sigma Chemical Co. (St. Louis, MO). Osmotic minipumps (model 2001) were purchased from Alza Corp. (Palo Alto, CA).

Results

Acute subcutaneous administration of NE to rats increased cardiac and aortic ODC activity (Fig. 1). This response was significantly attenuated after chronic (i.e., 6-day) infusion of NE (Table I). The ODC response was decreased to both submaximal and maximal challenge doses of NE in the thoracic and abdominal aorta. Decreased response in the heart was observed only with maximal challenge doses of NE. The response to other agents was characterized to determine whether the decreased response to NE was accompanied by altered responsiveness to other stimuli. Doses of angiotensin and vasopressin that caused a marked aortic response in control animals had no effect after NE infusion (Table I). Similarly, the response of cardiac ODC to angiotensin was decreased. In contrast, the response of cardiac ODC to vasopressin was not affected by NE infusion.

To determine whether or not the reduced ODC response was dependent on the continued infusion of NE, a challenge dose of NE was administered 2 hr after the termination of a 6-day NE infusion (Table II). The ODC response was reduced to the same extent as when the challenge dose was administered during the continuation of the 6-day NE infusion.

To determine whether the blunted ODC responses

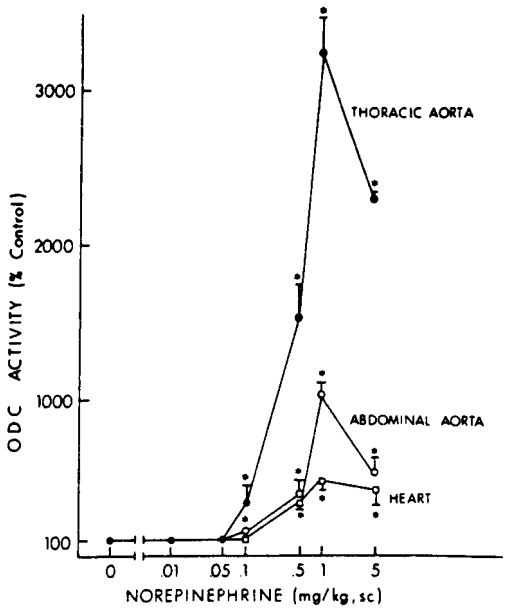

Figure 1. Stimulation of thoracic aorta, abdominal aorta, and heart ODC activity by actue subcutaneous injection of NE 4 hr earlier. Points and bars represent means ± SE of individual determinations from three to nine rats. Control values were 0.36 pmol/mg/hr for thoracic aorta, 0.41 pmol/mg/hr for abdominal aorta, and 0.59 pmol/mg/hr for heart. * $P < 0.01$ compared with vehicle-injected control.

reflected altered physiologic responses, dose relationships were established for the pressor response to the α-adrenergic agonist phenylephrine administered intravenously (Fig. 2). The ED_{50} was significantly increased after NE infusion, leading to reduced response throughout most of the dose range, although the maximal response was increased after NE infusion.

Radioligand binding studies were performed to determine whether changes in α- and β-adrenergic receptor number or affinity might account for altered responsiveness. Binding of the β-adrenergic antagonist [^{125}I]pindolol to cardiac membranes from control rats (6-day vehicle infusion) revealed a single population of sites with a density of 14.1 fmol/mg membrane protein (0.96 pmol/heart) (Fig. 3). The apparent dissociation constant was 60 p*M*. NE infusion for 6 days reduced the number of sites by 21% and 17% on a per milligram protein and per heart basis, respectively, with no change in affinity. Binding of [^{125}I]pindolol at a single concentration (0.12 n*M*) to cardiac membrane preparations from individual animals confirmed that the decrease in number of sites was statistically significant (Table III).

Binding of the α-adrenergic antagonist [^{3}H]prazosin to cardiac membranes from control rats revealed a single population of sites with a density of 47 fmol/mg membrane protein (3.2 pmol/heart) and an apparent dissociation constant of 0.25 n*M* (Fig. 4). NE infusion for 6 days reduced the number of sites by 29% and 19% on a per milligram protein and per heart basis, respectively. Binding at a single ligand concentration (1.7 n*M*) to cardiac membrane preparations from in-

Table I. Response of Aortic and Cardiac ODC Activity to Acute Subcutaneous Administration of NE, Angiotensin II, and Vasopressin during Day 6 of NE Infusion[a]

	Thoracic aorta		Abdominal aorta		Heart	
	Vehicle infusion	NE infusion	Vehicle infusion	NE infusion	Vehicle infusion	NE infusion
Control	100 ± 11	105 ± 14	100 ± 17	122 ± 15	100 ± 18	119 ± 10
NE (0.2 mg/kg)	1050[b] ± 94	100[c] ± 8	203[b] ± 31	125[c] ± 20	208[b] ± 12	213[b] ± 15
NE (1 mg/kg)	3190[b] ± 163	911[b,c] ± 210	2090[b] ± 341	681[b,c] ± 179	445[b] ± 42	275[b,c] ± 58
Angiotensin II (1 mg/kg)	1320[b] ± 215	150[c] ± 22	283[b] ± 54	145[c] ± 31	268[b] ± 17	151[c] ± 38
Vasopressin (75 IU/kg)	1150[b] ± 391	104[c] ± 37	562[b] ± 210	140[c] ± 38	306[b] ± 59	298[b] ± 35

[a] Values represent mean ± SE of individual determinations from four to seven rats. Control values were 0.35 pmol/mg/hr for thoracic aorta, 0.22 pmol/mg/hr for abdominal aorta, and 0.74 pmol/mg/hr for heart.
[b] $P < 0.05$ compared with control injection.
[c] $P < 0.05$ compared with vehicle infusion administered in the same injection.

Table II. Response of Thoracic and Abdominal Aorta ODC Activity to NE (0.2 mg/kg) Administered Subcutaneously 2 hr after the Termination of a 6-Day NE Infusion[a]

	Thoracic aorta		Abdominal aorta	
	Saline	NE	Saline	NE
Control infusion	100 ± 11	1543 ± 428[b]	100 ± 8	148 ± 10[b]
NE infusion	107 ± 9	116 ± 21[c]	105 ± 4	101 ± 7[c]

[a] Values are expressed as percentage of control and represent the mean ± SE of individual determinations from six rats. Control values were 0.28 pmole/mg/hr for thoracic aorta and 0.26 pmole/mg/hr for abdominal aorta.
[b] $P < 0.01$ compared with control infusion, saline injection.
[c] $P < 0.01$ compared with control infusion, NE injection.

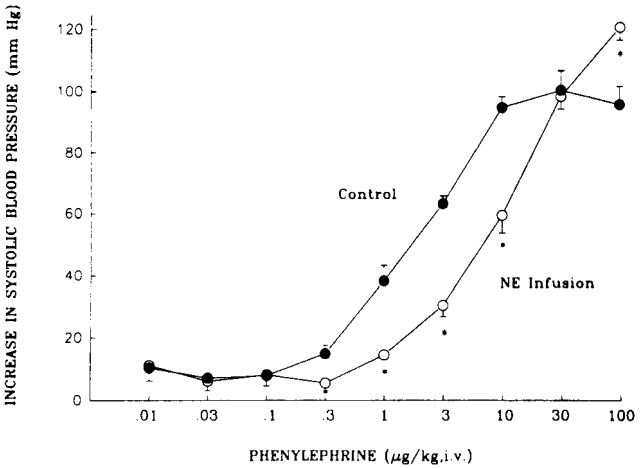

Figure 2. Increase in systolic blood pressure after acute intravenous administration of phenylephrine. Phenylephrine was administered 30 min after the termination of a 6-day NE infusion. Points and bars represent mean ± SD of individual determinations from three to five rats. ED_{50}s are significantly different by linear regression analysis (1.7 ± 0.1 for control; 6.0 ± 0.1 for NE infusion; $P < 0.001$). Maximal response is significantly different (94 ± 9 and 122 ± 6 mm Hg for control and NE infusion, respectively). Basal values were 74 ± 3 mm Hg for control and 73 ± 1 mm Hg for NE infusion. ●, Control (vehicle infusion); ○, NE infusion.

dividual animals confirmed that the decrease in number of sites was statistically significant (Table III).

Discussion

There is a marked decrease in the responsiveness of cardiovascular adrenergic receptors after 6 days of continuous NE infusion. The ability of the adrenergic agonist NE to stimulate cardiac and aortic ODC activity is substantially reduced after 6 days of NE infusion. Decreased responsiveness of ODC activity is not dependent on the continued presence of infused NE because the suppression persists for at least 2 hr after the termination of NE infusion. These results are consistent with a previous report from this laboratory demonstrating that NE infusion elicits a transient stimulation of ODC activity followed by a return of activity to control levels despite the continuation of NE infusion (20). Thus, the failure of NE infusion to elicit a sustained elevation of ODC activity can probably be attributed to desensitization. This is not simply a homologous desensitization of adrenergic receptors by NE because the response to other agents is also reduced. The response of aortic ODC to stimulation by angiotensin and vasopressin is blunted, as is the response of cardiac ODC to stimulation by angiotensin. The reason for an unchanged response of cardiac ODC to stimulation by vasopressin is not known.

Because stimulation of tissue ODC activity is frequently used as an index of the tissue's responsiveness to various agonists (11, 12), these results suggest that physiologic responses may be desensitized during NE infusion. However, we previously reported that the elevation of blood pressure during NE infusion does not attenuate, as would be expected if there was desensitization of cardiovascular adrenergic receptor responsiveness. Thus, the correlation between ODC activity and blood pressure breaks down under these conditions. Phenylephrine dose-response curves were measured

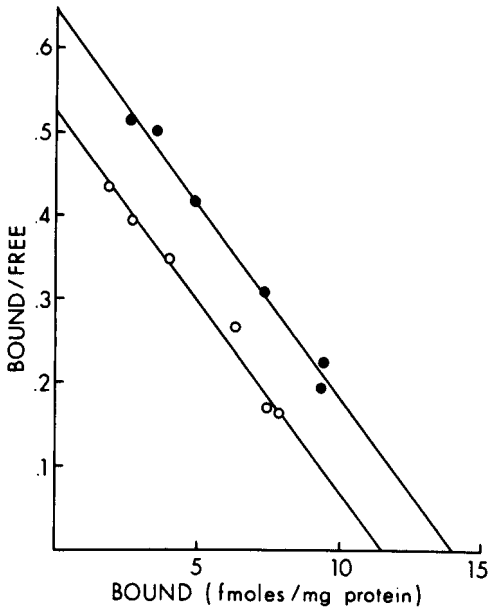

Figure 3. Scatchard analysis of [^{125}I]pindolol binding to cardiac membrane preparations. Each point represents the mean of triplicate determinations on membrane preparations pooled from 10 rats. Lines were fitted by least squares analysis. Intercept on the abcissa is significantly less in rats infused with NE for 6 days ($P < 0.001$). The slopes are not significantly different. The apparent K_d are 60 p*M* and 61 p*M* in control and NE-infused rats, respectively. Correlation coefficients are 0.99 for each group. ●, control (vehicle infusion); ○, NE infusion.

Table III. Effect of NE Infusion on Cardiac α- and β-Adrenoceptor Binding[a]

	n	[^{3}H]Prazosin (fmol/mg protein)	[^{125}I]Pindolol (fmol/mg protein)
Control	12	26.4 ± 1.2	6.8 ± 0.2
NE infusion	10	20.9 ± 1.1[b]	5.5 ± 0.2[c]

[a] Each value represents the mean ± SE of triplicate determinations from each of the indicated number of animals. Ligand concentrations were 1.7 n*M* for prazosin and 0.12 n*M* for pindolol.
[b] $P < 0.005$.
[c] $P < 0.001$ by Student's *t* test (two-tailed, unpaired).

after NE infusion in order to assess physiologic responsiveness directly. The pressor response to phenylephrine was significantly decreased as evidenced by an increase in the ED_{50} for both diastolic (data not shown) and systolic pressures. The persistence of alterations of the pressor response for at least 30 min after termination of NE infusion is consistent with the persistence of decreased ODC responses.

Increased activity of the sympathetic nervous system is thought to contribute to the initiation and maintenance of hypertension in humans and laboratory animals (21–23). The expected consequence of such an alteration in adrenergic activity is a reduced responsiveness to subsequent stimuli, yet most studies report increased vascular reactivity during hypertension. Increased sensitivity to vasopressor stimuli has been demonstrated in isolated vessels and in vascular beds of renal, DOCA-salt, and spontaneously hypertensive rats (24–27). The mechanism underlying these changes is controversial (28–30), but it is not an expected consequence of enhanced NE release from cardiovascular sympathetic nerves (31).

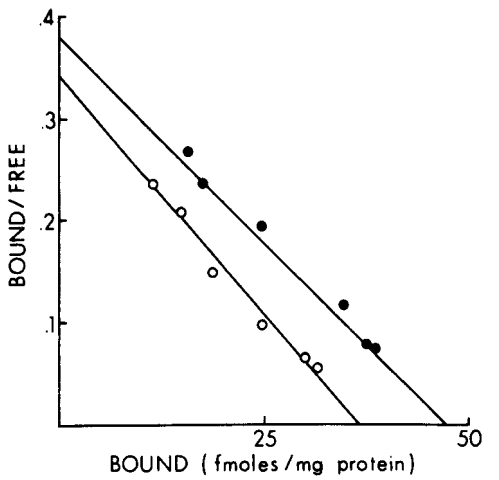

Figure 4. Scatchard analysis of [^{3}H]prazosin binding to cardiac membrane preparations. Each point represents the mean of triplicate determinations on membrane preparations pooled from 10 rats. Lines were fitted by least square analysis. Intercept on the abscissa is significantly less in rats infused with NE for 6 days ($P < 0.001$). The slopes are not significantly different. The apparent K_d are 0.25 n*M* and 0.22 n*M* for control and NE infused rats, respectively. Correlation coefficients are 0.99 for each group. ●, Control (vehicle infusion); ○, NE infusion.

The catecholamine infusion model of hypertension is fundamentally different from most other models in that elevated blood pressure is a consequence of pharmacologic stimulation of adrenoceptors. It is similar to human and experimental pheochromocytoma both in the levels of plasma catecholamines that are attained and in the degree of hypertension that is produced (32, 33). Thus, it permits a reasonably direct investigation of the perturbations that result from chronic stimulation of adrenergic receptors. One such perturbation is reduction in the number and/or affinity of cardiovascular adrenergic receptors. Others have reported large decreases (30–60%) in the number of cardiac α- and β-adrenergic receptors in DOCA-salt, renal, and spontaneously hypertensive rats (34–36). This is often attributed to down-regulation because adrenergic activity is thought to be elevated in these animals. Yet in catecholamine infusion hypertensive rats there is a relatively small decrease (15–30%) in cardiac α- and β-adrenergic receptor number despite the fact that in this model, elevated blood pressure is solely dependent on stimulation of adrenergic receptors. Factors other than enhanced receptor activation by NE may contribute to the receptor alterations in these other models of hypertension. Alternatively, longer periods of NE infusion may produce further reductions in receptor numbers.

The relatively small decrease in the number of cardiac adrenergic receptors during NE infusion is associated with a large decrease in ODC responsiveness. The loss of aortic and cardiac ODC responsiveness to stimulation by angiotensin and vasopressin further emphasizes the importance of postreceptor changes. The mechanism(s) whereby NE exposure decreases ODC responsiveness is not yet known. This could occur by direct NE effects on α- and β-receptors and could also involve indirect effects due to increased blood pressure. Further experiments will be necessary to distinguish between these possibilities. These results are consistent with recent reports that marked desensitization of *in vitro* vascular response to NE is accompanied by reduced thromboxane A_2 responsiveness, but not by a reduction in α-receptor number of affinity (9, 37).

The present studies demonstrate a pronounced decrease in α-receptor-mediated pressor responsiveness in rats made hypertensive by NE infusion. This is accompanied by reduced stimulation of ODC activity, which is often used as a biochemical index of tissue responsiveness. Reduced adrenergic receptor numbers can only partially account for the diminished responses. Thus, continuous NE infusion provides an animal model of hypertension in which the consequences of a prolonged hyperadrenergic state can be readily assessed.

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