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Enhanced endothelial nitric oxide activity contributes to the reduced responsiveness of vascular α_1-adrenoceptors following aortic barodenervation

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Abstract

We have recently shown that short-term aortic barodenervation diminishes constrictor responses to activation of α_1-adrenoceptors in rat aortic smooth muscle. This study investigated the potential role of vascular endothelium and its derived vasoactive substances, nitric oxide and prostaglandins, in the reduced α_1-adrenoceptor responsiveness after aortic barodenervation. Exposure of isolated aortic rings from aortic barodenervated and sham-operated rats 48 h after surgery to cumulative addition of phenylephrine (α_1-adrenoceptor agonist, 3×10^{-8}–1×10^{-4} M) resulted in concentration-related contractions that were significantly ($P < 0.05$) smaller in rings of denervated rats. Removal of the endothelium increased phenylephrine-mediated contractions in rings obtained from aortic barodenervated rats to near sham-operated levels as demonstrated by the similar contractile responses and slopes of the regression lines of the concentration–response curves. Pretreatment with indomethacin (cyclooxygenase inhibitor, 1×10^{-5} M) had no effect on contractile responses to phenylephrine in rings from both groups of rats. In contrast, N^G-nitro-L-arginine (nitric oxide synthase inhibitor, 3×10^{-5} M) elevated basal vascular tone and significantly ($P < 0.05$) increased α_1-adrenoceptor responsiveness, effects that were more evident in rings from denervated compared with sham-operated rats. N^G-nitro-L-arginine produced significantly ($P < 0.05$) greater increases in the slopes of the regression lines ($136.1 \pm 22\%$ vs. $73.0 \pm 8.6\%$ mg tension/mg tissue/log molar concentration) and maximum contractile response (E_{max}) to phenylephrine ($161.2 \pm 8.2\%$ vs. $76.7 \pm 6.1\%$) in rings from denervated compared with sham-operated rats suggesting an enhanced nitric oxide activity in aortas of denervated rats. This notion is further supported by the finding that cumulative i.v. administration of N^G-nitro-L-arginine (1, 2, 4 and 8 mg/kg) elicited significantly ($P < 0.05$) greater pressor responses in conscious barodenervated compared with sham-operated rats. These results suggest that the endothelium plays a major role in the reduced constrictor responses to α_1-adrenoceptor activation that occurs shortly after aortic barodenervation. This effect of the endothelium appears to involve, at least in part, enhancement of endothelial nitric oxide activity. © 1997 Elsevier Science B.V.

Keywords: Aortic barodenervation; α_1-Adrenoceptor; Endothelium; Nitric oxide (NO)

1. Introduction

In a recent study from our laboratory, we provided the first evidence that short-term (48 h) aortic barodenervation diminishes α_1-adrenoceptor responsiveness in rat aortic smooth muscle (El-Mas et al., 1997). Compared with sham operation, aortic barodenervation exhibited substantial reductions in contractile responses and maximal force generated by α_1-adrenoceptor activation in aortic ring preparations (El-Mas et al., 1997). The reduced responsiveness of α_1-adrenoceptors has been the consequence of the elevated sympathetic neural activity commonly seen in these rats (Abdel-Rahman, 1992; El-Mas et al., 1994a,b, 1997). Nonetheless, it is not clear whether a possible alteration in the functional activity of vascular endothelium after aortic barodenervation may play a contributory role in the attenuated α_1-adrenoceptor-mediated contractions. The role of vascular endothelium in the modulation of circulatory tone through releasing a variety of vasoactive relaxing and constricting factors has been documented (Furchgott, 1983; Vanhoutte et al., 1986). In effect, Hiremath et al. (1991) reported that reduced reactivity of α_1-adrenoceptors occurs in aortas of pheochromocytoma-bearing rats and removal of the endothelium restores α_1-adrenoceptor responsive-

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ness to normal levels. Similarly, attenuation of α_1-adrenoceptor responsiveness in aortic rings after in vitro exposure to α_1-adrenoceptor agonists is less remarkable in endothelium-denuded preparations (Hiremath et al., 1991; Hu et al., 1992). These findings suggested a major contribution of the endothelium in α_1-adrenoceptor-mediated smooth muscle contraction (Hiremath et al., 1991; Hu et al., 1992).

The main objective of the present study was to evaluate the role of the vascular endothelium in the reduced constrictor responses to α_1-adrenoceptor activation in aortic smooth muscle after selective aortic baroreceptor denervation. To this end, α_1-adrenoceptor responsiveness was assessed in aortas with intact or denuded endothelium obtained from aortic barodenervated and sham-operated rats 48 h after surgery. Because the results showed that removal of the endothelium increased α_1-adrenoceptor responsiveness of rings obtained from aortic barodenervated rats to near sham-operated values, further experiments were conducted to determine the potential role of the endothelium-derived vasoactive substances, nitric oxide and prostaglandins, in the diminished α_1-adrenoceptor responsiveness. This was achieved by investigating the effect of inhibition of the activity of nitric oxide synthase and cyclooxygenase by N^G-nitro-L-arginine and indomethacin, respectively, on α_1-adrenoceptor responsiveness in aortic rings obtained from aortic barodenervated and sham-operated rats. Whether aortic barodenervation alters the activity of endothelial nitric oxide in vivo was also investigated by evaluating hemodynamic responses to N^G-nitro-L-arginine in conscious freely moving aortic barodenervated and sham-operated rats.

2. Materials and methods

2.1. Preparation of the rats

Male Wistar rats (300–360 g; High Institute of Public Health, Alexandria, Egypt) were used in the present study. For measurement of blood pressure, the method described in our previous studies (El-Mas and Abdel-Rahman, 1992; El-Mas et al., 1994a,b) was adopted. Briefly, the rats were anesthetized by thiopental (50 mg/kg i.p.). Catheters (Polyethylene 50) were placed in the abdominal aorta and vena cava via the femoral artery and vein for measurement of blood pressure and i.v. administration of drugs, respectively. The catheters were inserted about 5 cm into the femoral vessels and secured in place with sutures. The arterial catheter was connected to a Gould-Statham pressure transducer (Oxnard, CA, USA) and blood pressure was displayed on a Grass polygraph (model 7D, Grass Instrument, Quincy, MA, USA). Heart rate was computed from blood pressure waveforms by a Grass tachograph and was displayed on another channel of the polygraph. Blood pressure and heart rate were monitored until aortic barodenervation or sham operation was completed. Experiments were performed in strict accordance with institutional animal use guidelines.

2.2. Aortic baroreceptor denervation

Aortic barodenervation was accomplished by bilateral transection of the superior laryngeal, cervical sympathetic and aortic depressor nerves following a midline incision in the cervical region as described in our previous studies (El-Mas and Abdel-Rahman, 1992; El-Mas et al., 1994a,b). Sham-operated rats were prepared by exposing the relevant nerve trunks without sectioning. A single dose of phenylephrine (8 μg/kg) was injected i.v. before and after aortic barodenervation or sham operation. A smaller decrease in heart rate of aortic barodenervated, compared with sham-operated, rats in response to phenylephrine-induced elevation in blood pressure indicated successful denervation (El-Mas and Abdel-Rahman, 1992; El-Mas et al., 1994a,b). Finally, the catheters were tunnelled subcutaneously and exteriorized at the back of the neck between the scapulae. The catheters were flushed with heparin (200 U/ml) and plugged with stainless steel pins. Incisions were closed by surgical clips and swabbed with povidone-iodine solution (Betadine). Each rat received an intramuscular injection of penicillin G procaine (60 000 U) and was housed in a separate cage with free access to food and water.

2.3. Rat isolated aortic ring preparation

Isolation of rat aortas and recording of isometric contraction were performed as described in previous studies including ours (Nagao et al., 1992; El-Mas et al., 1997). Rats were killed by decapitation and thoracic aortas were removed immediately. Thoracic aortas from both aortic barodenervated and sham-operated rats were trimmed free of connective tissue and cut into ring segments 3 mm in length. In experiments involving endothelium-denuded rings, the endothelium was removed mechanically by gentle rubbing of the intimal surface of the aorta with a fine forceps. The forceps was inserted into the lumen of the ring and rolled back and forth on a filter paper moistened with the physiological solution (Nagao et al., 1992). Proper removal of the endothelium was tested by the absence of vasorelaxant responses to acetylcholine in rings precontracted with phenylephrine (Nagao et al., 1992).

Aortic rings with and without endothelium were mounted in 10 ml organ baths containing physiological solution at 37°C and aerated with 95% O_2 and 5% CO_2. The physiological solution was composed of the following (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1. Aortic rings were mounted in the organ baths by means of two stainless steel wire hooks inserted through the lumen of the ring. One of the hooks was anchored to a stationary pin at the bottom of the organ bath and the other was connected to

an isometric force-displacement transducer (Grass FT-03C) which was connected to a Grass polygraph (Model 7d) for recording isometric contractions of the aorta. An optimum resting tension of 1 g was placed on the tissue and an equilibration period of 2 h was allowed before the start of the experiment, with the bath fluid being replaced every 20 min. A concentration of phenylephrine (3×10^{-6} M), which was found in preliminary experiments to produce 50–60% of the maximal response, was added to the bath on two separate occasions during the 2 h equilibration period. This procedure was found to stabilize the preparation, allowing the subsequent construction of more consistent concentration–response curves (Hamed et al., 1983; El-Mas et al., 1997).

2.4. Experimental protocols

2.4.1. Hemodynamic effects of aortic barodenervation

Changes in mean arterial pressure and heart rate evoked by aortic barodenervation and sham operation were investigated in all rats at 5 min (anesthetized state) and also 48 h after recovery from anesthesia (i.e., conscious state). The baroreflex sensitivity was measured at the same time intervals by the administration of a single dose of phenylephrine (8 μg/kg). Changes in mean arterial pressure and heart rate evoked by phenylephrine were measured and used for calculation of the baroreflex sensitivity as detailed later. A period of 30 min was allowed at the beginning of the experiment for stabilization of blood pressure and heart rate.

2.4.2. Effect of endothelium removal on α_1-adrenoceptor responsiveness in aortic barodenervated aortic rings

This experiment evaluated the possible role of vascular endothelium in the aortic barodenervation-evoked reduction in α_1-adrenoceptor responsiveness in aortic smooth muscle. Contractile responses to α_1-adrenoceptor activation (by phenylephrine) were evaluated in aortic rings, with intact or denuded endothelium, obtained from aortic barodenervated and sham-operated rats 48 h after surgery. After equilibration, concentration–contractile response curves of phenylephrine (3×10^{-8}–1×10^{-4} M) were established by the method of stepwise cumulative addition (Hamed et al., 1983; El-Mas et al., 1997). The concentration of phenylephrine was increased by a half log unit with each addition. Each new addition was made only after the response to the previous concentration had attained a steady state. At the end of the experiment, the aortic rings were dried on a filter paper and weighed. Contractile responses were expressed in terms of mg tension/mg tissue.

2.4.3. Role of the endothelium-derived vasoactive substances in the reduced α_1-adrenoceptor responsiveness in aortic barodenervated rats

This experiment investigated whether aortic barodenervation alters the functional activity of vasoactive substances, namely nitric oxide and prostaglandins, involved in the endothelium-mediated control of vascular tone. Cumulative concentration–contractile response curves of phenylephrine (3×10^{-8}–1×10^{-4} M) were constructed in rings with intact endothelium obtained from aortic barodenervated and sham-operated rats before and after incubation with indomethacin (cyclooxygenase inhibitor, 1×10^{-5} M) or N^G-nitro-L-arginine (nitric oxide synthase inhibitor, 3×10^{-5} M). After a control concentration–response curve, a wash period of 60 min was allowed. The tissues were then incubated with indomethacin (60 min) or N^G-nitro-L-arginine (30 min) before re-evaluation of phenylephrine-evoked responses.

2.4.4. Effect of aortic barodenervation on hemodynamic responses to N^G-nitro-L-arginine in conscious rats

Whether aortic barodenervation alters nitric oxide activity in vivo was investigated in this experiment. Hemodynamic responses evoked by inhibition of nitric oxide synthase (by N^G-nitro-L-arginine) in conscious freely moving aortic barodenervated and sham-operated rats were determined 48 h after surgery. Dose–response curves of increases in blood pressure and decreases in heart rate to cumulative i.v. doses of N^G-nitro-L-arginine (1, 2, 4 and 8 mg/kg, at 10 min intervals) were established in the two groups of rats.

2.5. Drugs

Phenylephrine hydrochloride (Sigma Chemical, USA), acetylcholine hydrochloride (BDH, England), N^G-nitro-L-arginine (RBI, USA), indomethacin sodium (lyophilized, EPICO Pharmaceutical, Egypt), thiopental (Triopental, Biochemie, Austria), povidone–iodine solution (Betadine, Nile Pharmaceutical, Egypt) and Penicid (Cid Pharmaceutical, Egypt) were purchased from commercial vendors. Drugs were prepared in saline and stored refrigerated.

2.6. Data analysis

Values are presented as mean ± S.E.M. Mean arterial pressure was calculated as diastolic pressure + one third pulse pressure (systolic–diastolic pressures). The baroreflex sensitivity tested with phenylephrine was determined by calculation of the ratio Δheart rate/Δmean arterial pressure (El-Mas and Abdel-Rahman, 1992, 1993). The increases in muscle tension of aortic rings evoked by cumulative addition of phenylephrine were calculated in terms of mg tension/mg tissue. Changes in α_1-adrenoceptor responsiveness was evaluated by determining the percentage changes in the maximal contraction (E_{max}) to phenylephrine slope of the curve derived from regression analysis of the linear portions (approximately 15–85%) of the concentration–response curves of phenylephrine for individual tissues (Hamed et al., 1983; El-Mas et al., 1997). Analysis of variance (ANOVA) followed by a

Newman–Keuls post-hoc analysis was used for multiple comparisons among means. Simple contrasts were made with *t*-test. Probability levels less than 0.05 were considered significant.

3. Results

3.1. Baseline hemodynamic data

Denervation of aortic baroreceptors resulted in immediate and statistically significant ($P < 0.05$) increases in mean arterial pressure (from 125 ± 2 to 145 ± 4 mmHg) and heart rate (from 391 ± 6 to 420 ± 6 beats/min) in anesthetized rats. Sham operation, on the other hand, had no effect on either variable (Fig. 1). Intravenous administration of a test dose of phenylephrine (8 μg/kg) before aortic barodenervation and sham operation and then 5 min later elicited increases in mean arterial pressure associated with decreases in heart rate (data not shown). The baroreflex sensitivity (Δheart rate/Δmean arterial pressure) measured 5 min after aortic barodenervation was significantly ($P < 0.05$) suppressed, compared with predenervation values (0.59 ± 0.02 vs. 1.05 ± 0.02 beats/min per mmHg). The baroreflex sensitivity tested by phenylephrine was not affected by sham operation (0.98 ± 0.05 vs. 1.10 ± 0.03 beats/min per mmHg). The mean arterial pressure and heart rate of conscious freely moving aortic barodenervated rats subsided to sham-operated levels at 48 h (Fig. 1) whereas the baroreflex sensitivity remained attenuated (0.89 ± 0.06 vs. 1.89 ± 0.03 beats/min per mmHg).

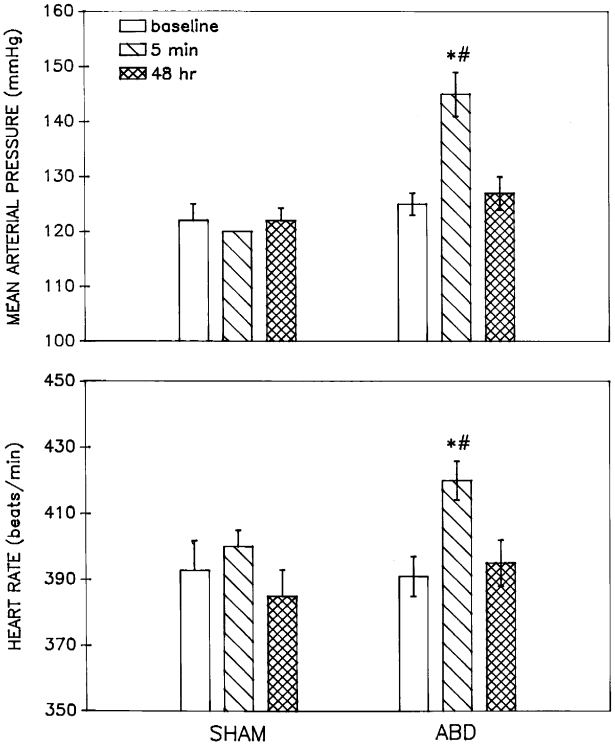

Fig. 1. The immediate (5 min, during thiopental anesthesia) and short-term (48 h, conscious rats) effects of aortic baroreceptor denervation (ABD) or sham operation on mean arterial pressure and heart rate. Values are means ± S.E.M. of observations obtained from rats of all groups used throughout the study. * and #$P < 0.05$ vs. pre-ABD and post-sham values, respectively.

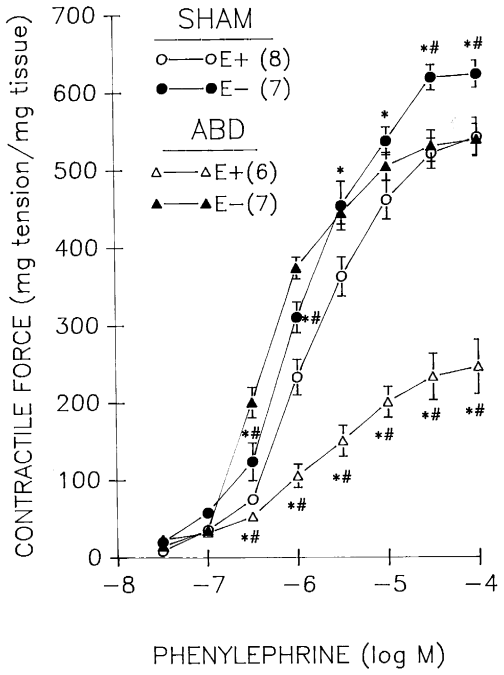

Fig. 2. Contractile responses evoked by cumulative addition of phenylephrine to aortic rings with intact (E+) or denuded (E−) endothelium obtained from aortic baroreceptor denervated (ABD) and sham-operated rats 48 h after surgery. Values are means ± S.E.M. and number of observations in each group is shown in parentheses. * and #$P < 0.05$ vs. sham E+ and ABD E− values, respectively.

3.2. Effect of endothelium removal on α_1-adrenoceptor responsiveness

Changes in α_1-adrenoceptor responsiveness in aortic rings with intact or denuded endothelium obtained from aortic barodenervated and sham-operated rats 48 h after surgery are shown in Fig. 2. Cumulative addition of phenylephrine (3×10^{-8}–1×10^{-4} M) resulted in concentration-related increases in the contractile force of aortic rings of both groups of rats (Fig. 2). In rings with intact endothelium, phenylephrine elicited significantly ($P < 0.05$) smaller contractile responses in rings from aortic barodenervated compared with sham-operated rats (Fig. 2). The slopes of the regression lines (86 ± 7 vs. 225 ± 15 mg tension/mg tissue/log molar concentration) and maximum contraction (245 ± 35 vs. 540 ± 25 mg tension/mg tissue) in response to phenylephrine were significantly ($P < 0.05$) smaller in rings from aortic barodenervated compared with sham-operated rats. Endothelium removal significantly ($P < 0.05$) augmented the contractile responses to phenylephrine and caused marked upward shift

Table 1
Maximum contractions (E_{max}, mg tension/mg tissue) and slopes (mg tension/mg tissue/log molar concentration) of the regression lines of the concentration–contractile response curves to phenylephrine to aortic rings with intact (E+) or denuded (E−) endothelium obtained from aortic barodenervated and sham-operated rats 48 h after surgery

Group	*n*	E_{max}	Slope
Sham-operated			
E+	8	540 ± 35	225 ± 15
E−	7	619 ± 17 [a]	260 ± 7 [a]
Aortic barodenervated			
E+	6	245 ± 35 [b]	86 ± 7 [b]
E−	7	537 ± 20 [a]	234 ± 14 [a]

Values are means ± S.E.M.
[a] and [b] $P < 0.05$ vs. E+ and sham-operated values, respectively.

in the concentration–response curves. This effect of endothelium removal was more evident in aortic rings obtained from denervated rats (Fig. 2). As shown in Table 1, the increase in E_{max} of phenylephrine in rings from aortic barodenervated rats after endothelium removal amounted approximately to 120% (from 245 ± 35 to 537 ± 20 mg tension/mg tissue) compared with only 15% in rings from sham-operated rats (from 540 ± 25 to 619 ± 17 mg tension/mg tissue). Similarly, endothelium removal elicited substantially greater increases in the slope of the regression line in rings from aortic barodenervated compared with sham-operated rats (170% vs. 15%) (Table 1). Except for a slightly higher E_{max} in rings from sham-operated rats, the contractile responses to phenylephrine were similar in rings from aortic barodenervated and sham-operated rats after endothelium removal (Fig. 2). Further, the slopes of the regression lines in endothelium-denuded rings from aortic barodenervated and sham-operated rats were not statistically different (Table 1).

3.3. Effect of indomethacin and N^G-nitro-L-arginine on phenylephrine-evoked contractile responses

The effects of inhibition of cyclooxygenase (by indomethacin) or nitric oxide synthase (by N^G-nitro-L-arginine) activity on α_1-adrenoceptor responsiveness in aortic rings with intact endothelium obtained from aortic barodenervated and sham-operated rats are illustrated in Figs. 3 and 4, respectively. Preincubation with indomethacin (1×10^{-5} M) for 1 h had no effect on basal vascular tone of the aorta and caused slight but insignificant increases in the contractile responses to phenylephrine in rings of both aortic barodenervated and sham-operated rats (Fig. 3). The percentage increases in the E_{max} and slopes of the regression lines were similar in rings obtained from both groups of rats (Table 2). In contrast, preincubation of the tissues with N^G-nitro-L-arginine (3×10^{-5} M) increased aortic basal tone to levels that were significantly ($P < 0.05$) higher in rings from aortic barodenervated compared with sham-op-

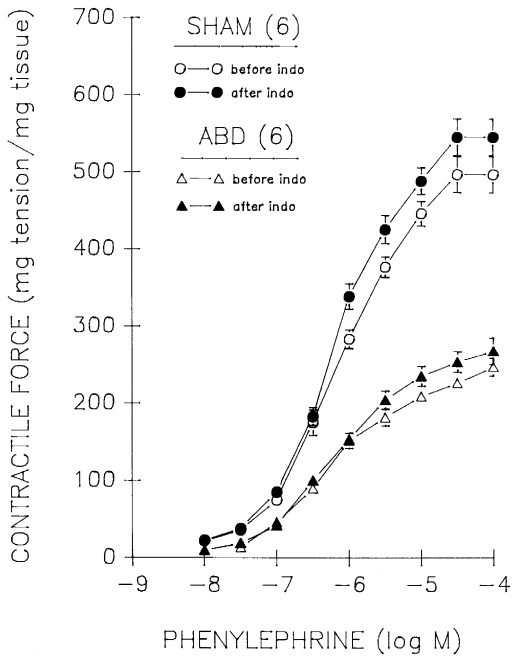

Fig. 3. Effect of indomethacin (indo, 1×10^{-5} M) on the contractile responses evoked by cumulative addition of phenylephrine to aortic rings obtained from aortic baroreceptor denervated (ABD) and sham-operated rats 48 h after surgery. Values are means ± S.E.M. and the number of observations in each group is shown in parentheses.

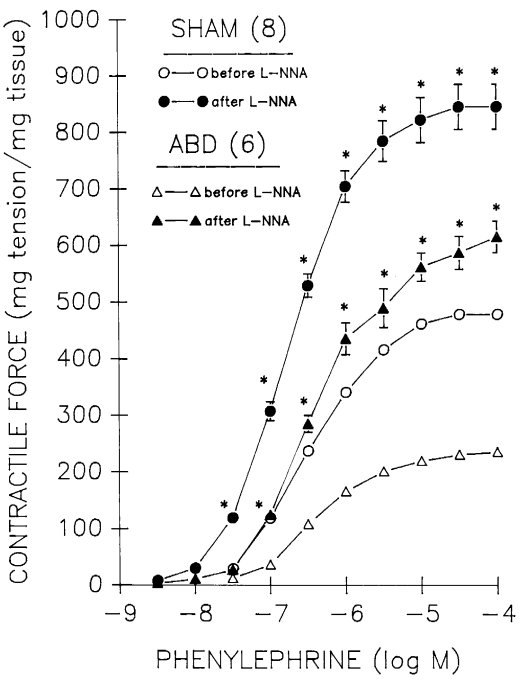

Fig. 4. Effect of N^G-nitro-L-arginine (L-NNA, 3×10^{-5} M) on the contractile responses evoked by cumulative addition of phenylephrine to aortic rings obtained from aortic baroreceptor denervated (ABD) and sham-operated rats 48 h after surgery. Values are means ± S.E.M. and number of observations in each group is shown in parentheses. * $P < 0.05$ vs. respective before L-NNA values.

erated rats (90 ± 7 vs. 59 ± 5 mg tension/mg tissue). Moreover, N^G-nitro-L-arginine elicited significant ($P < 0.05$) increases in phenylephrine-evoked contractions in rings from both aortic barodenervated and sham-operated rats (Fig. 4). The absolute increases in E_{max} (378 ± 15 vs. 366 ± 19 mg tissue/mg tissue) and slopes of the regression lines (138 ± 13 vs. 142 ± 9 mg tension/mg tissue/log molar concentration) were similar in rings obtained from aortic barodenervated and sham-operated rats. However, the percentage increases in E_{max} ($161.2 \pm 8.2\%$ vs. $76.7 \pm 6.1\%$) and slopes of the regression lines ($136.1 \pm 22\%$ vs. $73.0 \pm 8.6\%$ mg tension/mg tissue/log molar concentration) were significantly ($P < 0.05$) greater in rings from aortic barodenervated compared with sham-operated rats (Table 2) suggesting a higher capacity of N^G-nitro-L-arginine to potentiate α_1-adrenoceptor-mediated responses in rings from aortic barodenervated rats.

3.4. Effect of aortic barodenervation on hemodynamic responses to N^G-nitro-L-arginine in conscious rats

This experiment investigated whether short-term aortic barodenervation alters the modulatory effect of endothelial nitric oxide on vascular tone in vivo. The baseline mean arterial pressure (125 ± 3 vs. 122 ± 2 mmHg) and heart rate (389 ± 7 vs. 381 ± 8 beats/min) were similar in conscious freely moving aortic barodenervated and sham-operated rats. Cumulative i.v. administration of bolus doses (1, 2, 4, 8 mg/kg; at 10 min intervals) of N^G-nitro-L-arginine to conscious freely moving aortic barodenervated and sham-operated rats 48 h after surgery elicited dose-related increases in mean arterial pressure associated with reciprocal changes in heart rate (Fig. 5). Increases in mean arterial pressure evoked by individual doses of N^G-nitro-L-arginine were gradual and reached their maxima within 7 to 10 min. The pressor responses to N^G-nitro-L-arginine were significantly ($P < 0.05$) greater in aortic barodenervated compared with sham-operated rats whereas the bradycardic responses were similar in the two groups of rats (Fig. 5). The percentage increases in mean arterial pressure evoked by N^G-nitro-L-arginine (1, 2, 4, 8 mg/kg) were slightly but significantly ($P < 0.05$) higher in barodenervated rats (13.4 ± 1.1, 20.3 ± 1.9, 31.6 ± 2.2 and $38.2 \pm 1.3\%$, respectively) compared with sham-operated values (7.9 ± 1.1, 13.8 ± 2.1, 24.3 ± 1.9, $34.8 \pm 1.1\%$, respectively).

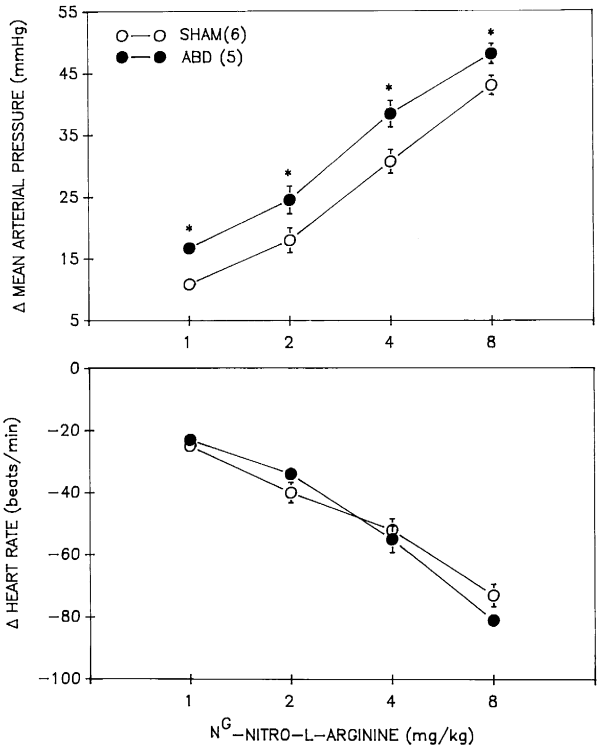

Fig. 5. Effects of cumulative i.v. injections of N^G-nitro-L-arginine on mean arterial pressure and heart rate in conscious freely moving aortic baroreceptor denervated (ABD) and sham-operated rats 48 h after surgery. Note that decreases in heart rate are similar in both groups of rats while rises in mean arterial pressure are significantly ($^*P < 0.05$) greater in ABD compared with sham-operated rats. Values are means ± S.E.M. and the number of observations in each group is shown in parentheses.

Table 2
Percentage increases, elicited by indomethacin and N^G-nitro-L-arginine, in E_{max} and slope of the regression line of the concentration–contractile response curve to phenylephrine to aortic rings of aortic barodenervated (ABD) and sham-operated rats 48 h after surgery

Group	*n*	E_{max} (%increase)	Slope (%increase)
Indomethacin			
Sham	6	10.2 ± 3.6	11.5 ± 5.2
ABD	6	9.9 ± 3.6	15.4 ± 6.6
N^G-nitro-L-arginine			
Sham	8	76.7 ± 6.1	73.0 ± 8.6
ABD	6	161.2 ± 8.2 [a]	136.1 ± 22.0 [a]

Values are means ± S.E.M.
[a] $P < 0.05$ vs. respective sham-operated values.

4. Discussion

The findings of the present study suggest a major role for vascular endothelium in the reduced responsiveness of α_1-adrenoceptors after aortic barodenervation. This view is supported by the observation that endothelium removal abolished the aortic barodenervation-evoked reduction in α_1-adrenoceptor responsiveness in aortic smooth muscle and restored phenylephrine-mediated contractions to near sham-operated values. This effect of the endothelium seems to involve, at least in part, enhancement of the vasorelaxant activity of endothelial nitric oxide in rings from aortic barodenervated rats as suggested by the findings that inhibition of nitric oxide synthase by N^G-nitro-L-arginine elicited: (i) remarkably greater increases in basal vascular tone and α_1-adrenoceptor responsiveness in aortic rings

obtained from aortic barodenervated compared with sham-operated rats and (ii) significantly greater pressor responses in conscious freely moving aortic barodenervated rats. Finally, the lack of an effect of indomethacin on contractile responses to phenylephrine eliminates a possible role for endothelial cyclooxygenase products in the reduced sensitivity of α_1-adrenoceptors in aortas of aortic barodenervated rats.

Our previous study was the first to demonstrate that short-term aortic barodenervation reduces α_1-adrenoceptor responsiveness in rat aortic smooth muscle (El-Mas et al., 1997). This reduction in aortic responsiveness to α_1-adrenoceptor activation has been interpreted to suggest a down-regulatory response to the elevated sympathetic activity (El-Mas et al., 1997) known to exist after elimination of aortic baroafferents (Abdel-Rahman, 1992; El-Mas et al., 1994a,b, 1997). However, the issue whether the reduction in α_1-adrenoceptor sensitivity involves aortic barodenervation-mediated changes in the functional activity of vascular endothelium has not been investigated. This may be important particularly in view of the increasing evidence that highlights the critical role of the endothelium in the modulation of α_1-adrenoceptor-mediated smooth muscle contraction (Hiremath et al., 1991; Hu et al., 1992). The present study addressed two questions pertinent to the possible role of vascular endothelium in aortic barodenervation-evoked reduction in α_1-adrenoceptor responsiveness in aortic smooth muscle and whether it involves an altered function of essential endothelial vasoactive substance such as nitric oxide and products of cyclooxygenase pathway; substances that play crucial roles in the regulation of vascular tone (Moncada and Vane, 1979; Vanhoutte et al., 1986; Nagao et al., 1992).

The present finding that α_1-adrenoceptor activation elicited lesser contractions in aortic rings with intact endothelium obtained from aortic barodenervated, compared with sham-operated, rats supports our hypothesis that short-term aortic barodenervation reduced α_1-responsiveness in vascular smooth muscle (El-Mas et al., 1997). Removal of the endothelium restored the α_1-adrenoceptor responsiveness in rings from aortic barodenervated rats to near sham-operated values as indicated by the similar contractile responses and slopes of the regression lines of the concentration–response curves of phenylephrine in endothelium-denuded rings of both groups. The presence of a slightly but significantly smaller E_{max} value for phenylephrine in aortic barodenervated, compared with sham-operated, aortic endothelium-denuded rings may presumably reflect changes at the level of the vascular smooth muscle (El-Mas et al., 1997). Nevertheless, these changes are relatively smaller compared to differences in α_1-adrenoceptor responses demonstrated in the presence of intact endothelium. The present results, therefore, highlight the importance of vascular endothelium in the blunted α_1-adrenoceptor responsiveness in aortas of aortic barodenervated rats. A similar role for vascular endothelium in the reduced α_1-adrenoceptor reactivity has also been documented in other rat models that expressed prolonged α_1-activation such as pheochromocytoma-bearing rats (Hiremath et al., 1991) and after in vitro exposure of vascular smooth muscle to α_1-adrenoceptor agonists (Hiremath et al., 1991; Hu et al., 1992).

The current study presents three findings that may suggest a role for nitric oxide, the major relaxing factor derived from the endothelium (Nagao et al., 1992), in the endothelium-dependent reduction of α_1-adrenoceptor responsiveness in aortic barodenervated aortas. First, inhibition of nitric oxide synthase activity by N^G-nitro-L-arginine produced significantly greater increases in basal vascular tone of aortic rings obtained from aortic barodenervated compared with sham-operated rats suggesting an increased basal activity of nitric oxide in denervated rings. Second, N^G-nitro-L-arginine elicited substantially greater increases in α_1-adrenoceptor responsiveness in rings obtained from aortic barodenervated compared with sham-operated rats. The percentage increases in the slope of the concentration–contractile response curve to phenylephrine and in E_{max} values after N^G-nitro-L-arginine pretreatment in aortic barodenervated rings were two-fold the corresponding increases in sham-operated rings. Third, intravenous administration of N^G-nitro-L-arginine to conscious freely moving rats produced dose-related pressor responses that were significantly greater in rats with surgically eliminated aortic baroafferents. These data are consistent with the established role of nitric oxide in the regulation of vascular tone (Vargas et al., 1990; Hiremath et al., 1991; Du et al., 1992) and suggest that an increased activity of endothelial nitric oxide may be involved in the diminished α_1-adrenoceptor responsiveness in aortas from aortic barodenervated rats. The demonstration, however, that phenylephrine-evoked contractions in N^G-nitro-L-arginine-pretreated tissues remained remarkably higher in rings from sham-operated compared with ABD rats infers that other endothelial factors, e.g., hyperpolarizing factor or endothelin, may have contributed to the reduced α_1-adrenoceptor responsiveness in denervated preparation. It is notable also that pressor responses to N^G-substituted L-arginine may involve inhibition of nitric oxide synthesis at non-endothelial sites such as the central nervous system (Togashi et al., 1992) and ganglia (Toda et al., 1993). The possibility, therefore, should be considered that alterations in nitric oxide synthase activity at these sites may have contributed to the reduced responses to α_1-adrenoceptor activation after aortic barodenervation. It is notable that in addition to reducing the constrictor responses to activation of α_1-adrenoceptors, the enhanced endothelial nitric oxide activity following aortic barodenervation may likely diminish responses to other vasoconstrictor agents as well. Further studies, however, are needed to ascertain this point.

It is not clear from the present findings whether an enhanced release and/or responsiveness to nitric oxide is responsible for the reduced responsiveness of α_1-adrenoc-

eptors in aortic smooth muscle of aortic barodenervated rats. A considerable body of evidence suggests that endogenous nitric oxide release is dependent upon the degree of vascular tone. This view is supported by the report of Vargas et al. (1990) that in vivo release of nitric oxide is markedly reduced by vasodilation and enhanced with increased vascular tone. Further, Kelm et al. (1992, 1995) found that basal nitric oxide formation and bradykinin-induced release of nitric oxide are higher in coronary circulation of spontaneously hypertensive, compared with Sprague–Dawley, rats. These investigators (Kelm et al., 1992, 1995) suggested that such an increase in the responses of spontaneously hypertensive rats may reflect a compensatory response to the elevated blood pressure and vascular resistance in these rats. Given that aortic barodenervated rats, even though normotensive, exhibit elevated sympathetic activity and peripheral vascular resistance (Abdel-Rahman, 1992; Sannajust et al., 1992; El-Mas et al., 1994a,b), it is conceivable to assume that prolonged contraction of vascular smooth muscle of denervated rats may have triggered an adaptive increase in nitric oxide release that served to attenuate vascular contractility. This increase in nitric oxide release may be necessary to maintain regional blood flow in the face of a sympathetically-mediated vasoconstriction. The notion should also be considered that α_1-adrenoceptors have been identified in vascular endothelial cells (Bevan and Duckles, 1975) whose prolonged activation by catecholamines may lead to changes in the release of nitric oxide (Hiremath et al., 1991).

Alternatively, the enhanced functional activity of the endothelium–nitric oxide system in aortic smooth muscle of barodenervated rats may not involve an increase in nitric oxide release but rather an increased responsiveness of smooth muscle to the relaxing effect of nitric oxide. Nitric oxide is known to relax vascular smooth muscle through a mechanism which involves stimulation of soluble guanylate cyclase and elevation of intracellular cyclic guanosine monophosphate (Moncada et al., 1991). Enhancement of the nitric oxide-generated cyclic guanosine monophosphate, if exists, may lead to impaired responsiveness of smooth muscle to vasoconstricting substances. This view is supported by the observation that reduced responsiveness of α_1-adrenoceptors in rat aortas after prolonged exposure to norepinephrine is associated with increases in guanylate cyclase activity and relaxant responses to sodium nitroprusside (Hu et al., 1992). Our own finding (El-Mas et al., 1997) that the depressor responses to nitroprusside are enhanced in aortic barodenervated rats may support the presence of an augmented guanylate cyclase activity after barodenervation. Sodium nitroprusside is a nitrovasodilator known to react with hemoprotein and sulphydryl-components in vascular smooth muscle cells to release nitric oxide which subsequently activates guanylate cyclase and causes vasodilation (Bates et al., 1991).

The finding in the present study that systemic administration of N^G-nitro-L-arginine elicited similar decreases in heart rate in aortic barodenervated and sham-operated rats deserves a comment. It has been shown that the bradycardia evoked by nitric oxide synthase inhibition is a reflex response to the elevation in blood pressure (Du et al., 1991, 1992). Since the results of this study and previous studies (El-Mas and Abdel-Rahman, 1992; El-Mas et al., 1994a, 1997) demonstrated a reduction in reflex bradycardia after aortic barodenervation, it would be anticipated that smaller decreases in heart rate and probably greater pressor responses to N^G-nitro-L-arginine should arise in denervated rats. The possibility should, however, be considered that inhibition of nitric oxide synthase activity exhibits direct effects on central and cardiac tissues that may have contributed to the lack of differences in heart rate responses to N^G-nitro-L-arginine in aortic barodenervated and sham-operated rats. For example, N^G-nitro-L-arginine has been shown to enhance baroreflex control of heart rate through a direct effect on medullary areas involved in baroreflex control (Liu et al., 1996; Tseng et al., 1996; Vasquez et al., 1994). Further, inhibition of nitric oxide synthase alters the physiological response of ventricular myocytes to sympathetic and parasympathetic activation (Balligand et al., 1993). These direct effects of nitric oxide synthase inhibition on central and peripheral tissues may have prevented the development of a lesser reflex bradycardia to N^G-nitro-L-arginine after aortic barodenervation.

In conclusion, findings of the present study demonstrate that vascular endothelium plays a major role in the reduction of α_1-adrenoceptor-mediated contractions of aortic smooth muscle subsequent to selective denervation of aortic baroreceptors. This effect of the endothelium appears to involve, at least in part, enhancement of the vasorelaxant activity of endothelial nitric oxide after barodenervation. The enhanced nitric oxide activity may result from an increase in nitric oxide release in compensation for the elevated vascular resistance in aortic barodenervated rats and/or an increase in the sensitivity of vascular smooth muscle to nitric oxide.

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