Vascular Injury Augments Adrenergic Neurotransmission

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**Background** We have observed persistent desensitization to exogenous norepinephrine after balloon injury. We postulated that this desensitization may be due to a local increase in the release of neuronal norepinephrine.

**Methods and Results** New Zealand White rabbits underwent left iliac artery angioplasty; 4 weeks later, both iliac arteries were harvested. Maximal response to exogenous norepinephrine was reduced in injured compared with uninjured vessels (12.3±1.0 g versus 10.3±1.5 g; n=7, P=.056). By contrast, response to electrical stimulation (to induce neuronal norepinephrine release) was significantly greater in injured tissues (36±7% versus 14±3%; values expressed as percent of maximal contraction to exogenous norepinephrine; P=.025). Direct measurement of tissue norepinephrine revealed a threefold increase 4 weeks after injury (1236±410 versus 466±97 pg/mg; injured versus noninjured). To determine if desensitization to exogenous norepinephrine was due to a persistent increase in neuronal norepinephrine release, the experiments were repeated after chemical sympathectomy using 6-hydroxydopamine (6-OHDA) (65 mg/kg). To determine if activation of vascular angiotensin II contributed to facilitation of adrenergic neurotransmission, other animals received ramipril (RAM; 1 mg/kg per day). Both treatments were initiated 7 days before angioplasty. In the 6-OHDA group there was no evidence of desensitization, judged by maximal response to exogenous norepinephrine (7.5±0.6 versus 7.5±0.8, noninjured versus injured). Similar results were obtained in RAM animals (9.9±0.8 versus 9.6±1.2, noninjured versus injured).

**Conclusions** This is the first study to demonstrate enhanced adrenergic neurotransmission after balloon injury. The facilitation of adrenergic neurotransmission may be due to increased local concentrations of angiotensin II and is associated with desensitization to exogenous norepinephrine. (*Circulation*, 1994;89:777-784.)

**Key Words** restenosis norepinephrine angiotensin

We and others have demonstrated that balloon injury induces alterations in vascular reactivity that are secondary to changes in vascular smooth muscle and endothelial function. Immediately after balloon injury, there is a marked reduction in the responsiveness to vasoconstrictor agents. Within several days, the vascular smooth muscle recovers some contractile function, and after 2 to 4 weeks, contractile responses to potassium chloride have largely recovered. However, we have shown previously that an aberration of adrenergic response persists up to 6 weeks after balloon injury and is manifested by a reduction in the sensitivity to norepinephrine as well as a suppression of the maximal response.

A desensitization to exogenous catecholamines is observed in tissues that are repeatedly stimulated. We hypothesized that the desensitization after vascular injury was due to a local and sustained increase in adrenergic neurotransmission in the area of injury. We further postulated that this local facilitation of adrenergic neurotransmission was due to activation of the tissue renin-angiotensin system. (It is known that after balloon injury, there is increased expression of vascular angiotensinogen and angiotensin-converting enzyme [ACE]; it is also known that angiotensin II [Ang II] facilitates the release of norepinephrine from adrenergic nerve terminals.) Accordingly, the present study was designed to confirm our previous observations that adrenergic response is downregulated after balloon injury. Furthermore, we tested the hypothesis that this abnormality was associated with an enhancement of adrenergic neurotransmission. Finally, we sought to determine if the persistent and local increase in catecholamine release may be due to activation of the vascular renin-angiotensin system.

**Methods**

**Animals**

Male New Zealand White rabbits (weight, 2.5 to 3.0 kg) were entered into the study after a 1-week period of acclimation in the housing facilities of the Stanford Department of Laboratory Animal Medicine (DLAM), during which time the animals were fed normal rabbit chow and received water ad libitum. All animals were inspected before the study by the DLAM veterinarian and monitored daily by DLAM technicians and the investigators. All experimental protocols were approved by the Administrative Panel on Laboratory Animal Care of Stanford University and were performed in accordance with the recommendations of the American Association for the Accreditation of Laboratory Animal Care.

Before balloon angioplasty, animals were anesthetized using a mixture of ketamine (5 mg/kg) and Rompun (35 mg/kg) given in an initial intravenous injection. The level of anesthesia was continuously monitored during the procedure, and additional anesthetic was given as needed. The left iliac artery was used for balloon injury to allow the use of the contralateral artery as a noninjured control vessel.

The left superficial femoral artery was exposed, isolated, and the distal portion was ligated with a small surgical clip. A 2F Fogarty arterial embolectomy catheter (Baxter Health Care Co, Santa Ana, Calif) was inserted into the artery and...
advanced proximally into the iliac artery past the bifurcation into the aorta. The balloon was inflated, then withdrawn while inflated, then deflated. Three successive withdrawals with the inflated balloon were made. We have demonstrated previously that this degree of injury completely denudes the endothelium and injures the underlying vascular smooth muscle.2 The proximal portion of the superficial femoral artery was then ligated, and the site was checked for hemostasis. Animals were allowed to recover from anesthesia before returning to their cages. Four weeks later, the animals were killed, and the iliac arteries were harvested for studies of vascular reactivity.

Vascular Reactivity

Twenty-eight days after balloon injury, animals were given an overdose of intravenous pentobarbital. Both iliac vessels were removed and immediately placed into oxygenated physiological saline solution (PSS) that was composed of the following (mmol/L): NaCl, 118.3; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25.0; EDTA, 0.026; glucose, 11.1. In some experiments, the KCl concentration was increased by an equimolar replacement of NaCl with KCl. Adherent fat and connective tissue was removed, and the iliac arteries were cut into rings of 3- to 4-mm width. Two pairs of rings (left and right iliac arteries) from the proximal and distal iliac artery were mounted horizontally on stainless steel stirrups through the lumen and connected to force transducers. The vascular rings were suspended in the organ chambers filled with oxygenated PSS at 37°C. Over a period of 60 minutes, rings were progressively stretched to the optimal point of their length-tension relation (determined previously to be 2 g).2,3

To determine the response to neuronally released endogenous norepinephrine, the iliac rings were placed between two parallel platinum plate electrodes to stimulate adrenergic nerve endings, as described previously.2,7 Frequency-response curves to electrical stimuli were obtained using electric impulses (square waves with amplitude of 9 V, duration of 2 milliseconds, and frequency of 0 to 16 Hz) provided by a DC amplifier triggered by a stimulator (Grass SD9). After electrical stimulation studies, the vessels were washed repeatedly with fresh oxygenated PSS and allowed to return to their resting tension. To determine the response of the vascular rings to exogenous norepinephrine, rings were used for response curves, which were obtained by adding to the bath increasing concentrations of norepinephrine (in half-log increments from 10^-10 to 10^-4 mol/L). After the maximal response to exogenous norepinephrine had been obtained, the vascular rings were washed repeatedly with fresh PSS for 60 minutes, by which time they had regained their resting tension. To determine the ability of the vascular smooth muscle to contract to a non-receptor-mediated agonist, the rings were exposed to increasing concentrations of KCl (40 to 80 mmol/L) in the presence of propranolol (10^-6 mol/L) and phentolamine (10^-6 mol/L) to block the action of any neuronally released norepinephrine. All drug dilutions were prepared in distilled water made fresh the day of the experiment and were allowed to sit on ice. Norepinephrine bitartrate and 6-OHDA were purchased from the Sigma Chemical Co (St Louis, Mo). Ramipril was a gift of the Upjohn Co (Kalamazoo, Mich).

Total Norepinephrine Assay

The initial vascular reactivity studies revealed that vascular injury was associated with a reduced response to exogenous norepinephrine. To test the hypothesis that vascular injury induces a persistent increase in local neuronal norepinephrine, tissue norepinephrine was measured. Iliac artery segments were homogenized in a glass Potter-Elvenhjem homogenizer with 1 mL of 0.1 mol/L HClO4 at 0°C to 4°C. The suspension was then centrifuged at 16 000g in a microfuge for 5 minutes. The resulting supernatant was then filtered through a 0.2-mm filter before analysis.

Norepinephrine content was determined using high-pressure liquid chromatography and electrochemical detection using an ESA coulochem 5100A detector equipped with a 3-mm C-18 reverse phase column. The mobile phase consisted of 98% 0.5 mol/L NaHPO4 · H2O, pH 3.6, 1.2 mmol/L 1-octane sulfonic acid, 0.2 mmol/L EDTA, 2% methanol, and was cycled at a flow rate of 1.5 mL/min. Graded concentrations of norepinephrine bitartrate were used as standards, and 3,4-dihydroxyamphetamine (DHBA) served as the internal standard. All standard solutions were prepared fresh the day of the analysis. Retention times were 5.2 minutes for norepinephrine and 14.8 minutes for DHBA.

Pharmacological Regimens. The initial in vitro studies of vascular reactivity revealed that 4 weeks after vascular injury, there was a desensitization to exogenous norepinephrine. This abnormality was associated with an increased response to stimulation of adrenergic nerve endings. Furthermore, the measurements of tissue norepinephrine revealed an increase 4 weeks after balloon injury. We therefore postulated that the desensitization to exogenous norepinephrine was due to persistently increased local release of neuronal norepinephrine after the vascular injury. To further test this hypothesis, we repeated the vascular injury in animals that were chemically denervated. We reasoned that if the desensitization to norepinephrine was due to a persistent increase in local catecholamine release induced by the vascular injury, this effect could be prevented by sympatholysis before the vascular injury. Accordingly, some animals received 6-hydroxydopamine (6-OHDA) as an initial intravenous bolus dose of 5 mg/kg dissolved in 15 mL of sterile water 1 week before balloon injury. Because of the sympathetic discharge that occurs during the infusion, the animals were sedated with ketamine during administration of 6-OHDA. The initial infusion was followed 24 to 48 hours later by a subsequent intravenous dose of 30 mg/kg 6-OHDA. This dose of 6-OHDA has previously been demonstrated to produce a chemical denervation of the sympathetic nervous system which persists for 7 to 10 days.2,24 To maintain the sympathetic denervation throughout the course of the study, an additional dose (30 mg/kg) was given 7 days after the balloon injury.

The above studies suggested that adrenergic desensitization 4 weeks after vascular injury was due to persistent and local increases in neurotransmitter release. A number of paracrine substances are known to facilitate adrenergic neurotransmission. Ang II activates its corresponding receptor on the prejunctional nerve membrane to facilitate neuronal release of norepinephrine.16-20 It is also known that the vascular renin-angiotensin system is activated after balloon injury.20-25 We reasoned that local elaboration of Ang II after balloon injury might play a role in facilitating adrenergic neurotransmission and thereby induce the desensitization to exogenous norepinephrine. Therefore, inhibition of converting enzyme activity should interfere with Ang II facilitation of neurotransmission and thereby restore the response to norepinephrine. Accordingly, ramipril was dissolved in the drinking water and given in a dose of 1 mg/kg per day. This dose was chosen because it did not result in a significant drop in the blood pressure. The animals were allowed to drink water ad libitum. Water consumption was monitored daily, and the concentration of ramipril was adjusted to administer the chosen dose. Ramipril treatment was initiated 7 days before balloon injury and was continued for the duration of the experiment. Blood pressure measurements at baseline, before balloon injury, and at 4 weeks after balloon injury were obtained. For these measurements, the central ear artery was cannulated, and the conscious animal was allowed to rest quietly for at least 5 minutes; when the blood pressure had stabilized, the intra-arterial pressure was recorded.

ACE Activity

To confirm that the oral administration of ramipril (1 mg/kg per day) had a measurable effect, plasma ACE activity was
determined. Blood samples were drawn directly from the heart after the animal was killed into heparinized collecting tubes. The plasma was centrifuged and stored at −70°C until analyzed for ACE activity.

ACE activity was measured by the inhibitor binding technique as described by Cheung and Cushman.25 Fifteen milliliters of plasma was aliquoted into 135 mL of distilled water. One hundred milliliters of the substrate hippuryl-L-histidyl-L-leucine (Hip-His-Leu) was added to each sample then incubated for 30 minutes at 37°C. The reaction was stopped with 1.45 mL of 0.28N NaOH at room temperature. A graded series of concentrations of histidyl-L-leucine (H-L) were used to generate a standard curve. To standards and all plasma samples, 100 µL O-phtaldialdehyde was added. The solution was then vortexed, incubated for 10 minutes at room temperature, and then covered with foil. The reaction was stopped with 200 µL of 3N HCl and incubated for 30 minutes at room temperature, then covered with foil. All samples and standards were centrifuged for 5 minutes at 2000g, and the supernatant was analyzed by spectrofluorimetry (excitation wavelength of 364 nm and emission wavelength of 486 nm). The ACE activity is expressed as nmol · min⁻¹ · mL⁻¹.

**Histological Studies**

On completion of the vascular reactivity studies, the iliac rings were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with an elastic van Gieson stain for light microscopy and histomorphometric measurements. Measurements of intimal and medial cross-sectional areas (millimeters squared) were made by a skilled observer blinded to the treatment groups. An eyepiece grid with 100 counting points (line intersections), each 0.4 mm apart, was used.26,27 Total grid area (millimeters squared) was determined by measurement of grid dimensions with a stage micrometer at the same microscope magnification used for point counts. Total area of the intima (or media) was defined as the number of grid points overlying the intima (or media). Area of each was calculated by the formula 0.01 (P × grid area, where P is the number of points counted over the intima (or media). At least five cross sections from each vascular segment were analyzed, and the values were averaged.

One segment from a control animal (injured), which contained thrombus that made assessment of intimal thickening difficult, was excluded from analysis.

**Data Analysis**

Data are expressed as mean±SEM. Dose-response curves to norepinephrine and frequency-response curves to electrical stimuli are expressed as contractions in grams above the resting tension. The dose and frequency response curves are characterized by determining the maximal response to the drug or stimulus. Statistical analysis to detect significant differences in vascular reactivity between injured and noninjured segments within the same experimental group was by Student's t test for paired observations (two-tailed). Comparisons between the three experimental groups were made by ANOVA.

**Results**

**Vascular Reactivity Studies**

**Response to Exogenous and Neuronally Released Norepinephrine**

Norepinephrine induced concentration-dependent increases in tension in both the injured and uninjured iliac arteries. The maximal response to exogenous norepinephrine was reduced in the injured vessels (12.3±1.0 versus 10.3±1.5; P=.056; Fig 1A).

By contrast, the response to neuronal stimulation was reversed. Electrical stimulation induced a frequency-dependent contraction caused by neuronally released norepinephrine, which tended to be greater in the injured tissues (Fig 1B). When the response to neuronally released norepinephrine was expressed as a percentage of the maximal response to exogenous norepinephrine, the differences between the injured and noninjured tissues were even more apparent (Fig 2). Expressed in this way, the maximal response to electrical stimulation in the injured tissues was significantly greater than that in the noninjured segments (36±7% versus 14±3%; n=7 in both groups; P=.025).

In contrast to the adrenergic response, there were no differences between the two groups in the responsiveness to potassium chloride, a nonadrenergic agonist of vascular smooth muscle contraction (Table 1).

**Tissue Norepinephrine**

The initial vascular reactivity studies suggested that the desensitization to exogenous norepinephrine after vascular injury was due to an increase in local release of neuronal norepinephrine. To confirm this hypothesis, tissue norepinephrine was directly measured in injured
and noninjured iliac arteries 4 weeks after balloon angioplasty. In the uninjured iliac artery, norepinephrine concentration was 466±97 pg/mg (n=12). By contrast, there was a threefold elevation in tissue norepinephrine in the injured artery to 1236±410 pg/mg (n=12, P<.05).

**Effect of Chemical Sympathectomy**

To determine if the reduced responsiveness to exogenous norepinephrine in the injured vessels was due to local increases in neurally released norepinephrine, the influence of neuronal norepinephrine was removed by chemical sympathectomy using 6-OHDA. In vascular segments from the 6-OHDA-treated animals, the response to electrical stimulation was essentially abolished in both the injured and noninjured segments. In the noninjured vascular segments, the maximal response to electrical stimulation was only 0.16±0.08 g (2.1% of the maximal response to exogenous norepinephrine in these tissues). In the injured segments, the maximal response to electrical stimulation was only 0.04±0.04 g (0.5% of the maximal response to norepinephrine in these tissues; Table 2). These studies confirmed that the 6-OHDA treatment had induced a successful chemical denervation of the sympathetic nerve endings in the vessel wall.

In the 6-OHDA-treated animals, contractions to exogenous norepinephrine induced concentration-dependent increases in tension in both the injured and noninjured tissues; the dose-response curves were identical with maximum contractions that were not different between the two groups (7.47±0.65 g versus 7.54±0.82 g, noninjured versus injured; n=5 in each group; P=NS; Fig 3).

**Role of Ang II in Adrenergic Alteration**

To determine if an activation of the vascular renin-angiotensin system in the vascular wall may be involved in the alteration of adrenergic response after balloon injury, one group of animals received ramipril before and after balloon injury until the vessels were harvested for study. The administration of ramipril reduced plasma ACE activity by 73% (from 76.6±5.1 to 21.3±3.9 nmol·min⁻¹·mL⁻¹; vehicle [n=5] versus ramipril [n=3], respectively). In vascular segments from ramipril-treated animals, exogenous norepinephrine induced concentration-dependent increases in tension in both injured and noninjured segments that were not different (maximal response, 9.99±0.86 g versus 9.59±1.2 g, noninjured versus injured; n=7 in each group; P=NS; Fig 4). Responses to electrical stimulation were not different between the injured and noninjured tissues (Table 2). Likewise, contractions to potassium chloride were not different between the two groups (Table 1).

**Histomorphometric Analysis**

In all experimental groups, the noninjured iliac arteries had no intimal thickening with intimal/medial ratios that approached zero. In all three groups, the balloon-injured arteries showed varying degrees of intimal thickening (Table 3). The intimal/medial ratio of injured segments from animals treated with ramipril was significantly reduced in comparison to 6-OHDA animals (0.97±0.15 versus 0.39±0.16, 6-OHDA treatment versus ramipril treatment; P=.03). This difference was largely due to a reduction in medial thickness in the 6-OHDA–treated group in comparison with the ramipril-treated animals (Table 3). There was no significant difference in intimal/medial ratio between the control (0.64±0.12) and either of the experimental groups.

**Discussion**

This is the first study to demonstrate a facilitation of adrenergic neurotransmission after balloon injury. This enhancement of adrenergic neurotransmission is associated with a desensitization to exogenous norepinephrine. Our data also suggest that local activation of the vascular renin-angiotensin system may play a role in the

**TABLE 1. Contractions to KCl in Injured and Noninjured Iliac Arteries**

<table>
<thead>
<tr>
<th>KCl, mmol/L</th>
<th>Control n=7</th>
<th>6-OHDA n=5</th>
<th>Ramipril n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noninjured</td>
<td>Injured</td>
<td>Noninjured</td>
</tr>
<tr>
<td>40</td>
<td>5.4±0.9</td>
<td>4.7±1.0</td>
<td>4.1±0.5</td>
</tr>
<tr>
<td>60</td>
<td>5.6±0.9</td>
<td>5.2±1.0</td>
<td>4.4±0.6</td>
</tr>
<tr>
<td>80</td>
<td>5.4±0.8</td>
<td>4.9±0.9</td>
<td>4.2±0.7</td>
</tr>
</tbody>
</table>

6-OHDA indicates 6-hydroxydopamine. Data are expressed as grams above resting tension and are shown as mean±SEM.
enhancement of adrenergic neurotransmission. Previous studies have examined the role of endothelial and smooth muscle cell dysfunction after balloon injury. The current investigation suggests a new avenue for exploration in understanding the pathophysiological mechanisms of vascular injury.

We have observed previously that balloon injury induces a persistent and progressive adrenergic dysfunction in the rat aorta. This was manifested by reduced contraction to exogenous norepinephrine of vascular rings harvested from injured rat aortas. This abnormality persisted for up to 6 weeks after balloon injury. This abnormality was not due to a generalized depression of vascular smooth muscle contractile function, because the sensitivity to potassium chloride was restored at a time when response to norepinephrine was depressed. The major hypothesis of the current investigation was that the desensitization to exogenous norepinephrine was secondary to local and sustained increases in neuronal release of norepinephrine. A desensitization to exogenous catecholamines can be seen in tissues that are repeatedly stimulated. Desensitization of adrenergic response may occur through several mechanisms including phosphorylation of the adrenergic receptor, sequestration and degradation of the receptor, and/or a reduction in its synthesis. We hypothesized that one or more of these processes may be initiated after balloon injury caused by a local and sustained increase in neurotransmission in the area of injury.

In the present study, we confirmed our previous observations (using a different animal model) that vascular injury results in a persistent desensitization to exogenous norepinephrine. Four weeks after balloon injury, the response of the injured segment to norepinephrine was reduced in comparison to the uninjured vascular segment (Fig 1A). By contrast, the response to potassium chloride was not altered 4 weeks after balloon injury. Potassium chloride induces a contraction of the vascular smooth muscle that is due to depolarization and influx of extracellular calcium; this contraction is independent of adrenoceptor function.

To stimulate the neuronal release of norepinephrine, we used well-established parameters of electrical stimulation. We were surprised by the marked enhancement of response to neuronal stimulation in the injured tissues (Fig 1B). When the response to electrical stimulation was expressed as a percent of maximal response of the tissues to exogenous norepinephrine (to take into account the desensitization of the vascular smooth muscle to norepinephrine), the maximal response in the injured tissues was greater than twice that of the uninjured vessels (Fig 2). These data suggest that the

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**Table 2. Effect of Balloon Injury on Response to Electrical Stimulation in Animals Treated With 6-OHDA or Ramipril**

<table>
<thead>
<tr>
<th>Electrical Stimulation, Hz</th>
<th>6-OHDA (n=5)</th>
<th>Ramipril (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noninjured</td>
<td>Injured</td>
</tr>
<tr>
<td>2</td>
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<td>0.01±0.01</td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>8</td>
<td>0.11±0.05</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>16</td>
<td>0.15±0.08</td>
<td>0.04±0.02</td>
</tr>
</tbody>
</table>

6-OHDA indicates 6-hydroxydopamine. Data are expressed as grams above resting tension and are shown as mean±SEM.

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**Fig 3.** Dose-response curves to exogenous norepinephrine in injured and noninjured iliac arteries from animals treated with 6-hydroxydopamine (6-OHDA). This sympatolytic agent abolished neuronal release of norepinephrine in these vascular segments, as determined by the response to electrical stimulation (Table 2). In the absence of neuronal influence, there is no difference between the injured and noninjured vascular segments. This is consistent with the hypothesis that the desensitization to exogenous norepinephrine in injured tissues (Fig 1) is due to chronic increases in the local release of catecholamines in the injured segments.

**Fig 4.** Dose-response curves to exogenous norepinephrine in injured and noninjured iliac arteries harvested from animals treated with ramipril. The responsiveness to norepinephrine in the injured tissues has been restored by the converting enzyme inhibitor ramipril. This is consistent with the hypothesis that activation of the renin-angiotensin system after vascular injury plays a role in the increased neuronal release of endogenous norepinephrine and subsequent desensitization to the neurotransmitter.
neuronal release of norepinephrine is facilitated after balloon injury. Our central hypothesis was further strengthened by direct measurement of tissue norepinephrine levels. Four weeks after vascular injury, we observed a threefold increase in the concentration of tissue norepinephrine. The increase in local catecholamine levels could be explained by a number of mechanisms including an increase in the number of presynaptic nerve terminals, an enhanced synthesis and release of neurotransmitter from individual nerve terminals, a reduction in the uptake or metabolism of locally released neurotransmitter, or from increased uptake of circulating norepinephrine.

If enhanced local release of norepinephrine is responsible for the desensitization to exogenous norepinephrine, then removal of this neuronal influence should restore the responsiveness to exogenous norepinephrine. To test this hypothesis, we induced a chemical sympathectomy by administering 6-OHDA to some animals. The efficacy of the denervation was established by studying the response of the vascular rings to electrical stimulation, which was essentially abolished. In the absence of neurally released norepinephrine, we found that the response to exogenous norepinephrine of the injured and uninjured vessels was not different (Fig 3). Generally, 6-OHDA treatment induces a supersensitivity to exogenous norepinephrine. We did not note an increased sensitivity to norepinephrine in the iliac arteries of chemically denervated rabbits. This probably is due to a countervailing effect on vascular smooth muscle reactivity, because the contractions to potassium chloride were also depressed in this group (Table 1). The direct effect of 6-OHDA treatment to reduce iliac artery contractility may be in part due to an effect on vascular smooth muscle growth, because the medial thickness was reduced in the injured vessels of these animals (Table 3). Our findings are similar to those of Fronek,28 who observed that chemical denervation with 6-OHDA was associated with increased sensitivity to exogenous norepinephrine in most vascular beds of the rabbit but not the auricular or iliac arteries. Nevertheless, in our study, adrenergic denervation was associated with an equalization of the response to exogenous norepinephrine in the injured and uninjured vessels. This is consistent with our central hypothesis that vascular injury induces a local increase in tissue catecholamines that is manifested by a desensitization to exogenous norepinephrine.

The mechanism by which adrenergic neurotransmission is facilitated after balloon injury may involve the vascular renin-angiotensin system. Ang II is known to facilitate the release of norepinephrine from adrenergic nerve endings. In the isolated portal vein and pulmonary artery of the rabbit, Ang II augments the release of tritiated norepinephrine evoked by electrical stimulation.16,17 Ang II enhances the appearance of adrenergic neurotransmitters in the venous effluent from canine skin and kidney during stimulation of the sympathetic nerves.18,19 This probably is due to direct interaction of Ang II with its corresponding receptor on the prejunc- tional adrenergic nerve terminal, the existence of which has been previously demonstrated by autoradiographic studies.20 Finally, it is known that after balloon injury, there is increased expression of vascular angiotensinogen and ACE.10,12 Evidence suggests that this activation of vascular renin-angiotensin contributes to the alterations in vascular structure after balloon injury.13-15

If the desensitization to exogenous norepinephrine is due to Ang II-facilitated release of neuronal norepinephrine, inhibitors of Ang II synthesis should restore the response to exogenous norepinephrine. Indeed, in the ramipril-treated animals, the response to exogenous norepinephrine is not different in the injured segments (Fig 4). This finding is consistent with the hypothesis that injury-induced activation of the vascular renin-angiotensin system leads to higher tissue levels of Ang II, which facilitates local norepinephrine release. However, we do not exclude other effects of ramipril (ie, on local kinin concentrations) that could be responsible for the observed effect. To summarize, the present investigation confirms our previous observation that balloon injury induces a persistent desensitization to exogenous norepinephrine. This aberration of adrenergic response appears to be due to a local facilitation of neurally released neurotransmitter. Injury-induced activation of the vascular renin-angiotensin system may play a role in the facilitated adrenergic neurotransmission.

In light of these observations, the importance of neurotransmitters in the pathophysiology of restenosis after balloon angioplasty must be considered. Catecholamines have long been known to stimulate smooth muscle cell growth. In vitro studies have revealed that catecholamines promote growth of cultured smooth muscle cells in association with increased expression of the proto-oncogene c-myc.29-31 In vivo studies suggest a role for local catecholamines in vascular growth. Bevan32,33 surgically denervated the central carotid artery of the rabbit and observed a reduction in medial growth manifested by reduced wall thickness and protein content.32,33 Hart et al34 found that the characteristic increase in vessel wall to lumen ratio that is observed in hypertensive arteries does not occur after sympathectomy. An infusion of Ang II increases DNA synthesis in vascular smooth muscle of the rat thoracic aorta; this effect is antagonized by the α1-adrenergic antagonist prazosin.35 The mitogenic effects of catecholamines on

<table>
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<tr>
<th>Measurement</th>
<th>Vehicle Group (n=7)</th>
<th>Ramipril Group (n=8)</th>
<th>6-OHDA Group (n=5)</th>
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<tr>
<td></td>
<td>Injured</td>
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<td>Intimal area, mm²</td>
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<td>I/M</td>
<td>0.64±0.12</td>
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<td>0.30±0.16</td>
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6-OHDA indicates 6-hydroxydopamine; I/M, intimal/medial ratio.
*Significantly different from value in ramipril group by ANOVA (P<.05).
the vessel wall may be due to regulation of growth-related gene expression by the α1-adrenergic receptor.36

To summarize, the evidence is strong that endogenous catecholamines modulate the growth of medial smooth muscle cells. It remains to be established whether or not endogenous catecholamines promote the growth of neointimal cells after vascular injury. However, Fingerle and colleagues37 have studied the effects of two adrenergic antagonists, prazosin and urapidil, on myointimal hyperplasia after balloon injury to rat carotid arteries. They observed a reduction in the size of the neointima in urapidil-treated animals as compared with control animals and reduced neointima formation in both prazosin-treated and urapidil-treated animals when intimal DNA content was measured. Their data suggest that antagonism of the adrenergic system can inhibit myointimal hyperplasia. In support of their findings, we found that inhibition of converting enzyme appeared to restore normal adrenergic neurotransmission and that this effect was associated with a trend toward reduced myointimal hyperplasia, an observation previously reported by others.10,38 By contrast, we did not observe a decrease in the degree of myointimal hyperplasia in the injured vessels of our chemically denervated animals. This result could be explained by a direct effect of 6-OHDA on vascular smooth muscle cell growth.

We and others have shown that the endothelium regenerating after balloon injury has altered function, manifested by reduced nitric oxide–dependent vasodilation.1,2,21 Exogenous nitric oxide can inhibit the neuronal release of norepinephrine29; therefore, it is possible that local catecholamine release may also be facilitated in the setting of balloon injury, where the synthesis and/or degradation of endogenous nitric oxide may be altered.

Summary

The present investigation provides the first evidence for a facilitation of adrenergic neurotransmission after balloon injury. This effect is associated with a desensitization of the vascular smooth muscle to norepinephrine. An injury-induced activation of the vascular renin-angiotensin system may be responsible for the facilitated release of adrenergic neurotransmitter.

Acknowledgments

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