Mechanisms of $\alpha_1$-Adrenergic Vascular Desensitization in Conscious Dogs

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To investigate the mechanisms of $\alpha_1$-adrenergic vascular desensitization, osmotic minipumps containing either saline ($n=9$) or amidephrine mesylate (AMD) ($n=9$), a selective $\alpha_1$-adrenergic receptor agonist, were implanted subcutaneously in dogs with chronically implanted arterial and right atrial pressure catheters and aortic flow probes. After chronic $\alpha_1$-adrenergic receptor stimulation, significant physiological desensitization to acute AMD challenges was observed, i.e., pressor and vasoconstrictor responses to the $\alpha_1$-adrenergic agonist were significantly depressed ($p<0.01$) compared with responses in the same dogs studied in the conscious state before pump implantation. However, physiological desensitization to acute challenges of the neurotransmitter norepinephrine (NE) (0.1 $\mu$g/kg per minute) in the presence of $\beta$-adrenergic receptor blockade was not observed for either mean arterial pressure (MAP) (30±7 versus 28±5 mm Hg) or total peripheral resistance (TPR) (29.8±4.9 versus 29.7±7.3 mm Hg/l per minute). In the presence of $\beta$-adrenergic receptor plus ganglionic blockade after AMD pump implantation, physiological desensitization to NE was unmasked since the control responses to NE (0.1 $\mu$g/kg per minute) before the AMD pumps were now greater ($p<0.01$) than after chronic AMD administration for both MAP (66±5 versus 32±2 mm Hg) and TPR (42.6±10.3 versus 23.9±4.4 mm Hg/l per minute). In the presence of $\beta$-adrenergic receptor, ganglionic, plus NE-uptake blockade after AMD pump implantation, desensitization was even more apparent, since NE (0.1 $\mu$g/kg per minute) induced even greater differences in MAP (33±5 versus 109±6 mm Hg) and TPR (28.1±1.8 versus 111.8±14.7 mm Hg/l per minute). The maximal force of contraction induced by NE in the presence or absence of endothelium was significantly decreased ($p<0.05$) in vitro in mesenteric artery rings from AMD pump dogs compared with saline control dogs. Furthermore, $\alpha_1$-adrenergic receptor density, as determined by $[^3H]$prazosin binding in membrane preparations from vessels in the mesentery, was decreased (8.2±1.0 versus 18.4±1.4 fmol/mg protein, $p<0.001$) without any change in $K_I$ in the AMD pump dogs compared with the saline pump dogs. In aortic membranes $\alpha_1$-adrenergic receptor density in AMD pump dogs did not differ from saline pump dogs, but the affinity of the aortic receptors for $[^3H]$prazosin binding was decreased ($K_I$, 0.29±0.07 versus 14.0±0.01 nM, $p<0.01$), and NE-induced displacement of $[^3H]$prazosin binding demonstrated a loss of high-affinity binding sites (12±9 versus 82±2 percent, $p<0.05$). Thus, although endothelial mechanisms do not appear important, both autonomic reflex and biochemical mechanisms are altered by chronic $\alpha_1$-adrenergic receptor stimulation in the conscious dog; the altered autonomic mechanisms affect the physiological expression of desensitization, whereas separate biochemical mechanisms observed in vessels of different caliber mediate the desensitization. (Circulation Research 1992;71:1185-1199)

KEY WORDS • $\alpha_1$-adrenergic receptor • desensitization • catecholamine • dogs

Catecholamine desensitization has been recognized for over 30 years and has been studied extensively, primarily through the use of in vitro systems. The majority of these studies focused on myocardial desensitization and consequently have examined $\beta$-adrenergic receptor mechanisms, which are the predominant catecholamine receptors for regulating myocardial contractility. In contrast, $\alpha_1$-adrenergic receptors are the primary catecholamine receptors regulating peripheral vascular tone. Mechanisms of $\alpha_1$-adrenergic vascular desensitization have also been examined, but again under in vitro conditions. The extent to which $\alpha_1$-adrenergic peripheral vascular desensitization occurs and the mechanisms involved in intact animals are not known. Accordingly, the overall goal of this study was to investigate the effects of chronic $\alpha_1$-adrenergic receptor stimulation on peripheral vascular responses in which physiological desensitization in chronically instrumented, conscious dogs could be exam-

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ined and then correlated with in vitro studies. In view of recent studies demonstrating that autonomic mechanisms complicate β-adrenergic receptor–mediated desensitization in the heart,25 our underlying hypothesis was that the expression of peripheral vascular α₁-adrenergic receptor desensitization, particularly to the physiological neurotransmitter norepinephrine (NE), would depend on autonomic reflex mechanisms as well as biochemical mechanisms. NE, which is the predominant α₁-adrenergic neurotransmitter at the nerve terminal and effector organ junction, increases arterial pressure and induces baroreflex buffering, resulting in diminished sympathetic neural tone and enhanced parasympathetic restraint. Furthermore, NE is taken up by nerves, which could affect concentrations of the α₁-adrenergic agonist at the postjunctional receptor site.

Accordingly, the primary goal of the present investigation was to determine whether chronic α₁-adrenergic receptor stimulation induces desensitization of peripheral vascular responses to acute α₁-adrenergic receptor challenges induced by the neurotransmitter NE and the selective α₁-adrenergic receptor agonist amidephrine mesylate (AMD)26–28 in the conscious dog. A second goal was to determine whether neural reflex or catecholamine reuptake mechanisms modify the expression of α₁-adrenergic receptor desensitization in the conscious animal. To address these goals, conscious dogs were studied before and 2–3 weeks after implantation of osmotic minipumps containing AMD, which release the α₁-adrenergic receptor agonist continuously and induce chronic α₁-adrenergic receptor stimulation. A third goal was to determine whether endothelial mechanisms mediate α₁-adrenergic receptor desensitization. A fourth goal was to determine the vascular biochemical mechanisms responsible for desensitization, e.g., whether they involved downregulation of α₁-adrenergic receptor density. The final goal was to determine whether different mechanisms of α₁-adrenergic receptor desensitization apply to vessels of different caliber. To address these latter two goals, ligand binding techniques were used in membranes prepared from the mesentry, which contains a mix of smaller vessels ranging from intermediate sized arterioles to capillaries, and from a large conductance vessel, the aorta. Thus, the approach to understanding mechanisms mediating α₁-adrenergic receptor desensitization involved studies in intact, conscious animals coupled with in vitro studies using isolated vessel rings and in vitro studies using vascular membrane preparations.

Materials and Methods

Preparation of the Model

Eighteen adult mongrel dogs of either sex were anesthetized with halothane (0.5–1.5 vol/100 ml in oxygen) and ventilated with a Harvard respirator after induction with thiamyyl sodium (10–15 mg/kg i.v.). A left thoracotomy was performed through the fifth intercostal space using sterile technique. Tygon catheters (Norton Co., Akron, Ohio) were placed in the descending thoracic aorta and right atrium, and a solid-state pressure gauge (model P22, Konigsberg Instruments, Inc., Pasadena, Calif.) was inserted into the left ventricle via an apical stab wound. To measure ascending aortic flow (cardiac output), an aortic flow probe (Transonic Systems, Ithaca, N.Y.) was implanted around the root of the ascending aorta. The incision was closed in layers, the pneumothorax was reduced, and the animals were allowed to recover. After 2–3 weeks of recovery and after control experiments were completed, anesthesia was induced locally with lidocaine to implant an osmotic minipump (Alza Corp., Palo Alto, Calif.) subcutaneously in the neck. In nine animals, 2 ml saline was placed in the pump, while in the other nine animals AMD was placed in the pump to infuse at a rate of 1 μg/kg per minute. A second pump was implanted 10 days later, and a third pump was implanted 3 weeks after the first pump so that the elevated AMD levels were sustained for 3–4 weeks. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the “Guide for the Care and Use of Laboratory Animals” of the Institute of Laboratory Animal Resources, National Research Council (DHHS publication No. [NIH] 85–23, revised 1985).

Measurements

Hemodynamic measurements were recorded on a multichannel tape recorder (Honeywell, Denver, Colo.) and played back on a direct-writing oscillograph (Gould-Brush, Cleveland, Ohio). Fluid-filled catheters in the aorta and right atrium were connected to strain-gauge manometers (Statham Instruments, Oxnard, Calif.) for the measurement of arterial and right atrial pressures. A transonic flowmeter was used to measure cardiac output. The flow probes were calibrated in vitro using timed blood collections in a gravity flow system. Zero aortic flow was assumed to occur during mid and late diastole. Mean values for arterial pressure and aortic flow, i.e., cardiac output, were obtained continuously using resistance–capacitance circuits. Mean values, which are less susceptible to artifact than instantaneous values, were used to assess the data. Calculations of total peripheral resistance (TPR) were used to assess peripheral vasconstriction. Total peripheral resistance was calculated as the quotient of mean arterial pressure (MAP) minus mean right atrial pressure and cardiac output.

Protocol

First, the α₁-adrenergic selectivity of AMD was investigated. To accomplish this, acute challenges to NE (0.4 μg/kg per minute), which stimulates both α₁- and α²-receptors, and to AMD (4 μg/kg per minute) were examined in the conscious state in the presence of β-adrenergic receptor blockade with propranolol (1 mg/kg). This dose of propranolol abolished the effects of isoproterenol (0.1 μg/kg). The effects of NE or AMD were then examined in the presence of β- and α₁-adrenergic receptor blockade with prazosin (1.5 mg/kg), which abolished the effects of phenylephrine (PE) (5 μg/kg). Baseline hemodynamics before pump implantation and at 1 day, 4 days, 1 week, 2 weeks, and 3 weeks after AMD pumps were examined. The time course of physiological desensitization to the α₁-adrenergic receptor agonist was assessed in three dogs, i.e., acute AMD challenges (1, 2, and 4 μg/kg per minute) were examined at 1 day, 4 days, 1 week, and 2 weeks after AMD pump implantation. All dogs were studied before implantation of the pumps in the conscious state, 2–3
weeks after recovery from surgery, and then again 2–3 weeks after implantation of the osmotic infusion pump. In all nine dogs with AMD pumps, stepwise 5-minute infusions of NE (0.05, 0.1, 0.2, and 0.4 µg/kg per minute) and AMD (1, 2, and 4 µg/kg per minute) in the presence of β-adrenergic receptor blockade with propranolol (1 mg/kg) were administered while measurements of phasic and mean arterial pressure, right atrial pressure, cardiac output, and heart rate were recorded continuously. On a separate day the 5-minute infusions of NE (0.05, 0.1, and 0.2 µg/kg per minute) and AMD (0.5, 1, and 2 µg/kg per minute) in the presence of β-adrenergic receptor blockade with propranolol (1 mg/kg) plus ganglionic blockade with hexamethonium bromide (30 mg/kg) and atropine (0.1 mg/kg) were repeated in all nine dogs with AMD pumps. Absence of reflex heart rate change in response to changes in arterial pressure induced by PE (2.5 µg/kg) and nitroglycerin (5 µg/kg) confirmed the adequacy of the blockade. Further, NE (0.02, 0.05, and 0.1 µg/kg per minute) and AMD (0.5, 1, and 2 µg/kg per minute) infusions in the presence of β-adrenergic receptor blockade plus NE-uptake blockade with desmethylimipramine (1 mg/kg), which abolished the effects of tyramine (50 µg/kg), were examined in three dogs with AMD pumps. Finally, NE (0.02, 0.05, and 0.1 µg/kg per minute) and AMD (0.5, 1, and 2 µg/kg per minute) infusions in the presence of β-adrenergic receptor, ganglionic, plus NE-uptake blockade were examined in three dogs with AMD pumps. In two dogs with AMD pumps, acute PE (0.5, 1, and 2 µg/kg per minute) challenges were also examined in the presence of β-adrenergic receptor blockade alone, β-adrenergic receptor plus ganglionic blockade, β-adrenergic receptor plus NE-uptake blockade, and β-adrenergic receptor plus ganglionic plus NE-uptake blockade.

Membrane Preparation

At 5–7 days after the third pump implantation, the animals were anesthetized with sodium pentobarbital (30 mg/kg) and the entire aorta and mesenteric vascular tree were removed quickly for membrane preparation. The aorta and mesenteric vascular tree were placed in ice-cold buffer A (250 mM sucrose, 10 mM HEPES, 10 mM PMSF, and 10 mM benazidine, pH 7.5). The mesentery was cleaned on the top of a glass plate kept cold on ice. The trimming procedure involved careful separation and removal of fat and lymph nodes from arteries. The removal of the mesenteric connective tissues and its surrounding fat tissue from the small vessels was performed carefully from branch to branch with scissors and forceps so that most of the smaller vessels could be separated intact. The endothelium in the aorta was removed by scraping the intimal surface. The aorta and mesenteric vessels were then minced and diluted with buffer A (1:10 weight/vol). Aortic and mesenteric membrane preparations were obtained as follows. First, the minced tissue was disrupted with two 15-second bursts with the use of a Brinkman polytron (setting 10) followed by filtration through 14-gauge silk screen. Undisrupted aggregates were then pelleted by centrifugation at 1,000g for 20 minutes (model RC-5B, Sorvall), and the supernatant was centrifuged at 12,000g for 20 minutes. The supernatant was next centrifuged at 210,000g (Sorvall ultracentrifuge) to obtain the microsomal pellet. The microsomal pellet was resuspended in buffer B (100 mM Tris, 5 mM EGTA, 10 mM PMSF, and 10 mM benazidine, pH 7.5) by homogenization and subsequently repelleted and rehomogenized into the same buffer to a protein concentration of 4–6 mg/ml in the aorta and 1.5 mg/ml in the mesentery before storing at −70°C. The yield of membrane protein in the aorta was 1.5 mg/g wet wt, and the yield of membrane protein in the mesentery was 0.5 mg/g wet wt. To determine whether significant material was discarded after the first 1,000g centrifugation, the pellet from the first 1,000g centrifugation was examined. In the six samples analyzed, reliable α₁-adrenergic receptor binding data could not be obtained because of high nonspecific binding and data scatter. This fraction appears to contain mostly fat and connective tissue and is unlikely to contain many α₁-receptors.

Binding Studies

α₁-Adrenergic receptor binding studies in aortic and mesenteric preparations were performed in all saline and AMD pump dogs using eight concentrations of 25 µl [³H]prazosin ranging from 0.01 to 2.4 nM, 25 µl phentolamine (10 µM) or buffer, and 100 µl of the membrane protein (35 µg assay in aortic membrane preparations and 50 µg assay in mesenteric artery preparations). The α₁-adrenergic receptor assays were incubated at 37°C for 30 minutes since equilibrium binding studies demonstrated a plateau after 20 minutes. The incubation was terminated by filtration through Whatman GF/C filters. The filters were placed in 5 ml Hydrofluor (National Diagnostics, Manville, N.J.) and counted for 5 minutes in a liquid scintillation counter (model 6985, Beta Trac, TM Analytic, Elk Grove Village, Ill.). Specific binding of [³H]prazosin to the α₁-adrenergic receptor binding was greater than 75%.

Competitive inhibition agonist binding curves were performed using 100 µl of aortic membrane preparation, 25 µl [³H]prazosin (0.2 nM), and 25 µl NE (1 mM–10 nM), with 21 concentrations of NE or buffer. The assays were performed using aortic membranes from each of three dogs with saline and AMD pumps. The assay mixtures were incubated at 37°C for 30 minutes, since equilibrium binding studies demonstrated a plateau after 20 minutes. The incubation was terminated by filtration through Whatman GF/C filters. The filters were placed in 5 ml Hydrofluor and counted for 5 minutes in a 6985 Beta Trac liquid scintillation counter. The binding data were analyzed by the LIGAND computer program of Munson and Rodbard. In the computer analysis the F test was used to compare the best fit for the ligand binding competition data.

α₂-Adrenergic receptor binding studies in aortic membrane preparations were performed in all saline and AMD pump dogs using eight concentrations of 25 µl [³H]rauwolscine ranging from 1 to 25 nM, 25 µl rauwolscine (1 µM) or buffer, and 100 µl of the membrane protein (50 µg assay). The α₂-adrenergic receptor assay mixtures were incubated at 37°C for 30 minutes. The incubation was terminated by filtration through Whatman GF/C filters. The filters were placed in 5 ml Hydrofluor and counted for 5 minutes in a 6985 Beta Trac liquid scintillation counter.

β-Adrenergic receptor antagonist binding studies in aortic membrane preparations were performed in seven
saline pump dogs and five AMD pump dogs using eight concentrations of 25 µl 125I-cyanopindolol ranging from 0.02 to 1.0 nM, 25 µl isoproterenol (0.1 mM) or buffer, and 100 µl of the membrane protein (20 µg/assay). Assay mixtures were incubated at 37°C for 40 minutes, terminated by rapid filtration on Whatman GF/C filters, and counted in a Tracer gamma counter for 1 minute. The dissociation constant (Kd) and receptor density were calculated from Scatchard analysis and from the nontransformed binding data with the mass action law—based, curve-fitting program LIGAND.29 Na,K-ATPase activity was determined by the method of Jones and Besch.30 Protein levels were assayed by the method of Lowry et al.31 Plasma catecholamine samples were taken before and 3–4 weeks after pump implantation. At all times the samples were taken with the animals in the baseline state, before the acute administration of drugs. Plasma catecholamine levels were measured by the radioenzymatic assay of Peuler and Johnson.32 In this assay NE and epinephrine are converted to their O-H-methylated derivatives with catechol-O-methyl transferase derived from rat liver. These derivatives are then extracted and purified by thin-layer chromatography on silica gel and converted by periodate oxidation (requiring an α-OH group) to the aromatic aldehyde, which is then extracted and counted. To determine whether AMD was measured as NE in the assay, increasing concentrations of AMD, from 1 to 1,000 µg/ml, were added to samples from five normal dogs. AMD did not affect the measurement of NE significantly.

Isolated Mesenteric Artery Studies

At the time of death, the mesenteric artery from six AMD pump dogs and six saline pump dogs was excised and placed in Krebs-Ringer bicarbonate buffer bubbled with 95% O2–5% CO2 and containing (mM) NaCl 117, KCl 5.0, NaHCO3 25.0, CaCl2 1.25, MgSO4 1.26, KH2PO4 1.0, and dextrose 10.0. While maintained at room temperature in this buffer, the adherent adventitia and fatty tissue were removed. Mesenteric artery rings 2 mm wide in the absence and presence of endothelium were mounted in a 25-ml jacketed tissue bath kept at 37.5–38°C. A basal tension was applied as described by Angus et al.33 and the rings were allowed to equilibrate for 1 hour before exposure to drugs. Responses were recorded as changes in grams of tension on a physiological recorder with a strain-gauge force displacement transducer. Cumulative dose–response curves for NE (10−8–10−4 M), PE (10−8–10−4 M), AMD (10−7–10−4 M), and KCl (10–70 mM) were generated by producing a stepwise increase in concentration of each drug as soon as a steady response was obtained from each preceding dose. Relaxation elicited by acetylcholine (10−5–10−5 M) in preparations precontracted with 30 mM KCl were used to ascertain the integrity of the endothelium.

Data Analysis

Mean values and standard errors were calculated with an IBM PC/AT computer (IBM Instruments, Inc., Danbury, Conn.). The data collected from the same dogs before and after pump implantation or before and after infusion of NE, AMD, and PE were tested statistically with Student’s t test for paired data, whereas the comparison between the groups with saline and AMD pumps was made by Student’s t test for grouped data.34 The relations between the doses of NE and AMD and their responsiveness were examined by regression analysis. Comparison of regression lines was performed by determining the significance of differences in both the slopes and elevations of the lines by the F test.35 For the studies in isolated vascular rings, statistical comparison of maximal contraction between saline and AMD dogs was performed using both Student’s t test for grouped data and the multigroup repeated measurements analysis of variance, whereas comparisons with and without endothelium were performed using Student’s t test for paired data. A value of p<0.05 was considered statistically significant.

Results

α1-Adrenergic Selectivity of AMD

The pressor and vasoconstrictor responses to NE were not abolished but only attenuated by α1-adrenergic receptor blockade with prazosin. In contrast, the pressor and vasoconstrictor responses to AMD were abolished by α1-adrenergic receptor blockade, indicating that AMD is a selective α1-adrenergic receptor agonist. We selected AMD as the agonist rather than PE because the latter also possesses β-adrenergic receptor properties.

Effects of AMD and Saline Pumps on Baseline Hemodynamics

As shown in Table 1, there were no significant differences in MAP, cardiac output, TPR, and heart rate between the two groups of dogs before implanta-
tion of pumps. The saline pumps elicited no change in hemodynamics.

Increases in MAP and TPR and decreases in heart rate and cardiac output were evident at 1 day after AMD pump implantation and reached a steady state at 1 week after pump implantation (Figure 1). At 2–3 weeks after AMD pump implantation, MAP and TPR were significantly increased (p<0.05) and cardiac output and heart rate were significantly decreased (p<0.05) when compared with values observed in the dogs with saline pumps (Table 1).

**Time Course of Desensitization**

Figure 2 shows the time course of physiological desensitization to AMD. AMD challenges induced similar increases in MAP and TPR at 1 or 4 days after pump implantation compared with responses before pump implantation, i.e., no desensitization, but at 1 week after implantation of AMD pumps, physiological desensitization to AMD was observed. Thus, despite evidence of effects of AMD on baseline values of MAP and TPR at 1 day after pump implantation, desensitization was not observed until 1 week after pump implantation.

**Effects of α-Adrenergic Receptor Challenges in the Presence of β-Adrenergic Receptor Blockade Before and After AMD Pumps**

Physiological desensitization was assessed by comparing α-adrenergic receptor–mediated pressor and vasoconstrictor responses, i.e., increases in MAP and TPR, before and after the AMD pumps were implanted. In the dogs studied with chronic α-adrenergic receptor stimulation, challenges with low and intermediate doses of NE (0.05, 0.1, and 0.2 μg/kg per minute) in the presence of β-adrenergic receptor blockade increased MAP and TPR by similar amounts before and after the pumps, i.e., desensitization to NE was not observed. However, challenge with the highest dose of NE in the presence of β-adrenergic receptor blockade induced smaller increases (p<0.05) in MAP (57±7 versus 71±5
blockade induced smaller increases \((p<0.01)\) in MAP \((32.2±2\text{ versus }66.5\text{ mm Hg})\) and TPR \((23.9±4.4\text{ versus }42.6±10.3\text{ mm Hg/l per minute})\) compared with responses in the control state \((\text{Table 3})\). These differences were apparent at all doses studied \((\text{Figure 4})\). After pumps there was a significant difference \((p<0.01)\) in the elevation of the regression lines in response to NE \((\text{Figure 4})\), but the slopes of the lines were not different. Acute AMD challenge \((2\mu g/kg\text{ per minute})\) in the presence of \(\beta\)-adrenergic receptor plus ganglionic blockade induced smaller increases \((p<0.01)\) in MAP \((27.9±4.2\text{ versus }90.4±4\text{ mm Hg/l per minute})\) and TPR \((18.2±2.9\text{ versus }55.2±5.6\text{ mm Hg/l per minute})\) after pumps compared with before pumps. Thus, in the presence of ganglionic blockade, physiological desensitization to NE as well as AMD and PE was apparent.

**Effects of \(\alpha\)-Adrenergic Receptor Challenges in the Presence of \(\beta\)-Adrenergic Receptor Plus Ganglionic Blockade Before and After AMD Pumps**

After chronic \(\alpha\)-adrenergic receptor stimulation, acute NE challenge \((0.1\mu g/kg\text{ per minute})\) in the presence of \(\beta\)-adrenergic receptor plus ganglionic blockade induced smaller increases \((p<0.01)\) in MAP \((32.2±2\text{ versus }66.5\text{ mm Hg})\) and TPR \((23.9±4.4\text{ versus }42.6±10.3\text{ mm Hg/l per minute})\) compared with responses in the control state \((\text{Table 3})\). These differences were apparent at all doses studied \((\text{Figure 4})\). After pumps there was a significant difference \((p<0.01)\) in the elevation of the regression lines in response to NE \((\text{Figure 4})\), but the slopes of the lines were not different. Acute AMD challenge \((2\mu g/kg\text{ per minute})\) in the presence of \(\beta\)-adrenergic receptor plus ganglionic blockade induced smaller increases \((p<0.01)\) in MAP \((27.9±4.2\text{ versus }90.4±4\text{ mm Hg/l per minute})\) and TPR \((18.2±2.9\text{ versus }55.2±5.6\text{ mm Hg/l per minute})\) after pumps compared with before pumps. Thus, in the presence of ganglionic blockade, physiological desensitization to NE as well as AMD and PE was apparent.

**Effects of \(\alpha\)-Adrenergic Receptor Challenges in the Presence of \(\beta\)-Adrenergic Receptor Plus NE-Uptake Blockade Before and After AMD Pumps**

After chronic \(\alpha\)-adrenergic receptor stimulation, acute NE challenge \((0.1\mu g/kg\text{ per minute})\) in the presence of \(\beta\)-adrenergic receptor plus NE-uptake
Table 2. Effects of Ganglionic Blockade and Norepinephrine-Uptake Blockade on Hemodynamic Responses to Amidephrine Before and After Chronic a1 Stimulation

<table>
<thead>
<tr>
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<th>Before pump</th>
<th>After pump</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Change</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
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<tr>
<td>β-Blockade (n=9)</td>
<td>97±3</td>
<td>48±6</td>
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<td>β+Ganglionic blockade (n=9)</td>
<td>84±5</td>
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<td>β+NE uptake blockade (n=3)</td>
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<td>β+Ganglionic+NE-uptake blockade (n=3)</td>
<td>90±6</td>
<td>95±3</td>
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<tr>
<td>Cardiac output (l/minute)</td>
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<tr>
<td>β-Blockade (n=9)</td>
<td>2.23±0.12</td>
<td>-0.52±0.07</td>
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<tr>
<td>β+Ganglionic blockade (n=9)</td>
<td>2.27±0.17</td>
<td>-0.35±0.13</td>
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<td>β+NE uptake blockade (n=3)</td>
<td>1.73±0.41</td>
<td>-0.58±0.24</td>
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<td>β+Ganglionic+NE-uptake blockade (n=3)</td>
<td>1.92±0.30</td>
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<td>Total peripheral resistance (mm Hg/l per minute)</td>
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<tr>
<td>β-Blockade (n=9)</td>
<td>44.50±2.62</td>
<td>45.00±7.92</td>
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<td>39.21±3.94</td>
<td>55.16±5.64</td>
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<td>67.32±15.05</td>
<td>99.75±10.91</td>
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<td>49.07±7.62</td>
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<td>Heart rate (beats per minute)</td>
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<td>β-Blockade (n=9)</td>
<td>92±5</td>
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<td>β+Ganglionic blockade (n=9)</td>
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<td>β+NE uptake blockade (n=3)</td>
<td>68±11</td>
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<tr>
<td>β+Ganglionic+NE-uptake blockade (n=3)</td>
<td>116±4</td>
<td>2±2</td>
</tr>
</tbody>
</table>

Change, change from baseline; β-blockade and β-, β-adrenergic receptor blockade; NE, norepinephrine.
†p<0.05 difference before and after pump.
*tp<0.01 difference before and after pump.

Blockade induced smaller increases (p<0.05) in MAP (39±2 versus 86±2 mm Hg) and TPR (31.2±3.1 versus 107.2±20.6 mm Hg/l per minute) compared with responses in the control state (Table 3). After pumps there was a significant difference (p<0.01) in the elevation of the regression lines in response to NE, indicating that the regression lines were shifted downward, but the slopes of the lines were not different. After chronic a1-adrenergic receptor stimulation, acute AMD challenge (2 μg/kg per minute) in the presence of β-adrenergic receptor plus NE-uptake blockade induced smaller increases (p<0.05) in MAP (13±2 versus 77±6 mm Hg) and TPR (23.9±5.3 versus 99.8±10.9 mm Hg/l per minute) compared with responses in the control state (Table 2). There were significant differences (p<0.01) in both the slopes and elevations of the regression lines in response to AMD before and after pumps. Acute PE challenge (2 μg/kg per minute) also induced smaller increases in MAP (45 versus 83 mm Hg) and TPR (28.4 versus 55.4 mm Hg/l per minute) after pumps compared with before pumps. Thus, in the presence of NE-uptake blockade, physiological desensitization was even more apparent.

Effects of α-Adrenergic Receptor Challenges in the Presence of β-Adrenergic Receptor, Ganglionic, and NE-Uptake Blockade Before and After AMD Pumps

After chronic α1-adrenergic receptor stimulation, acute NE challenge (0.1 μg/kg per minute) in the presence of β-adrenergic receptor, ganglionic, and NE-uptake blockade induced smaller increases (p<0.05) in MAP (33±5 versus 109±6 mm Hg) and TPR (28.1±1.8 versus 111.8±14.7 mm Hg/l per minute) compared with responses in the control state (Table 3). There were significant differences (p<0.01) in both the slopes and elevations of the regression lines in response to NE before and after pumps (Figure 5). After chronic α1-adrenergic receptor stimulation, acute AMD challenge (2 μg/kg per minute) in the presence of β-adrenergic receptor, ganglionic, and NE-uptake blockade induced smaller increases (p<0.05) in MAP (10±7 versus 95±3 mm Hg) and TPR (13.2±5.9 versus 66.3±9.3 mm Hg/l per minute) compared with responses in the control state (Table 2). There were significant differences (p<0.01) in both the slopes and elevations of the regression lines in response to AMD before and after pumps (Figure 5). Acute PE challenge (2 μg/kg per minute) also induced smaller increases in MAP (62 versus 114 mm Hg) and TPR (30.2 versus 70.3 mm Hg/l per minute) after pumps compared with before pumps. Thus, NE-uptake blockade also enhanced the expression of physiological desensitization in the presence of ganglionic blockade since the pressor and vasoconstrictor responses to NE were buffered effectively by reflex mechanisms in the control state before pumps, but to a much lesser extent after chronic α1-adrenergic receptor stimulation.
TABLE 3. Effects of Ganglionic Blockade and Norepinephrine-Uptake Blockade on Hemodynamic Responses to Norepinephrine Before and After Chronic \( \alpha \)-Stimulation

<table>
<thead>
<tr>
<th></th>
<th>Before pump</th>
<th>After pump</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-Blockade (<em>n=9</em>)</td>
<td>100±3</td>
<td>28±5</td>
</tr>
<tr>
<td>( \beta )-Ganglionic blockade (<em>n=9</em>)</td>
<td>86±5</td>
<td>66±5</td>
</tr>
<tr>
<td>( \beta )-NE-uptake blockade (<em>n=3</em>)</td>
<td>102±6</td>
<td>86±2</td>
</tr>
<tr>
<td>( \beta )-Ganglionic+NE-uptake blockade (<em>n=3</em>)</td>
<td>90±6</td>
<td>109±6</td>
</tr>
<tr>
<td>Cardiac output (1/minute)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-Blockade (<em>n=9</em>)</td>
<td>2.14±0.16</td>
<td>-0.34±0.08</td>
</tr>
<tr>
<td>( \beta )-Ganglionic blockade (<em>n=9</em>)</td>
<td>2.33±0.17</td>
<td>-0.22±0.04</td>
</tr>
<tr>
<td>( \beta )-NE-uptake blockade (<em>n=3</em>)</td>
<td>1.70±0.36</td>
<td>-0.56±0.31</td>
</tr>
<tr>
<td>( \beta )-Ganglionic+NE-uptake blockade (<em>n=3</em>)</td>
<td>1.97±0.27</td>
<td>-0.70±0.26</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg/l per minute)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-Blockade (<em>n=9</em>)</td>
<td>48.17±3.27</td>
<td>28.93±7.25</td>
</tr>
<tr>
<td>( \beta )-Ganglionic blockade (<em>n=9</em>)</td>
<td>39.22±4.25</td>
<td>42.63±10.32</td>
</tr>
<tr>
<td>( \beta )-NE-uptake blockade (<em>n=3</em>)</td>
<td>63.61±9.01</td>
<td>107.23±20.63</td>
</tr>
<tr>
<td>( \beta )-Ganglionic+NE-uptake blockade (<em>n=3</em>)</td>
<td>78.17±9.01</td>
<td>111.75±14.65</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )-Blockade (<em>n=9</em>)</td>
<td>87±4</td>
<td>-10±3</td>
</tr>
<tr>
<td>( \beta )-Ganglionic blockade (<em>n=9</em>)</td>
<td>114±6</td>
<td>0±0</td>
</tr>
<tr>
<td>( \beta )-NE-uptake blockade (<em>n=3</em>)</td>
<td>68±11</td>
<td>-6±3</td>
</tr>
<tr>
<td>( \beta )-Ganglionic+NE-uptake blockade (<em>n=3</em>)</td>
<td>116±4</td>
<td>4±2</td>
</tr>
</tbody>
</table>

Change, change from baseline; \( \beta \)-blockade and \( \beta \), \( \beta \)-adrenergic receptor blockade; NE, norepinephrine.

\( ^*p<0.05 \) difference before and after pump.

\( {\dagger}p<0.01 \) difference before and after pump.

**Responsiveness of Isolated Mesenteric Artery Rings**

With intact endothelium the relaxations induced by acetylcholine were essentially identical in rings from AMD pump dogs and saline pump dogs (Figure 6). In contrast, the maximal force of contraction induced by AMD was significantly decreased (\( p<0.05 \)) in isolated mesenteric artery rings from the AMD pump group compared with the saline pump group (2.73±0.63 versus 5.07±0.57 g) (Figure 7). In mesenteric artery rings without endothelium, acetylcholine induced equivalent constriction in both groups (Figure 6), whereas AMD still induced less (\( p<0.05 \)) force of contraction in the AMD pump group compared with the saline pump group (3.56±0.41 versus 6.18±0.79 g) (Figure 7). In contrast, the KCl-induced contraction, which is nonspecific, i.e., not mediated by receptor stimulation, was not different between the two groups whether or not the endothelium was removed (Figure 7). The maximal force of contraction induced by NE and PE was also reduced in isolated mesenteric artery rings in the presence of endothelium in the AMD pump group compared with the saline pump group (Figure 8). These studies indicate that desensitization to \( \alpha \)-adrenergic receptor agonists were \( \alpha \)-adrenergic receptor–specific and not dependent on the endothelium.

**\( \alpha \)-Adrenergic Receptor Analyses in Membrane Preparations**

Figure 9 shows a Scatchard analysis of \([3H]Prazosin binding to aortic membranes and membranes from vessels in the mesentery. The \( K_d \) of \([3H]Prazosin binding to aortic membranes was increased (0.29±0.07 versus 0.14±0.01 nM, \( p<0.01 \)) without decreases in \( \alpha \)-adrenergic receptor density (Table 4). There were no significant differences between the two groups in \( \alpha_1 \)- (3.16±0.6 versus 86±15 fmol/mg protein) and \( \beta \)-adrenergic receptor densities (14±1 versus 16±2 fmol/mg protein) in the aorta. On the other hand, in membrane preparations from vessels in the mesentery, which contained intermediate-sized vessels to capillaries, \( \alpha \)-adrenergic receptor density was decreased (8.2±1.0 versus 18.4±1.4 fmol/mg protein, \( p<0.001 \)) without any change in \( K_d \) in the AMD pump group compared with the saline pump group (Table 4).

**NE-Induced Displacement of \([3H]Prazosin Binding in Aortic Membranes**

In the three animals studied with saline pumps, the use of a model for two classes of receptor sites resulted in a significant improvement of the fit of the data, as compared with a model for a single class of receptor sites. The parameter estimates for the affinity constants were \( K_d=4.3±0.6 \) \( \mu \)M and \( K_d=130±50 \) \( \mu \)M with proportions of 82±2% and 18±2% for the high-affinity.
state and the low-affinity state, respectively. The average percent of α1-adrenergic receptors binding NE with high affinity was significantly reduced (p<0.05) in animals with AMD pumps (12±9%) compared with animals with saline pumps (82±2%) (Figure 10). In one of three animals studied with AMD pumps, the agonist competition binding data were best fit to a single low-affinity site model with a K_{H} of 880 μM, whereas a two-site model was preferred in the other two animals. The parameter estimates for the affinity constants were K_{H}=2.4 μM and K_{L}=60 μM.

Na,K-ATPase

As an index of the consistency of the membrane preparations, the membrane marker Na,K-ATPase was measured in aortic and mesenteric membrane preparations and was not found to be significantly different in the saline pump versus the AMD pump groups (Table 4).

Catecholamine Levels

Baseline plasma NE levels in the AMD pump group were significantly increased (p<0.01) compared with values in the saline pump group (318±31 versus 198±9 pg/ml) 3–4 weeks after pump implantation. However, there were no differences in plasma epinephrine levels between the saline pump group and the AMD pump group.

Discussion

While most previous studies examining catecholamine desensitization have focused on the β-adrenergic receptor and the heart, peripheral vascular α1-adrenergic receptor desensitization has also been demonstrated. However, the mechanisms mediating desensitization have been examined primarily in acute or in vitro preparations. It is important to examine those mechanisms in a chronic, physiological preparation to understand more fully pathophysiological states with chronically elevated catecholamines or sympathetic tone. The current investigation is the first to study chronic α1-adrenergic desensitization in the conscious animal. In conscious dogs with reflex mechanisms intact but β-adrenergic receptors blocked, α1-adrenergic receptor desensitization to the specific agonists, AMD, or PE was readily apparent.

α1-Adrenergic receptor desensitization was also demonstrated in vitro by examining dose–response relations in isolated vascular rings, and the cellular mechanisms of desensitization were explored in vascular smooth muscle membrane preparations. Interestingly, physiological desensitization to NE was more difficult to demonstrate. Desensitization to NE was observed only at the highest dose tolerated by the conscious dogs. However, after reflex mechanisms were eliminated, physiological desensitization to NE was clearly demonstrated. Thus, autonomic reflex and catecholamine uptake mechanisms effectively buffered responses to NE in the control state but were less effective after chronic α1-adrenergic receptor stimulation. Had the mechanisms been equally effective after chronic α1-adrenergic receptor stimulation, physiological desensitization to
FIGURE 5. Graphs showing the effects of acute challenges to norepinephrine (NE) (0.02, 0.05, and 0.1 µg/kg per minute) (top panels) and amidephrine (AMD) (0.5, 1, and 2 µg/kg per minute) (bottom panels) in the presence of β-adrenergic receptor, ganglionic, and NE-uptake blockade on increases in mean arterial pressure (MAP) (left panels) and total peripheral resistance (TPR) (right panels) before (○, ■) and 2–3 weeks after (△, ●) AMD pump implantation (n=3). Data are expressed as mean±SEM. *Significant (p<0.01) differences in both the slopes and elevations of the regression lines in responses to both NE and AMD before and after pumps.

NE would have been readily apparent under all conditions; but because of the impairment in reflex and uptake control mechanisms, physiological desensitization was only apparent after these autonomic mechanisms were eliminated. Therefore, the different extent of physiological expression of desensitization observed under these conditions was due to different responses to acute NE challenges observed before AMD pump implantation.

Several interlocking neural mechanisms appear to be responsible for the differences in the desensitization observed with different α-adrenergic receptor agonists. Since these studies were conducted in the presence of β-adrenergic receptor blockade, complicating β-adren-

FIGURE 6. Graphs showing acetylcholine- (Ach) (10^{-9}–10^{-5} M) induced relaxations in isolated mesenteric artery rings precontracted with 30 mM KCl in the presence (+, left panel) and absence (−, right panel) of endothelium in the saline pump group (○, n=6) and the amidephrine (AMD) pump group (△, n=6). Data are expressed as mean±SEM. With intact endothelium the Ach-induced relaxations were essentially identical in rings from the saline and AMD pump groups. In mesenteric artery rings without endothelium Ach induced equivalent constriction in both groups.
buffering and observed case, expression of to implantation pressure influences α1-adrenergic agonists and α1-adrenergic receptor and E-4. 

FIGURE 7. Dose–response curves of amidephrine- (AMD) (left panels) and KCI- (right panels) induced contractile responses in isolated mesenteric artery rings in the presence (+, top panels) and absence (−, bottom panels) of endothelium in the saline pump group (○, n=6) and the AMD pump group (△, n=6). Data are expressed as mean±SEM. The AMD-induced contraction, which was mediated by stimulation of the α1-adrenergic receptor, was significantly attenuated in the AMD pump group compared with the saline pump group whether or not the endothelium was removed. In contrast, the KCl-induced contraction, which is not mediated by receptor stimulation but by depolarization of plasma membranes, did not differ between the two groups whether or not the endothelium was removed.

FIGURE 8. Dose–response curves of norepinephrine- (NE) (left panels) and phenylephrine- (PE) (right panels) induced contractile responses in isolated mesenteric artery rings in the presence (+, top panels) and absence (−, bottom panels) of endothelium in the saline pump group (○, n=6) and the amidephrine pump group (△, n=6). Data are expressed as mean±SEM. The NE- and PE-induced contractions were significantly attenuated in the amidephrine pump group compared with the saline pump group.

ergic influences could not be implicated. All three agonists used, i.e., NE, PE, and AMD, increased arterial pressure and consequently induced reflex buffering. In the conscious animal, the values obtained from MAP and TPR reflect the direct action of the vasoconstrictor minus the reflex buffering. It is conceivable that chronic α1-adrenergic receptor stimulation could affect reflex buffering and cause the responses after AMD pump implantation to appear artificially large, i.e., mask the expression of physiological desensitization. If that were the case, the physiological desensitization would only be observed in the presence of ganglionic blockade, which eliminated the complicating influences of reflex buffering. However, since AMD and PE responses were desensitized either in the presence or absence of ganglionic blockade, but desensitization to NE was uncovered only after ganglionic blockade, it suggests that some mechanism other than simple reflex buffering was responsible for the differences. One potential difference is the uptake of NE that occurs at the nerve ending, but that may occur to a lesser extent for AMD and PE. In the presence of desmethylimipramine, a neural NE-uptake blocker, clear desensitization to NE was evident after chronic α1-adrenergic stimulation, even in the absence of ganglionic blockade. This suggests that under conditions of chronic α1-adrenergic receptor stimu-
lation, reflex mechanisms, which include neuronal NE reuptake, mask the expression of physiological desensitization to an acute NE challenge.

In contrast to the NE responses, physiological desensitization was apparent with acute PE and AMD challenges even in the presence of only β-adrenergic receptor blockade. The desensitization was more apparent after addition of ganglionic blockade and further augmented after addition of NE-uptake blockade. One potential mechanism to explain this is that α1-adrenergic receptor agonists not only induce reflex buffering but also enhance the release of endogenous NE after chronic α1-adrenergic receptor stimulation. Thus, in the absence of AMD pumps, acute AMD challenges in the presence of β-adrenergic receptor plus NE-uptake blockade induced greater increases in MAP and TPR compared with responses in the presence of β-adrenergic receptor blockade, suggesting that the pressor and vasoconstrictor effects of AMD are due to both a direct action and to an indirect action involving release of endogenous NE.56–58 The indirect action of AMD may be due to its ability to induce carrier-mediated outward transport of NE.59 In support of this postulate, plasma NE levels in the AMD pump group were higher than in the saline pump group.

It is important to note that the absolute increases in MAP and TPR induced by acute NE challenges after chronic α1-adrenergic receptor stimulation were similar, whether examined in the presence of β-adrenergic receptor blockade alone, when β-blockade was combined with ganglionic blockade, or when it was combined with both ganglionic and NE-uptake blockade (Table 2). However, the increases in MAP and TPR induced by acute NE challenges before chronic α1-adrenergic receptor stimulation increased progressively with addition of ganglionic and NE-uptake blockade, respectively. Therefore, the different extent of physiological desensitization observed under these conditions was due to different responses to acute NE challenges observed before AMD pump implantation. Thus, both reflex mechanisms in general and uptake mechanisms in particular were responsible for the abnormally low

**TABLE 4. α1-Adrenergic Receptor Density and Na,K-ATPase in Aortic Membrane Preparations and Membrane Preparations From the Mesentery**

<table>
<thead>
<tr>
<th></th>
<th>Saline pumps</th>
<th>Amidephrine pumps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[3H]Prazosin</td>
<td>[3H]Prazosin</td>
</tr>
<tr>
<td>βmax (fmol/mg)</td>
<td>Kd (nM)</td>
<td>βmax (fmol/mg)</td>
</tr>
<tr>
<td>Aortic preparation</td>
<td>177±14</td>
<td>191±39</td>
</tr>
<tr>
<td>0.14±0.01</td>
<td>0.29±0.07*</td>
<td>1.01±0.11</td>
</tr>
<tr>
<td>Na,K-ATPase</td>
<td>1.08±0.11</td>
<td>1.55±0.13</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Mesenteric preparation</td>
<td>18.4±1.4</td>
<td>8.2±1.0†</td>
</tr>
<tr>
<td>0.46±0.03</td>
<td>0.39±0.07</td>
<td>6.0</td>
</tr>
<tr>
<td>Na,K-ATPase</td>
<td>1.65±0.14</td>
<td>1.55±0.13</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

βmax, Receptor density; Kd, dissociation constant. Na,K-ATPase is measured in μmol/P, per hour per milliliter.

*p<0.05 difference between amidephrine and saline pump.

†p<0.01 difference between amidephrine and saline pump.
responses to NE challenge in the absence of ganglionic plus uptake blockade. The reflex mechanisms and uptake mechanisms obviously induced lesser effects after chronic α₁-adrenergic receptor stimulation and thereby permitted expression of desensitization to the NE challenges. If the reflex and uptake mechanisms had both been intact after chronic α₁-adrenergic receptor stimulation, desensitization would have been apparent in the absence of ganglionic or catecholamine-reuptake blockade. Furthermore, if these mechanisms had both been intact, it is likely that baseline levels of MAP and TPR would not have remained elevated after chronic α₁-adrenergic receptor stimulation. These results suggest that chronic α₁-adrenergic receptor stimulation impairs reflex and uptake mechanisms, which play a role in the manifestation of physiological desensitization. Impaired reflex buffering could result from the chronic increase in pressure, whereas impaired uptake may be due to binding of AMD to the neuronal NE transporter, which would make it unavailable for inward NE transport.39

The studies in the isolated mesenteric artery rings provided further support for the concept that reflex and uptake mechanisms play a critical role in masking desensitization to NE challenges. In these in vitro experiments, in which vasoconstrictor responses occur in the absence of reflex buffering and uptake mechanisms, depressed responsiveness of isolated mesenteric artery rings to α₁-adrenergic receptor stimulation with acute challenges of NE, as well as with AMD and PE, were readily apparent. Maze et al19 infused epinephrine acutely into rabbits and examined this hyperadrenergic model for in vivo and in vitro evidence of desensitization. Their results also indicated that after an epinephrine infusion of 2 hours, a significant subsensitivity in the pressor response to PE is produced in conscious rabbits. In the corresponding in vitro experiments, they found a marked and prolonged loss in sensitivity of rabbit aortic rings to α₁-adrenergic receptor–mediated vascular contraction.19

Recently, Hiremath et al24 found desensitization of α₁-adrenergic receptor–induced vascular smooth muscle contraction both in the aortas from pheochromoctoma-bearing rats and also in aortic ring segments exposed for 6 hours in vitro to PE. In that study it was demonstrated that removal of the endothelium fully restored maximal responsiveness in both models, suggesting a major contribution of the endothelium in desensitization of α₁-adrenergic receptor–mediated smooth muscle contraction.24 In contrast, in the present study there were no significant differences in the maximal contractile responses of isolated mesenteric artery rings whether prepared with or without endothelium. Furthermore, responses of isolated mesenteric rings to acetylcholine were not different in the group with chronic α₁-adrenergic receptor stimulation compared with saline controls in either the presence or absence of endothelium. Thus, endothelial mechanisms do not appear to be critical in mediating desensitization in this model. The reason for the discrepancy between our study and that of Hiremath et al24 is not clear but may be related to
differences in species, in model, or to the exposure period to the \( \alpha_1 \)-adrenergic receptor agonist.

Another major goal of the present study was to determine the potential biochemical mechanisms responsible for the physiological desensitization, e.g., reduced \( \alpha_1 \)-adrenergic receptor density. Strittmatter et al.\(^{41} \) showed that the rapid desensitization, i.e., on the order of minutes, of the \( \alpha \)-adrenergic receptor of the rat parotid gland is associated with a decrease in \(^3\)H-dihydroergocryptine binding. Another difference in the results of the current investigation was that in contrast to what has been published in acute and isolated experiments,\(^{11,19,23,24} \) \( \alpha_1 \)-adrenergic receptor desensitization as assessed physiologically in the conscious animal is not a rapid phenomenon. Actually, physiological desensitization was not apparent for at least 4 days after chronic \( \alpha_1 \)-adrenergic receptor exposure. Colucci et al.\(^{13} \) demonstrated that mesenteric artery preparations from rats treated with epinephrine for 4 days had fewer numbers of \( \alpha \)-adrenergic receptor binding sites than did preparations from normal rats. More recently, Izzo et al.\(^{40} \) demonstrated rapid desensitization of \( \alpha_1 \)-adrenergic receptors in smooth muscle after exposure to NE. Gengo et al.\(^{41} \) reported that chronic PE infusion in rats for 6 days resulted in a decrease of cardiac \( \alpha_1 \)-adrenergic receptor density. Regulation of the density of \( \alpha_1 \)-adrenergic receptors on a vascular smooth muscle cell must be a function of the relative rates of receptor synthesis and degradation and also likely involves a cycling of receptors between cell surface and nonsurface accessible sites.\(^{41} \)

Another major finding of the current investigation was that there are different mechanisms of \( \alpha_1 \)-adrenergic receptor desensitization in large (conductance) vessels and small (resistance) vessels. The \( \alpha_1 \)-adrenergic receptor density, as determined by \(^3\)Hprazosin binding in membrane preparations from vessels in the mesentery, which contains intermediate-sized vessels to capillaries, was decreased without any change in \( K_d \) after chronic AMD infusion. On the other hand, the \( K_d \) of \(^3\)Hprazosin binding in aortic membranes was increased without decreases in \( \alpha_1 \)-adrenergic receptor density, suggesting a reduction in the affinity of the receptor. This concept was supported by the agonist binding experiments, which demonstrated a reduced number of \( \alpha \)-adrenergic receptors binding agonists with high affinity. There may be regional differences in the quantitative relation between the physiological responses of vascular smooth muscle and the number and affinity of its receptors, e.g., the dog thoracic aorta is known to have a large receptor reserve and many small arteries do not.\(^{42,43} \) Thus, not only the reduction of receptors but also other agonist-induced alterations in coupling to second messengers may contribute to the mechanism of desensitization to \( \alpha_1 \)-adrenergic receptor--mediated pressor and vasoconstrictor responses.

Another potential explanation for the separate mechanisms of \( \alpha_1 \)-adrenergic receptor desensitization in vessels of different caliber is the possibility that NE concentration at the receptor in these vessels differs during chronic \( \alpha_1 \)-adrenergic receptor stimulation. Chronic \( \alpha_1 \)-adrenergic receptor stimulation may induce the release of endogenous catecholamines,\(^{36} \) a phenomenon that was also observed in this study. The concentration of NE is greater in the mesenteric artery than the aorta, whereas the uptake of NE is less in the mesenteric artery than the aorta.\(^{44} \) Furthermore, in the aorta the distance from the nerve ending to the vascular smooth muscle is relatively large, implying that the neurotransmitter NE would reach the vascular smooth muscle in lower concentrations.\(^{43} \) All these factors taken together could act to produce higher concentrations of NE at the receptor site in mesenteric vessels than the aorta and could explain why \( \alpha_1 \)-adrenergic receptor downregulation occurs in mesenteric but not in aortic membranes. Finally, it is also possible to speculate that different \( \alpha_1 \)-adrenergic receptor subtypes\(^{45,46} \) respond differently to chronic \( \alpha_1 \)-adrenergic receptor stimulation.

In summary, chronic \( \alpha_1 \)-adrenergic receptor stimulation results in physiological desensitization to selective \( \alpha_1 \)-adrenergic receptor agonists and to the neurotransmitter NE in vitro. The in vitro studies did not demonstrate an important role for the endothelium in mediating desensitization. In the conscious animal, physiological desensitization to the neurotransmitter NE was more difficult to demonstrate, since it was masked by reflex mechanisms and neuronal uptake mechanisms that act to reduce the concentration of NE at the postjunctional receptor site. Thus, although endothelial mechanisms do not appear important, both autonomic reflex and biochemical mechanisms are altered by chronic \( \alpha_1 \)-adrenergic receptor stimulation in the conscious dog; the altered autonomic mechanisms affect the physiological expression of desensitization, whereas separate biochemical mechanisms observed in vessels of different caliber mediate the desensitization. These complex mechanisms are important to consider in order to understand peripheral vascular control in pathophysiological states involving chronically elevated catecholamines or sympathetic tone.

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