Preserved Cardiac \( \beta \)-Adrenergic Sensitivity in Early Renovascular Hypertension

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SUMMARY To determine the mechanism of blunted sympathetic reflex responses in early renovascular hypertension, we measured inotropic and chronotropic responses of the heart to \( \beta \)-adrenergic stimulation in vivo and myocardial \( \beta \)-adrenergic receptor number and adenylate cyclase activity in 10 dogs during an early stage of one-kidney renal hypertension. Mean aortic pressure was higher in the hypertensive dogs (152 ± 4 mm Hg) than in eight sham-operated dogs (122 ± 1 mm Hg; \( p < 0.001 \)), but heart rate, cardiac output, and left atrial pressure did not differ between the two groups. Blood pressure reduction with a direct-acting vasodilator, pinacidil, resulted in marked increases in heart rate (+97 ± 12 beats/min) and rate of change of left ventricular pressure (dP/dt; +1447 ± 367 mm Hg/sec) in normotensive dogs but only blunted heart rate (+54 ± 12 beats/min) and minimal left ventricular dP/dt (+376 ± 264 mm Hg/sec) responses in hypertensive dogs. In contrast, intravenously administered isoproterenol produced similar increases in heart rate and left ventricular dP/dt in the two groups. These two groups also did not differ in either left ventricular \( \beta \)-adrenergic receptor number and affinity or basal, isoproterenol-stimulated, and fluoride-stimulated adenylate cyclase activity. Thus, despite blunted reflex responses to blood pressure reduction, hypertensive dogs showed neither reduction in chronotropic and inotropic responses to direct \( \beta \)-adrenergic stimulation nor \( \beta \)-adrenergic desensitization of the myocardium, as assessed by \( \beta \)-adrenergic receptor number and adenylate cyclase activity. Blunted reflex responses in this model of early hypertension must be due to factors operating at some locus other than the \( \beta \)-adrenergic receptor-adenylate cyclase complex.

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KEY WORDS • myocardial \( \beta \)-adrenergic receptor number • cardiac \( \beta \)-adrenergic sensitivity • renovascular hypertension • isoproterenol • norepinephrine

W E1 have shown recently that sympathetically mediated hemodynamic responses to blood pressure reduction are reduced in dogs with early renovascular hypertension. Blunted baroreceptor responsiveness and myocardial responses to sympathetic stimulation have been demonstrated in animals with established renovascular hypertension2,3 and correlated with reduced myocardial \( \beta \)-adrenergic receptor number.4,5 However, whether similar changes occur during early hypertension is not known. The purpose of the present study was to determine whether myocardial \( \beta \)-adrenergic desensitization contributed to the blunted reflex responses observed in early renal hypertension. Experiments were performed in two groups of chronically instrumented dogs, soon after the establishment of one-kidney renal hypertension in one group and sham operations in the other normotensive control group. We measured responses of heart rate and rate of left ventricular pressure rise (left ventricular dP/dt) to isoproterenol and to pinacidil, a direct-acting vasodilator; cardiac \( \beta \)-adrenergic receptor number; cardiac adenylate cyclase activity; and ventricular norepinephrine content. Knowledge of the mechanisms contributing to the blunted reflexes present in early renal hypertension may enhance our understanding of the pathophysiology of hypertension.

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Materials and Methods

Surgical Preparation

Adult male beagles (weight, 8.8-13.6 kg), purchased from licensed suppliers, were anesthetized with sodium pentobarbital (25 mg/kg i.v.) and mechanically ventilated with a respirator (Harvard Apparatus, S. Natick, MA, USA). A sterile left-sided thoracotomy was performed through the fifth intercostal space for placement of Tygon catheters (inside diameter, 1.02 mm; Norton, Plastics and Synthetics Division, Akron, OH, USA) in the main pulmonary artery, left atrium, and descending aorta. The chest was closed, and catheters were exteriorized at the nape of the neck. The left renal artery was then exposed through a left flank incision. In one group of dogs, the renal artery was plicaded to reduce renal blood flow to 40% of baseline as measured by an electromagnetic flowmeter (Carolina Medical Electronics, King, NC, USA). In another group, the renal artery was exposed, but no arterial plication performed. After recovery, animals were trained to lie quietly on a table. Two weeks later, animals were reanesthetized and a right nephrectomy performed. Animals in which severe uremia developed, as manifested by weakness, refusal to eat, hematocrit less than 28%, or serum creatinine greater than 5 mg/dl, were excluded.

Hemodynamic Measurements

Hemodynamic studies were performed 5 to 8 days after the second operation. After subcutaneous injection of morphine sulfate (0.5 mg/kg) for sedation, animals were placed in a lateral decubitus position. With local anesthesia using 0.5% lidocaine and under fluoroscopic guidance, a transducer-tip catheter (Millar Instruments, Houston, TX, USA) was placed into the left ventricle for measuring left ventricular pressure through a femoral artery. The previously implanted catheters were connected to Statham P23Db transducers (Oxnard, CA, USA). Pressures from both fluid-filled and Millar catheters were recorded continuously on a multichannel Brush 4800 recorder (Gould Instruments Division, Cleveland, OH, USA) along with a limb lead electrocardiogram. Left ventricular dp/dt was measured by an electronic differentiator. The ratio of left ventricular dp/dt to a ventricular pressure during isovolumetric contraction at a developed pressure of 50 mm Hg (dp/dt/P) was measured as an index of myocardial contractility. Cardiac output was measured by an electromagnetic flowmeter (Carolina Medical Electronics, King, NC, USA) dye dilution technique with a cardiac output system (Model 140; Gilford Instrument Laboratories, Oberlin, OH, USA).

Forty-five minutes after acute catheterization, baseline hemodynamic values were recorded in triplicate over a 20-minute period. Neither heart rate nor aortic pressure differed from precatheterization values. To assess cardiac responses to direct β-adrenergic stimulation, we measured the peak responses of heart rate, aortic pressure, and maximum left ventricular dp/dt after four doses of intravenously administered isopropenol (0.1, 0.2, 0.4, and 0.8 μg). Doses were spaced to allow heart rate and aortic pressure to return to baseline before the next dose. Isopropenol doses producing a 25 beats/min increase in heart rate (CD25) and a 2000 mm Hg/sec increase in left ventricular dp/dt (ID2000) were determined by linear regression of the dose-response data for each animal.

After returning to a baseline hemodynamic state, dogs received four serial doses of intravenous pinacidil (0.05, 0.05, 0.1, and 0.2 mg/kg). Doses were administered 30 minutes apart to assess cardiac reflex responses to progressive reduction of aortic pressure. Each dose was administered over 5 minutes, and systemic hemodynamic measurements were taken every 5 minutes thereafter. Because a steady state response was achieved within 15 minutes after each dose of pinacidil, we averaged the four values obtained between 15 and 30 minutes of each interval and used the average as the response to the cumulative dose.

After completion of the hemodynamic study, animals were killed with lethal doses of pentobarbital and hearts were quickly removed and sectioned. Muscle samples were taken from the left ventricular free walls and frozen immediately in liquid nitrogen for subsequent biochemical analyses. The experimental procedures were in accordance with guidelines of the University of Rochester.

β-Adrenergic Receptor and Adenylate Cyclase Activity

Left ventricular β-adrenergic receptor characteristics and adenylate cyclase activity were measured in a washed membrane preparation made with minor modifications of previously reported methods. Ventricular tissue (1-1.5 g) trimmed of epicardium and endocardium was minced in 15 volumes of ice-cold 50 mM Tris HCl buffer containing 1 mM EGTA, pH 7.4. The coarse suspension was homogenized at 4°C with a Brinkmann Polytron (Westbury, NY, USA) at setting 9 for three 8-second bursts separated by 1 minute. The suspension was filtered through 250-μm nylon mesh and centrifuged at 500 g for 10 minutes. The supernatant was then spun at 30,000 g for 15 minutes. The resulting pellet was resuspended in 10 ml of buffer, using the Brinkmann Polytron as before, and centrifuged at 30,000 g for 15 minutes. This pellet was resuspended for all binding and cyclase assays in 50 volumes of Tris buffer. Average final membrane protein concentration was 0.20 mg/ml.

Saturation binding experiments were conducted with tritiated dihydroalprenolol ([3H]DHA; 104.8 Ci/mmol; New England Nuclear, Boston, MA, USA). The procedure was similar to that described previously. A 460-μl aliquot of membrane suspension was combined with 20 μl of [3H]DHA and 20 μl of either water or 2 x 10^-6 M l-alprenolol for a final reaction volume of 500 μl. Final DHA concentrations ranged from 0.3 to 9 nM. After incubation at 25°C for 20 minutes, reaction mixtures were diluted rapidly with 4 ml of ice-cold Tris buffer and filtered under vacuum through Whatman GF/B fiberglass filters (Clifton, NJ, USA). Each filter was rinsed three times with 4 ml of Tris buffer. Filters were dried under a heat lamp for
20 minutes and then counted in an aqueous scintillation cocktail. Receptor number (B$_{max}$) and dissociation constant (K$_D$) for DHA were determined by Scatchard analysis. Binding was saturable and reversible and demonstrated appropriate stereospecificity; specific binding at K$_D$ was 50 to 60% of total binding. Representative binding data are depicted in Figure 1.

Adenylate cyclase activity was measured under basal conditions, during stimulation with isoproterenol (10$^{-8}$–10$^{-5}$ M), and in the presence of 10 mM sodium fluoride. The reaction mixtures and incubation conditions were as previously described. Cyclic adenosine 3', 5'-monophosphate (cAMP) generated was assayed by the method of Tovey et al. Peak adenylate cyclase stimulation (V$_{max}$) by isoproterenol and the isoproterenol concentration producing half-maximal cyclase stimulation (K$_{ac}$) were determined graphically. Protein concentration was measured by the method of Lowry et al., using bovine serum albumin as a standard. All reagents were obtained from Sigma Chemical Company (St. Louis, MO, USA).

**Ventricular Norepinephrine**

Left ventricular norepinephrine content was measured in washed, acidified extracts of 0.2 to 0.4 g of tissue using methods described previously. Final extracts containing 3,4-dihydrobenzylamine as an internal standard were injected into a C18 reversed-phase column (Bioanalytical Systems, West Lafayette, IN, USA), and the output of the electromechanical detector was recorded on a strip chart recorder (Hewlett-Packard, Waltham, MA, USA). Catecholamine concentrations were determined by comparing the peak height of unknown sample curves with those of known standards. The coefficient of variation of duplicate samples was 4.8%.

**Statistics**

Results are given as means ± SE. Hemodynamic data were analyzed by two-way analysis of variance for independent groups with trend analysis. The statistical significance of the differences between control and experimental values were determined by Dunnett's test. Group means were compared with Student's t test. Differences were considered statistically significant at a p level of less than 0.05.

**Results**

**Hemodynamics**

Studies were completed in 10 hypertensive dogs (weight, 10.8 ± 0.3 kg) and eight sham-operated normotensive dogs (weight, 10.5 ± 0.6 kg). As expected, mean aortic pressure was significantly higher in the former group. However, there were no differences between the groups in heart rate, cardiac output, or mean left atrial pressure at baseline (Figure 2). Baseline left ventricular dP/dt was significantly higher in the hypertensive group (4772 ± 413 mm Hg/sec) than in sham-operated dogs (3553 ± 297 mm Hg/sec), but the difference in left ventricular dP/dt/P between the two groups (59 ± 7 vs 47 ± 6 sec$^{-1}$) was not statistically significant.

Isoproterenol administration increased heart rate and left ventricular dP/dt in both the hypertensive and normotensive groups (Figure 3). The changes were similar between the two groups, as was the reduction in aortic pressure produced by isoproterenol. Calculated CD$_{25}$ doses of isoproterenol were 0.56 ± 0.24 μg in sham-operated dogs and 0.46 ± 0.15 μg in hypertensive dogs. Similarly, ID$_{500}$ values were comparable in sham-operated dogs (0.45 ± 0.09 μg) and hyperten-
Figure 3. Responses of heart rate and rate of change of left ventricular pressure (dP/dt) in six normotensive and five hypertensive dogs to serial injections of isoproterenol. Each point represents the mean ± SE for the peak observed response to each dose. No differences were detected between the groups at any dose of isoproterenol.

Figure 4. Responses of heart rate and rate of change of left ventricular pressure (dP/dt) in eight normotensive and 10 hypertensive dogs to blood pressure reduction produced by intravenously administered pinacidil (0.05, 0.05, 0.1, and 0.2 mg/kg). Doses were separated by 30 minutes; each point represents a mean ± SE. Responses are plotted against the cumulative dose administered (0.05, 0.1, 0.2, and 0.4 mg/kg). Asterisk (p<0.05) indicates significant difference from baseline. Dagger (p<0.05) indicates significant difference compared with values in normotensive dogs.

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Pinacidil produced similar reductions in mean aortic pressure in the two groups. Mean aortic pressure decreased from 122 ± 1 to 94 ± 4 mm Hg in normotensive animals and from 153 ± 4 to 126 ± 6 mm Hg in hypertensive animals after the highest dose of pinacidil. Normotensive dogs demonstrated brisk reflex responses to blood pressure reduction produced by pinacidil (Figure 4). A significant increase in heart rate was noted at a cumulative dose of pinacidil of 0.1 mg/kg. The heart rate increased further as additional doses of pinacidil were administered. Similarly, left ventricular dP/dt increased after each dose of pinacidil. In contrast, hypertensive dogs demonstrated no significant increase in heart rate until 0.2 mg/kg (cumulative dose) of pinacidil was administered, and the peak response (+54 ± 12 beats/min) was significantly less than that in normotensive dogs (+97 ± 12 beats/min). Left ventricular dP/dt in hypertensive dogs showed no significant change from baseline. Likewise, left ventricular dP/dt/P increased in normotensive dogs (13.6 ± 4.6 sec⁻¹ after the highest dose of pinacidil) but did not increase significantly in the hypertensive group (1.4 ± 2.8 sec⁻¹; p<0.05 vs values in normotensive dogs).

Myocardial β-Adrenergic Receptor Number and Adenylate Cyclase Activity

β-Adrenergic receptor number (Bmax) in left ventricular myocardium did not differ between sham-operated dogs (205 ± 19 fmol/mg protein) and hypertensive animals (192 ± 14 fmol/mg protein). There was also no significant difference in Kd (2.57 ± 0.27 and 2.31 ± 0.13 nmol, respectively) between the groups. Protein content of the final cardiac membrane fraction per gram of wet tissue was similar for both groups. Figure 5 shows that isoproterenol stimulated cAMP production in membrane suspensions from the two groups at concentrations ranging from 10⁻⁷ to 10⁻³ M. No differences were apparent at any concentration. Basal adenylate cyclase activity was unchanged in renal hypertension compared with sham-operated dogs (Table 1). Similarly, there was no difference between the groups in Vmax or Km for isoproterenol stimulation or response to 10 mM sodium fluoride.

Discussion

In our present study, animals underwent hemodynamic assessment approximately 1 week after nephrectomy and establishment of hypertension. At this early stage, peripheral vascular resistance and plasma renin activity were both increased. However, cardiac output, heart rate, and left atrial pressure were unchanged, suggesting that left ventricular systolic function was not significantly affected. Nor was left ven-
AMP production represents the increase over basal adenylate cyclase activity. Each point represents a mean ± SE. No differences were detected between groups at any concentration of isoproterenol.

**Figure 5.** Isoproterenol-stimulated cyclic AMP (cAMP) production by adenylate cyclase in cardiac membrane preparations from six normotensive and seven hypertensive dogs. Cyclic AMP production represents the increase over basal adenylate cyclase activity. Each point represents a mean ± SE. No differences were detected between groups at any concentration of isoproterenol.

**Table 1.** Adenylate Cyclase Activity in Cardiac Membrane Preparations of Myocardium of Normotensive and Renal Hypertensive Dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive (n = 6)</th>
<th>Hypertensive (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal adenylate cyclase activity (pmol cAMP/mg/min)</td>
<td>56 ± 10</td>
<td>57 ± 9</td>
</tr>
<tr>
<td>Isoproterenol stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{\text{max}}$ (Δpmol cAMP/mg/min)</td>
<td>+59 ± 13</td>
<td>+62 ± 8</td>
</tr>
<tr>
<td>$K_{\text{EC}}$ (nM isoproterenol)</td>
<td>136 ± 36</td>
<td>158 ± 49</td>
</tr>
<tr>
<td>NaF-stimulated adenylate cyclase activity (Δpmol cAMP/mg/min)</td>
<td>358 ± 49</td>
<td>469 ± 93</td>
</tr>
</tbody>
</table>

Values are means ± SE. $n$ = number of experiments. $V_{\text{max}}$ = maximal increase in rate of cAMP accumulation (pmol cAMP formed/mg protein/min) above basal adenylate cyclase activity. $K_{\text{EC}}$ = concentration calculated to produce half-maximal adenylate cyclase activity. NaF-stimulated adenylate cyclase activity was obtained with 10 mM NaF; values are those in excess of basal adenylate cyclase activity. There was no significant difference in any of the parameters between the normotensive and hypertensive dogs.

Cardiac responses to blood pressure reduction, but other mechanisms cannot be excluded.
Acknowledgments

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References

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