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Short-term aortic barodenervation diminishes α_1-adrenoceptor reactivity in rat aortic smooth muscle

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Abstract

Our previous studies have shown that aortic baroreceptor denervation elicits acute increases in blood pressure and significant elevations of sympathetic activity and peripheral vascular resistance. This study investigated the short-term (3 and 48 h) effect of aortic barodenervation and associated sympathetic hyperactivity on the functional activity of α_1-adrenoceptors in rat aortic smooth muscle. Compared with sham operation, aortic barodenervation caused acute rises in blood pressure and heart rate and reductions in baroreflex sensitivity. Blood pressure and heart rate remained elevated when measured in conscious aortic barodenervated rats 3 h after surgery but subsided to sham-operated levels at 48 h; the baroreflex sensitivity, however, remained attenuated. Hexamethonium (0.5–4 mg/kg, i.v.) elicited significantly ($P < 0.05$) greater depressor responses in conscious aortic barodenervated rats than in sham-operated rats at both 3 and 48 h, suggesting a higher sympathetic activity in denervated rats. Exposure of aortic rings from aortic barodenervated and sham-operated rats to cumulative addition of phenylephrine (α_1-adrenoceptor agonist, 3×10^{-8}–1×10^{-4} M) resulted in concentration-related contractile responses that were similar in the two groups of rats at 3 h in contrast to significantly ($P < 0.05$) smaller contractions in rings from denervated rats at 48 h. The maximum contraction developed (E_{max}) at 48 h showed approximately 50% reduction in rings from aortic barodenervated compared with sham-operated rats (239 ± 16 vs. 558 ± 15 mg tension/mg tissue). The pA_2 value for prazosin (α_1-adrenoceptor antagonist) was not altered by aortic barodenervation at 3 h but showed significant ($P < 0.05$) increases, compared with sham-operated values, at 48 h. It is concluded that short-term aortic barodenervation results in an elevation of sympathetic activity that coincides with reduced responsiveness of aortic smooth muscle to α_1-adrenoceptor activation. The aortic barodenervation-induced α_1-adrenoceptor desensitization is not a result of decreased receptor affinity but may involve an alteration of receptor density or in the post-receptor activation events.

Keywords: Aortic barodenervation; α_1-Adrenoceptor; Aorta; Sympathetic activity; Receptor desensitization

1. Introduction

The arterial baroreflex arc plays an important role in the regulation of arterial blood pressure (Kumada et al., 1990; Chalmers and Pilowsky, 1991). Impaired baroreflex activity is known to exist in hypertensive animals (Takeda et al., 1989) and humans (Goldstein, 1983) and after partial (El-Mas and Abdel-Rahman, 1992; El-Mas et al., 1994b) or complete (Alexander and Morris, 1986) denervation of baroreceptors. Reported findings including our own have shown that transection of the aortic baroafferent nerves elicits an immediate increase in blood pressure followed within 48 h by full recovery to sham-operated levels (Osborn and England, 1990; El-Mas and Abdel-Rahman, 1992, 1995) or the blood pressure remains slightly elevated (Fink et al., 1980, Patel et al., 1981). The mechanism of pressure normalization in barodenervated rats has been attributed to the assumption that the initial sympathetic hyperactivity induced by barodenervation is not chronically maintained (Patel et al., 1981; Osborn and England, 1990). An alternative mechanism for pressure normalization may involve the occurrence of pressure diuresis and subsequent plasma volume contraction in response to the barodenervation-evoked hypertension (Guyton et al., 1974; Cowley, 1992; El-Mas et al., 1993). In a previous study (El-Mas et al., 1994a), we have shown that restoration of normal blood pressure levels after aortic baroreceptor denervation is due to a reduction in cardiac output which acts to offset increases in sympathetic activity and peripheral vascular resistance.

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It has been reported that noradrenergic pathways in the brain and spinal cord contribute to the central integration and processing of baroreflexes. This is supported by the observation that the nucleus tractus solitarii, the primary central synapse of baroafferents (Cowley, 1992), and the rostral ventrolateral medulla, the vasopressor area of the brain stem, have high catecholamine levels (Fuxe, 1965) and abundant catecholaminergic nerve terminals (Korner, 1971). Moreover, Haeusler (1973) reported that the baroreflex arc contains α-adrenoceptors and their activation facilitates baroreflexes. Evidence for alterations in sympathetic activity at central and peripheral sites after baroreceptor denervation has also been demonstrated. For instance, surgical elimination of baroreflexes has been shown to elevate indices of catecholaminergic activity in the brain (Alexander and Morris, 1988) and peripheral organs (Patel et al., 1981). Further, ganglionic (Abdel-Rahman, 1992) or total autonomic blockade (Sannajust et al., 1992) resulted in greater decreases in blood pressure of aortic barodenervated compared with sham-operated rats. Our recent study (El-Mas et al., 1994a) showed that plasma norepinephrine levels and total peripheral vascular resistance were substantially increased as early as 3 h after aortic barodenervation and remained so when measured 48 h later.

Interruption of baroreceptor afferent activity has also been shown to modify the binding activity of α-adrenoceptors in medullary sites that play a critical role in the control of cardiovascular function. MacLean et al. (1990) have demonstrated an increase in the affinity of α_1-adrenoceptors in the nucleus tractus solitarii of sinoaortic denervated rats. In a recent autoradiographic study, we have reported an upregulation of α_2-adrenoceptors in the rat brain stem subsequent to aortic barodenervation (submitted for publication). These changes seem to be correlated with the elevated sympathetic neural activity commonly seen in these rats (Abdel-Rahman, 1992; El-Mas et al., 1994a,b). There has been no study that evaluated the consequence of surgical elimination (partial or complete) of baroreceptor afferents and associated elevations in sympathetic activity and peripheral vascular resistance (Sannajust et al., 1992; El-Mas et al., 1994a,b) on the functional activity of α-adrenoceptors in vascular smooth muscles.

The present study was, therefore, designed to investigate whether partial attenuation of baroreceptor afferent activity evoked by aortic barodenervation alters the functional responsiveness of isolated aortic smooth muscle to α_1-adrenoceptor activation. In vitro studies were conducted to evaluate α_1-adrenoceptor reactivity to phenylephrine (α_1-adrenoceptor agonist) in isolated thoracic aortas obtained from aortic barodenervated rats during the pressor phase of barodenervation (3 h) and when normotensive levels were restored (48 h). Prazosin, an α_1-adrenoceptor antagonist, was used to determine changes in receptor affinity by the dissociation constant method (Hamed et al., 1983). Depressor responses to hexamethonium in conscious freely moving aortic barodenervated and sham-operated rats were used as a measure of peripheral sympathetic activity (Abdel-Rahman, 1992). In all groups of rats, the time-related changes in blood pressure, heart rate and baroreflex sensitivity were monitored before aortic barodenervation or sham operation and then at 5 min (anesthetized state) and 3 and 48 h (conscious state) later.

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 the sham or aortic barodenervation 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; Fahim et al., 1994). Rats were killed by decapitation and thoracic aortas were removed, trimmed free of connective tissue and cut into ring segments 3 mm in length. Aortic rings 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; Fahim et al., 1994).

2.4. Experimental protocols

2.4.1. Time-course of hemodynamic effects of aortic barodenervation

The acute (5 min, anesthetized state) and short-term (3 and 48 h, conscious state) effects of aortic barodenervation and sham operation on mean arterial pressure and heart rate were investigated. The baroreflex sensitivity was measured at the same time intervals by i.v. 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 to allow for stabilization of blood pressure and heart rate. The acute hemodynamic measurements were made in all groups of aortic barodenervated and sham-operated rats used throughout the study. Each rat group (aortic barodenervated or sham-operated) was then allocated for short-term hemodynamic measurement and subsequent in vitro studies at either 3 or 48 h.

2.4.2. Effect of aortic barodenervation on sympathetic activity

Two groups of rats, one aortic barodenervated and one sham-operated ($n = 6–8$), were used in this experiment to investigate whether partial attenuation of baroreflex function evoked by aortic denervation alters peripheral sympathetic activity. This was achieved by measuring depressor responses to hexamethonium, a ganglion blocker, in conscious freely moving rats at 3 and 48 h after surgery. After blood pressure stabilization, cumulative bolus i.v. doses of hexamethonium (0.5, 1, 2, and 4 mg/kg) were administered and peak changes in mean arterial pressure were calculated and compared in the two groups of rats. Each dose of hexamethonium was administered at the peak depressor response to previous dose.

2.4.3. Effect of aortic barodenervation on baroreflex-mediated control of heart rate

This experiment investigated the effect of 48 h aortic barodenervation on pressor and depressor responses to phenylephrine and nitroprusside, respectively, and the associated changes in heart rate. Two separate groups of rats (one aortic barodenervated and one sham-operated, $n = 7–9$) were used in this experiment. A series of bolus i.v. doses (1, 2, 4, 8, and 16 μg/kg) of phenylephrine or nitroprusside were administered randomly at 5-min intervals. Changes in mean arterial pressure and heart rate were measured and compared in aortic barodenervated and sham-operated rat groups.

2.4.4. Effect of aortic barodenervation on α_1-adrenoceptor-mediated contractions of rat aortic rings

This experiment investigated whether short-term aortic barodenervation alters the vascular responsiveness of aortic smooth muscle preparations to α_1-adrenoceptor activation. Contractile responses to α_1-adrenoceptor activation (by phenylephrine) were evaluated in aortic rings obtained from aortic barodenervated and sham-operated rats 3 h (during the pressor phase of aortic barodenervation) and 48 h (when aortic barodenervated rats were normotensive) after surgery. After equilibration, concentration-contractile response curves of phenylephrine ($3 \times 10^{-8}–1 \times 10^{-4}$ M) were established by the method of stepwise cumulative addition (Hamed et al., 1983). The concentration of phenylephrine was increased by half-log units with each addition. Each new addition was made only after the response to the previous concentration had attained a steady state. To investigate changes in α_1-adrenoceptor affinity, another phenylephrine concentration-response curve was constructed in the same rings after the addition of prazosin (α_1-adrenoceptor antagonist, 5×10^{-9} M) (Hamed et al., 1983). A wash period of 60 min was allowed after the first curve to help the muscle relax to baseline tension. Prazosin was then added and the second curve was established after an additional 30 min. The pA_2

value of prazosin was determined by the dissociation constant method as described later. In a preliminary control experiment, two consecutive phenylephrine concentration-response curves were constructed in five aortic rings, in the absence of prazosin, and exhibited similar contractile responses, thus eliminating the role of time as a factor that may alter the responses (data not shown). 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.

2.5. Drugs

Phenylephrine hydrochloride, prazosin hydrochloride, sodium nitroprusside, hexamethonium bromide (Sigma, St. Louis, MO, USA), thiopental (Triopental, Biochemie, Vienna, Austria), povidone-iodine solution (Betadine, Nile Pharmaceutical, Egypt) and Penicid (Cid Pharmaceutical, Egypt) were purchased from commercial vendors. Prazosin was prepared by levigation with a drop of glycerol and then a 5% dextrose solution was added slowly under vigorous stirring. Other 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 measured by calculation of the ratio Δheart rate/Δmean arterial pressure (El-Mas and Abdel-Rahman, 1992). The increases in muscle tension (mg tension/mg tissue) of aortic rings evoked by cumulative addition of phenylephrine were calculated. The contractile force evoked by a particular concentration of phenylephrine was expressed as a percentage of the maximum response (E_{max}) to phenylephrine (Hiremath et al., 1991). The concentrations of phenylephrine producing 50% of the maximal contraction (EC_{50}) were determined by regression analysis of the linear portions (approximately 15–85%) of the concentration-response curves for individual tissues (Hamed et al., 1983). The pA_2 value of prazosin at the α_1-adrenoceptors was determined by the dissociation constant method described by Hamed et al. (1983). The agonist dose ratios were calculated at the EC_{50} and used for calculation of the dissociation constant (K_B) according to the equation: K_B = [antagonist]/[agonist dose ratio − 1]. The pA_2 value was then calculated from the formula: $pA_2 = -\log K_B$. 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. Time-course of hemodynamic effects of aortic baroderervation

Section of the aortic depressor nerves while the rats were anesthetized with thiopental resulted in an immediate and statistically significant ($P < 0.05$) increase in mean arterial pressure (from 122 ± 2 to 143 ± 2 mmHg) and heart rate (from 384 ± 8 to 412 ± 10 beats/min). These increases in mean arterial pressure and heart rate remained until the wounds were clipped (Fig. 1). In sham-operated rats, no significant change in either variable was demon-

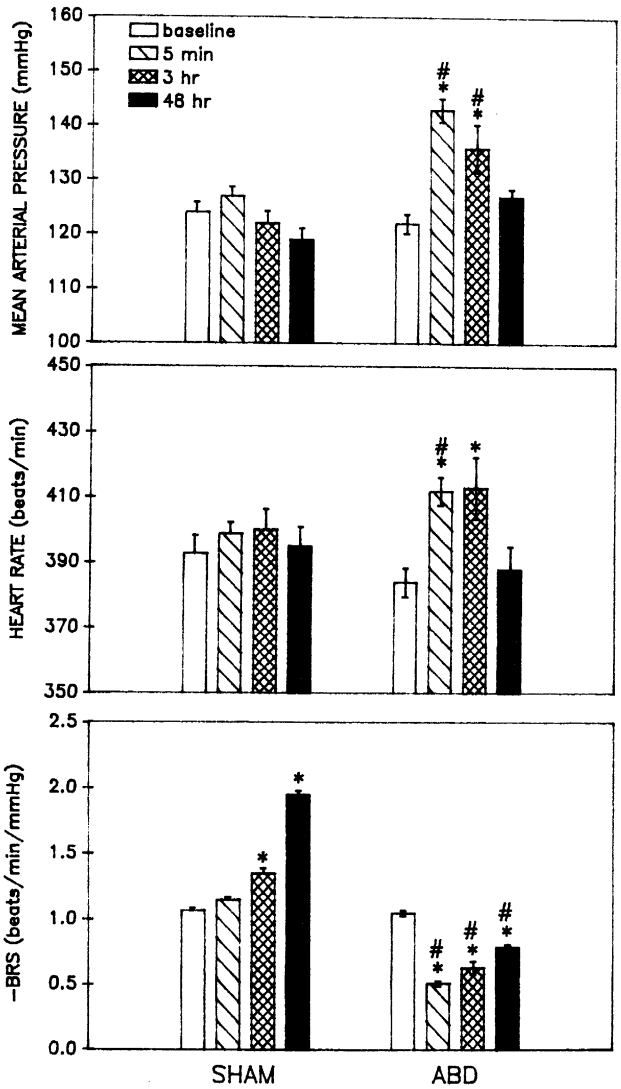

Fig. 1. The immediate (5 min, during thiopental anesthesia) and short-term (3 and 48 h, conscious rats) effects of aortic baroreceptor denervation (ABD) or sham operation on mean arterial pressure (top panel), heart rate (middle panel) and baroreflex sensitivity (BRS, lower panel). Values are means ± S.E.M. of observations obtained from rats of all groups used throughout the study. $^{*,\#}P < 0.05$ vs. pre-ABD (or pre-sham) and post-sham values, respectively.

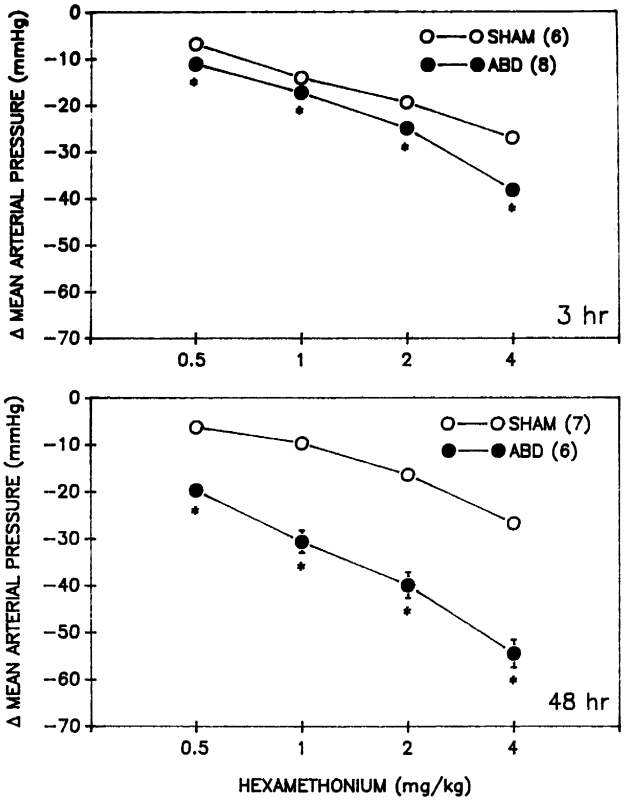

Fig. 2. Effects of cumulative i.v. injections of hexamethonium on mean arterial pressure in conscious aortic baroreceptor-denervated (ABD) and sham-operated rats 3 h (top panel) and 48 h (lower panel) after surgery. Values are means ± S.E.M. and the number of rats in each group is shown in parentheses. * $P < 0.05$ vs. respective sham values.

strated (Fig. 1). The mean arterial pressure and heart rate of conscious aortic barodenervated rats remained significantly ($P < 0.05$) elevated when measured 3 h after surgery and subsided to near predenervation levels at 48 h (Fig. 1). Intravenous administration of a test dose of phenylephrine (8 μg/kg) before aortic barodenervation and sham operation and then 5 min and 3 and 48 h 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 with phenylephrine 5 min after aortic barodenervation was significantly ($P < 0.05$) suppressed, compared to predenervation and respective sham-operated values, and remained so at 3 and 48 h (Fig. 1). The baroreflex sensitivity was not affected by sham operation at 5 min in anesthetized rats and showed significant increases at 3 and 48 h in conscious rats (Fig. 1).

3.2. Effect of aortic barodenervation on sympathetic activity

Depressor responses to hexamethonium, as a measure of sympathetic activity, were determined in conscious freely moving aortic barodenervated and sham-operated rats. Intravenous administration of cumulative doses of hexamethonium (0.5, 1, 2 and 4 mg/kg) to aortic barodenervated and sham-operated rats 3 and 48 h after surgery produced dose-related decreases in mean arterial pressure that were significantly ($P < 0.05$) greater in aortic baro-

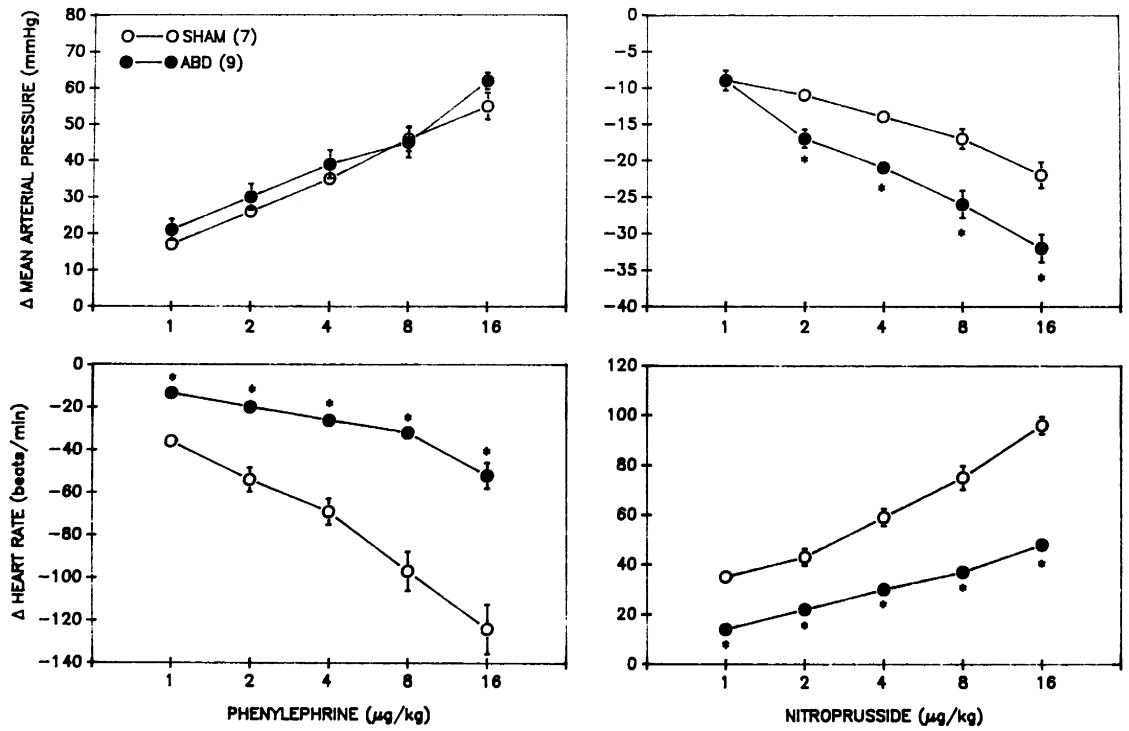

Fig. 3. Line graphs showing phenylephrine (right panels) and nitroprusside (left panels) evoked increases and decreases, respectively, in mean arterial pressure and associated changes in heart rate in conscious aortic baroreceptor-denervated (ABD) and sham-operated rats 48 h after surgery. Values are means ± S.E.M. and the number of rats in each group is shown in parentheses. * $P < 0.05$ vs. respective sham values.

denervated rats at both time intervals. The depressor response to a particular dose of hexamethonium started immediately after i.v. administration and reached its nadir within 1–2 min. As shown in Fig. 2, differences between the depressor responses to hexamethonium in aortic barodenervated and sham-operated rats were more evident at 48 h. Maximal depressor responses of 54 ± 3 and 27 ± 2 mmHg were obtained in aortic barodenervated and sham-operated rats, respectively, at 48 h.

3.3. Effect of aortic barodenervation on baroreflex-mediated control of heart rate

The effects of aortic barodenervation and sham operation on increases and decreases in mean arterial pressure evoked by phenylephrine and nitroprusside, respectively, and the associated changes in heart rate of conscious rats 48 h after surgery are shown in Fig. 3. In the two groups of rats, intravenous administration of bolus doses (1, 2, 4, 8 and 16 μg) of phenylephrine and nitroprusside at 5 min intervals elicited dose-related increases (in case of phenylephrine) and decreases (in case of nitroprusside) in mean arterial pressure associated with reciprocal changes in heart rate (Fig. 3). The pressor effects elicited by phenylephrine were similar in the two groups of rats whereas significantly ($P < 0.05$) greater depressor responses to nitroprusside were obtained in aortic barodenervated rats (Fig. 3). The baroreflex-mediated heart rate responses were greatly attenuated by denervation, as demonstrated by the significantly ($P < 0.05$) smaller decreases in heart rate in aortic barodenervated compared with sham-operated rats (Fig. 3).

3.4. Effect of aortic barodenervation on α_1-adrenoceptor-mediated contractions of rat aortic rings

Changes in α_1-adrenoceptor responsiveness in aortic rings obtained from aortic barodenervated and sham-operated rats 3 and 48 h after surgery are shown in Figs. 4 and 5. Cumulative addition of phenylephrine (3×10^{-8}–1×10^{-4} M) resulted in concentration-related increases in the contractile force of aortic rings from both groups of rats (Fig. 4). Aortic barodenervation had no effect on α_1-adrenoceptor responsiveness at 3 h as the contractile responses to phenylephrine in aortic rings obtained from aortic barodenervated and sham-operated rats were similar (Fig. 4). In contrast, phenylephrine elicited significantly ($P < 0.05$) smaller contractile responses in rings from aortic barodenervated rats compared with sham-operated rats at 48 h (Fig. 4). The maximum contraction (E_{max}) in response to phenylephrine in rings from aortic barodenervated rats amounted to 239 ± 16 mg tension/mg tissue compared with 558 ± 15 mg tension/mg tissue in rings from sham-operated rats, i.e. aortic barodenervation caused approximately 50% reduction in α_1-adrenoceptor responsiveness.

Exposure to prazosin (5×10^{-9} M) caused parallel

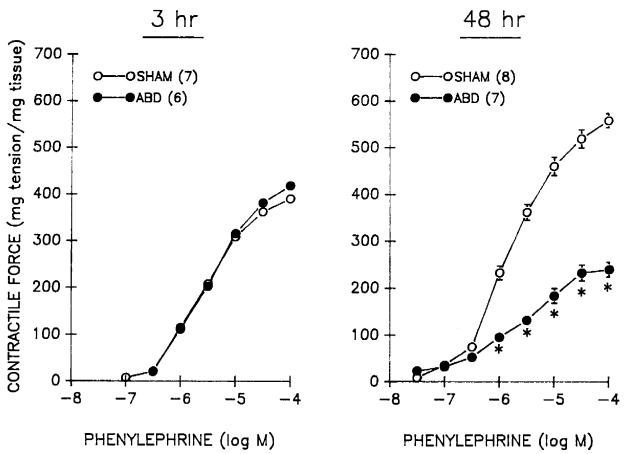

Fig. 4. Contractile responses evoked by cumulative addition of phenylephrine to aortic rings obtained from aortic baroreceptor-denervated (ABD) and sham-operated rats 3 h (left panel) and 48 h (right panel) after surgery. Values are means ± S.E.M. and the number of rings in each group is shown in parentheses. * $P < 0.05$ vs. respective sham values.

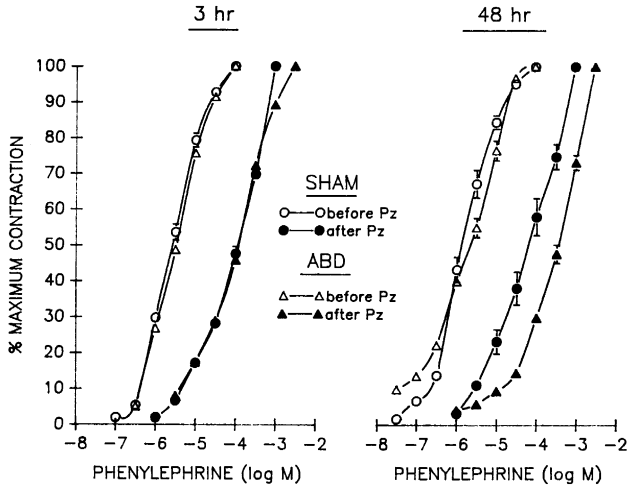

Fig. 5. Effect of prazosin (Pz, 5×10^{-9} M) on the contractile responses evoked by cumulative addition of phenylephrine to aortic rings obtained from aortic baroreceptor-denervated (ABD) and sham-operated rats 3 h (left panel) and 48 h (right panel) after surgery. Values are means ± S.E.M. and the number of rings in each group is shown in parentheses.

Table 1
The EC_{50} and E_{max} values of phenylephrine before and after addition of prazosin (Pz, 5×10^{-9} M) to aortic rings obtained from aortic barodenervated and sham-operated rats 3 and 48 h after surgery

Group	EC_{50} ($\times 10^{-5}$ M)		E_{max} (mg tension/mg tissue)	
	Before Pz	After Pz	Before Pz	After Pz
Sham operated				
3 h	0.28 ± 0.02	7.78 ± 0.39	390 ± 11	418 ± 45
48 h	0.17 ± 0.02	4.89 ± 0.85	558 ± 15	642 ± 49
Aortic barodenervated				
3 h	0.32 ± 0.01	8.77 ± 0.35	417 ± 11	450 ± 17
48 h	0.17 ± 0.02	26.30 ± 1.60	240 ± 16	271 ± 18

Values are means ± S.E.M. of 6–8 observations.

Table 2
pA_2 values of prazosin at postjunctional α_1-adrenoceptors assessed against phenylephrine-induced contractions of aortic rings obtained from aortic barodenervated and sham-operated rats 3 and 48 h after surgery

Group	*n*	pA_2
Sham operated		
3 h	7	9.73 ± 0.02
48 h	8	9.59 ± 0.12
Aortic barodenervated		
3 h	6	9.74 ± 0.03
48 h	7	10.56 ± 0.08 [a]

Values are means ± S.E.M. [a] $P < 0.05$ versus respective sham value.

rightward shifts in the concentration-response curves of phenylephrine in aortic rings from aortic barodenervated and sham-operated rats at 3 and 48 h (Fig. 5) without significant changes in the E_{max} values (Table 1). The magnitudes of the shifts in the curves evoked by prazosin were similar in rings from aortic barodenervated and sham-operated rats at 3 h but a markedly greater shift was demonstrated in rings from barodenervated rats at 48 h (Fig. 5). Comparison of the EC_{50} values of phenylephrine before and after prazosin showed a similar increase (approximately 30-fold) in rings from aortic barodenervated and sham-operated rats at 3 h (see Table 1 for the EC_{50} values). At 48 h, prazosin pretreatment caused an approximately 150-fold increase in the EC_{50} value of phenylephrine in rings from aortic barodenervated rats versus only a 30-fold increase in rings from sham-operated rats (Table 1). The pA_2 values determined by the dissociation constant method revealed no differences in α_1-adrenoceptor affinity for prazosin at 3 h in rings from aortic barodenervated and sham-operated rats (Table 2). In contrast, the pA_2 value of prazosin in rings from aortic barodenervated rats was significantly ($P < 0.05$) higher compared with that in rings from sham-operated rats at 48 h (10.56 ± 0.08 vs. 9.59 ± 0.12).

4. Discussion

The main finding of the present study was that surgical elimination of aortic baroafferent nerves significantly enhanced peripheral sympathetic activity that was associated with time-dependent changes in α_1-adrenoceptor reactivity in aortic smooth muscle. The α_1-adrenoceptor reactivity of aortic rings was not altered 3 h after aortic barodenervation but it exhibited significant attenuation at 48 h compared with sham operation. The pA_2 value of prazosin at α_1-adrenoceptors showed significant increases in aortic rings from aortic barodenervated, compared with sham-operated, rats suggesting that mechanisms other than modifications in receptor affinity are involved in the reduced aortic α_1-adrenoceptor reactivity. These mechanisms may include a decrease in α_1-binding sites triggered by the elevated sympathetic activity in denervated rats or an alteration in the post-receptor activation events.

The arterial baroreceptor reflex plays a crucial role in the regulation of sympathetic tone and central control of the circulation (Kumada et al., 1990; Chalmers and Pilowsky, 1991). Surgical elimination of baroreceptor afferents has been shown to elevate indices of sympathetic activity at central and peripheral sites (Patel et al., 1981; Alexander and Morris, 1988; Abdel-Rahman, 1992; Sannajust et al., 1992; El-Mas et al., 1994a). Little is known, however, about the consequence of baroreceptor denervation on the binding and functional activities of adrenoceptors at these sites. In an autoradiographic study, MacLean et al. (1990) have shown that total baroreceptor denervation (sinoaortic denervation) increases the affinity of α_1-adrenoceptor binding sites in the nucleus tractus solitarii. The present study investigated, for the first time, the effect of selective elimination of aortic baroafferent nerves on the functional activity of peripheral α_1-adrenoceptors in aortic smooth muscle. The hypothesis was tested that elevation of sympathetic activity and peripheral vascular resistance as a consequence of aortic barodenervation (Sannajust et al., 1992; El-Mas et al., 1994a,b) may diminish α_1-adrenoceptor reactivity in vascular smooth muscle.

The finding of the present study that the depressor responses to hexamethonium were enhanced in aortic barodenervated, compared with sham-operated, rats supports earlier reports (Abdel-Rahman, 1992; Sannajust et al., 1992; El-Mas et al., 1994a) that sympathetic activity and peripheral vascular resistance are higher in these rats. The increase in sympathetic activity, indicated by depressor responses to hexamethonium, was demonstrated as early as 3 h after aortic barodenervation and became more evident at 48 h. It is notable, however, that the dose of hexamethonium used in the present study (7.5 mg/kg) was lower than the standard dose (20 mg/kg) used in rats to completely block autonomic ganglia (Santajuliana et al., 1996). Nonetheless, substantially greater depressor responses were clearly demonstrated in aortic barodenervated rats with this submaximal dose range of hexamethonium. Reported findings revealed similar differences between aortic barodenervated and sham-operated rats after complete autonomic blockade with hexamethonium (32 mg/kg) (Abdel-Rahman, 1992). Our recent findings that plasma norepinephrine levels and peripheral vascular resistance are significantly elevated at 3 h after aortic barodenervation and remained so at 48 h (El-Mas et al., 1994a,b) provide further support for the presence of a higher sympathetic activity in aortic barodenervated rats.

The in vitro studies conducted in the current investigation showed that aortic barodenervation elicited time-dependent changes in α_1-adrenoceptor reactivity in isolated aortas. At 3 h, when blood pressure and sympathetic activity were elevated, aortic barodenervation had no affect on α_1-adrenoceptor reactivity since the contractile responses to phenylephrine in aortic rings from barodener-

vated and sham-operated rats were similar. Further, α_1-adrenoceptor affinity (pA_2) determined by prazosin was not different in aortas from the two groups of rats. The pA_2 values of prazosin at aortic α_1-adrenoceptors in this study are similar to those reported by others (Digges and Summers, 1983; Muramatsu et al., 1990). Studies of experimental and genetic hypertension have shown the influence of elevated blood pressure and sympathetic activity on the binding and functional activities of vascular α_1-adrenoceptors varied from an increase (Perry and Webb, 1988; Michel et al., 1990), a decrease (Wilson, 1991) or no change (Smith et al., 1987; Perry and Webb, 1988). These discrepancies have been attributed to factors including animal models of hypertension, vascular bed under investigation, and time elapsed between induction of hypertension and α_1-adrenoceptor reactivity determination (McElroy and Zimmerman, 1989; Michel et al., 1990; Wilson, 1991). Because elevations in blood pressure and sympathetic activity are expected to be associated with adrenoceptor desensitization, Michel et al. (1992) pointed out that the demonstration of increased or unchanged α-adrenergic reactivity in some studies may suggest that the mechanisms of adrenoceptor regulation are impaired in hypertension.

The current results showed that α_1-adrenoceptor reactivity in isolated aortas was significantly decreased, compared with sham-operated values, when tested 48 h after aortic barodenervation, as manifested by the substantial decreases in the contractile responses and maximal force generated by α_1-adrenoceptor activation. It is notable that at 48 h the blood pressure of aortic barodenervated rats was restored to predenervation levels whereas the sympathetic activity remained elevated. The reduced responsiveness of α_1-adrenoceptors may presumably represent receptor desensitization in response to the sympathetic overactivity and increased levels of norepinephrine being released at nerve terminals and/or in circulating plasma (Lefkowitz, 1981; Wilson, 1991). In fact, it is generally accepted that prolonged exposure of a tissue to an agonist drug or hormone results in a decreased responsiveness of this tissue to subsequent stimulation by the agonist (Goldstein et al., 1974). This phenomenon has been proved true in in vivo and in vitro studies that tested the effect of prolonged exposure of tissues to catecholamines. For example, Hiremath et al. (1991) found that desensitization of α_1-adrenoceptor-mediated vascular smooth muscle contraction occurs in aortas from pheochromocytoma-bearing rats and also in vitro aortic rings exposed to phenylephrine for 6 h. Desensitization of α-adrenoceptor-mediated smooth muscle contraction has also been demonstrated in blood vessels after in vitro exposure to epinephrine (Carrier et al., 1978; Lurie et al., 1985) as well as after in vivo infusion of the same drug (Maze et al., 1985).

Surprisingly, the present study demonstrated that α_1-adrenoceptor desensitization in aortic rings of aortic barodenervated rats was associated with a marked increase in receptor affinity, as evidenced by the significantly higher pA_2 value of prazosin in rings from aortic barodenervated compared with sham-operated rats. This finding may suggest that the diminution of α_1-adrenoceptor reactivity in rings from aortic barodenervated aortic rats is independent of a change in receptor affinity. The alteration in α-adrenoceptor reactivity has been generally attributed to changes in receptor density or affinity (Perry and Webb, 1988; Kiuchi et al., 1992). It is possible, therefore, that a decrease in α_1-adrenoceptor density, rather than affinity, due to aortic barodenervation-evoked prolonged sympathoexcitation may have contributed to α_1-adrenoceptor desensitization in aortic smooth muscle. In support of this assumption, elevated plasma levels of catecholamines in humans have been associated with decreased density of leukocytic adrenoceptors (Fraser et al., 1981). Similarly, Colucci et al. (1981) and Gengo et al. (1987) demonstrated a decrease in α_1-adrenoceptor density in mesenteric artery and cardiac tissues after prolonged α_1-adrenoceptor activation. An alternative mechanism for the occurrence of α_1-adrenoceptor desensitization may involve alterations in the post-receptor activation events. This view is supported by the notion that prolonged activation of α_1-adrenoceptors is associated with attenuation of α_1-adrenoceptor-induced stimulation of polyphosphoinositol turnover in cultured smooth muscle cells (Leeb-Lundberg et al., 1987) and in intact vessels (Lurie et al., 1985).

It is notable that attenuation of baroreflex sensitivity is usually associated with greater responsiveness to vasoactive (pressor and depressor) agents (Shepherd et al., 1983; Page, 1978). Similarly, diminished baroreflex sensitivity in pre-eclampsia is parallelled by increased responsiveness to angiotensin and norepinephrine (Talledo et al., 1968; Wasserstrum et al., 1989). The finding in the present study that attenuation of the baroreflex-mediated tachycardia in aortic barodenervated rats coincided with enhanced depressor responses to nitroprusside further supports the role of baroreflexes in suppressing responses to vasoactive agents. Given that baroreflex-mediated bradycardia was also attenuated in aortic barodenervated rats, the lack of an expected increase in the pressor responses to phenylephrine may infer that physiological desensitization of vascular α_1-adrenoceptors occurred in these rats and served to mask remarkable increases in α_1-adrenoceptor-mediated pressor responsiveness. This conclusion, however, appears to contradict findings of this and previous (Abdel-Rahman, 1992; Sannajust et al., 1992; El-Mas et al., 1994a) studies that peripheral resistance remains elevated as does sympathetic activity after aortic barodenervation. A higher peripheral resistance implies that a reduction in α_1-adrenergic responsiveness of resistance vessels does not occur. In effect, the enhanced depressor responses to hexamethonium in aortic barodenervated rats indicates that elevation of vascular resistance after aortic barodenervation is sympathetically mediated. Further, it has been emphasized that physiological desensitization is more difficult to demon-

strate since it is masked by central and peripheral neuronal mechanisms (Kiuchi et al., 1992). Future studies are needed to investigate (i) the influence of elimination of aortic baroafferents on pressor responsiveness to α_1-adrenoceptor activation in the absence of autonomic reflexes, and (ii) whether aortic barodenervation exerts differential effects on α_1-adrenoceptor responsiveness in conduit and resistance vessels. These studies will help explain the role of aortic baroafferents in the regulation of vascular tone and whether alterations in vascular responsiveness to α_1-adrenoceptor activation contribute to blood pressure normalization after aortic barodenervation.

Reported findings concerning the effect of sympathetic hyperactivity on α_1-adrenoceptor responsiveness in conduit and resistance vessels have been conflicting. Wilson (1991) reported that elevation of sympathetic activity in experimental models of hypertension (deoxycorticosterone-salt and two-kidney, one-clip hypertension) elicits significant reductions in the binding activity of aortic and mesenteric α_1-adrenoceptors. McElroy and Zimmerman (1989) demonstrated an increase in the affinity of the intrarenal arterial but not in the aortic α_1-adrenoceptor in two-kidney, one-clip hypertension. Kiuchi et al. (1992) proposed different mechanisms of α_1-adrenoceptor desensitization after chronic activation of α_1-adrenoceptors in large (reduction in receptor affinity) and small (reduction in receptor number) vessels. These discrepancies have been attributed to factors including differences in animal species, duration of sympathetic hyperactivity, and regional differences in vascular α_1-adrenoceptor reserve (Bevan, 1979; McElroy and Zimmerman, 1989; Wilson, 1991; Kiuchi et al., 1992).

In addition to sympathoexcitation, baroreceptor denervation is known to elicit other humoral changes that may contribute to the elevated peripheral resistance in aortic barodenervated rats. The reduced water intake (Werber and Fink, 1981; El-Mas and Abdel-Rahman, 1992) and plasma volume contraction (Fink et al., 1980; Werber and Fink, 1981) that develop after aortic barodenervation may elicit higher plasma levels of angiotensin and vasopressin (Cowley, 1992), which elicit vasoconstriction and elevate peripheral resistance. In effect, increases in plasma vasopressin levels after baroreceptor denervation have been documented (Bond and Trank, 1972; Alexander and Morris, 1986). Further, the elevation of sympathetic activity that follows aortic barodenervation may trigger the release of renin and hence increase the plasma angiotensin levels. A mutual interaction exists between the sympathetic nervous system and the renin-angiotensin system, with the activation of one amplifying the activity of the other (Zimmerman et al., 1984).

In conclusion, the findings of the present study support the hypothesis that the sympathetic hyperactivity that follows short-term aortic barodenervation results in a reduced reactivity of aortic smooth muscle to α_1-adrenoceptor activation. The diminution of aortic α_1-adrenoceptor reactivity cannot be attributed to changes in receptor affinity since the pA_2 value of prazosin at α_1-adrenoceptors showed significant increases in aortic rings from aortic barodenervated compared with sham-operated rats. Other mechanisms including alterations in receptor density and/or in the post-receptor activation events may explain the development of α_1-adrenoceptor desensitization in vascular smooth muscle after aortic barodenervation.

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