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EXPERIMENTAL STUDIES

Myocardial Sympathetic Denervation Prevents Chamber-Specific Alteration of Beta-Adrenergic Transmembrane Signaling in Rabbits With Heart Failure

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Objectives. **The purpose of this study was to assess the effect of myocardial sympathetic denervation on the chamber-specific alteration of beta-adrenergic signaling in left ventricular failure in rabbits.**

Background. **Local abnormalities in sympathetic nerve terminals, including the neuronal reuptake of norepinephrine, are thought to be responsible for the chamber-specific regulation of beta-adrenergic signaling in heart failure.**

Methods. **Sixteen rabbits were given 6-hydroxydopamine, 25 mg/kg body weight intravenously on days 1 and 2 and 50 mg/kg intravenously on days 7 and 8. Another 16 rabbits received vehicle. Aortic regurgitation was induced in eight of the 6-hydroxydopamine–treated and eight of the vehicle-treated rabbits on day 14. Another eight of the 6-hydroxydopamine–treated and eight of the vehicle-treated rabbits underwent a sham operation. The hearts were excised for biochemical analysis on day 21.**

Results. **Hemodynamic characteristics on day 21 showed left ventricular failure in both the aortic regurgitation groups. The plasma norepinephrine concentration on day 21 was higher in both the aortic regurgitation groups than in the sham groups. The beta-adrenoceptor densities and isoproterenol plus 5′-guanylylimidodiphosphate–, 5′-guanylylimidodiphosphate– and sodium fluoride–stimulated adenylyl cyclase activities were decreased only in the failing left ventricle of the vehicle-pretreated aortic regurgitation group, but in both ventricles of the 6-hydroxydopamine-pretreated aortic regurgitation group. The basal and forskolin-stimulated adenylyl cyclase activities were similar in both the aortic regurgitation groups and in the sham groups.**

Conclusions. **Sympathetic denervation prevented chamber-specific alterations in beta-adrenergic signaling in acute left ventricular failure. Local loss of sympathetic nerve endings, and especially the defective neuronal norepinephrine reuptake, are likely to be responsible for the chamber-specific alteration of the beta-adrenoceptor–G protein–adenylyl cyclase system in heart failure in rabbits.**

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An increase in plasma norepinephrine in patients with heart failure indicates a poor prognosis and is implicated as a cause of beta-adrenoceptor subsensitivity and downregulation in congestive heart failure (1). We previously reported that myocardial norepinephrine concentration and beta-adrenoceptor density were reduced in the left and right ventricles of rabbits with chronic biventricular heart failure produced by the prolonged administration of adriamycin. However, these changes occurred only in the left ventricle of rabbits with acute left ventricular failure induced by aortic regurgitation, despite a similar increase in plasma norepinephrine in both models (2). Bristow et al. (3), studying the human heart, demonstrated many chamber-specific beta-adrenergic neuroeffector abnormalities, including beta-adrenoceptor downregulation, tissue norepinephrine depletion and decreases in adenylyl cyclase activity stimulated by isoproterenol, zinterol, 5′-guanylylimidodiphosphate and forskolin, occurred in the failing right ventricle derived from primary pulmonary hypertension, while the abnormalities occurred in both ventricles with idiopathic dilated cardiomyopathy. A local abnormality in sympathoneuronal regulation is thought to be involved in these phenomena.

Delehanty et al. (4), using ^{3}H-norepinephrine tracer in pacing-induced heart failure, observed a significant negative correlation between interstitial norepinephrine and beta-adrenoceptor density. A local increase in interstitial norepinephrine concentration has been suggested to cause chamber-specific abnormalities. In fact, a local reduction of neuronal norepinephrine uptake has been postulated to increase the local interstitial norepinephrine (5,6). In contrast, the localized regulation of norepinephrine release by the afferent fibers that originate in the failing ventricle is an alternative explanation

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Abbreviations and Acronyms

ANOVA = analysis of variance
cAMP = cyclic 3′,5′-adenosine monophosphate
EGTA = ethylene glycol-bis(beta-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid

for chamber-specific regulation (7). Thus, the precise mechanism of the chamber-specific regulation in beta-adrenoceptor density is still unknown.

Myocardial denervation can interfere with aforementioned neuronal function of the sympathetic nerve terminals and affect the chamber-specific regulation following the induction of acute left ventricular overload. Chemical sympathectomy by 6-hydroxydopamine, which causes selective destruction of adrenergic nerve terminals, has been reported to decrease the neuronal uptake of tritiated norepinephrine (8,9). We therefore hypothesized that if the local release of norepinephrine is responsible for the chamber-specific subsensitivity of beta-adrenergic signaling, pretreatment with 6-hydroxydopamine prevents the downregulation of beta-adrenoceptor in the failing myocardium. However, if a local defect of neuronal uptake of norepinephrine is responsible for the chamber specificity, a marked downregulation would occur in both ventricles and the chamber-specific phenomenon would be abolished by pretreatment with sympathetic denervation, because sympathetic denervation by 6-hydroxydopamine induces a severe defect of neuronal uptake of norepinephrine in both ventricles. We thus investigated the effect of sympathetic denervation on the chamber-specific regulation in beta-adrenergic signaling after the induction of left ventricular failure by aortic regurgitation to assess which mechanism is responsible for the phenomenon.

Methods

Animals. We studied 32 Japanese female white rabbits (3 months old) with a body mass ~3 kg. The experiments conformed to the "Position of the American Heart Association on Research Animal Use" adopted by the Association in November 1984.

Chemical denervation. The agent 6-hydroxydopamine hydrobromide (Sigma Chemical) was dissolved in physiologic saline that contained ascorbic acid (1 mg/ml). Sixteen rabbits were administered two doses of 6-hydroxydopamine (25 mg/kg body weight intravenously) 24 h apart, and two doses (50 mg/kg intravenously) on days 7 and 8 according to the method of Maling et al. (10). As controls, 16 weight-matched rabbits received saline that contained ascorbic acid (1 mg/ml) in the same schedule.

Blood sampling for plasma norepinephrine determinations. A polyethylene catheter was placed in the jugular vein 1 week before the administration of 6-hydroxydopamine or vehicle. A 5-ml blood sample was obtained without anesthesia before the initial injection and again 1 week postoperatively. Blood was centrifuged at 1,500*g* for 15 min, and plasma was stored at −80°C until needed for assay. The norepinephrine concentration was assayed by high performance liquid chromatography (11).

Induction of acute left ventricular failure. A total of 32 rabbits were anesthetized with chloral hydrate (150 mg/kg intravenously). Aortic regurgitation was induced in eight rabbits with 6-hydroxydopamine and in eight rabbits with vehicle pretreatment, as previously described (2). In brief, the right carotid artery was isolated, and the aortic root pressure was measured with a 5F micromanometer-tipped catheter (PC-350, Millar Instruments Inc.). A 5F metal catheter was then introduced into the right carotid artery and advanced to the aortic root, where the motion of the aortic valve was felt through the catheter. The catheter was pushed toward the left ventricle and the aortic valve was perforated. Diastolic murmur was audible in this setting. Aortic pressure was again measured to assess the severity of aortic regurgitation; an immediate decrease in aortic diastolic pressure predicts the volume overloading to the left ventricle (12). In the remaining eight rabbits treated with 6-hydroxydopamine and in the eight rabbits treated with vehicle, the right carotid artery was isolated and the aortic pressure was measured, but aortic regurgitation was not induced by a sham operation. Antibiotics were injected for the next 2 days.

Hemodynamic measurements. Hemodynamic measurements were obtained 1 week postoperatively in the open-chest condition under anesthesia with chloral hydrate. Ventilation was controlled through an endotracheal tube (internal diameter 4.0 mm) introduced through a tracheal incision. Tidal volume was adjusted to 50 ml, with a respiratory rate of 20 beats/min. Aortic root pressure was monitored with a 5F micromanometer-tipped catheter introduced from the left carotid artery. Aortic flow was measured with an electromagnetic flow probe (Nihon-Kohden, Tokyo, Japan) placed around the aortic root. Left ventricular pressure was monitored with a 3F micromanometer-tipped catheter (model PC-330, Millar Instruments Inc.) that was introduced through an apical incision. The right ventricular pressure was measured by a Teflon catheter introduced through its free wall. Left ventricular diameter was measured using a pair of ultrasonic microcrystals attached to the anterior and posterior epicardial surfaces. Hemodynamic data were collected with a thermal array recorder (Nihon-Kohden, Tokyo, Japan) at a paper speed of 200 mm/s.

Determination of ventricular mass and myocardial norepinephrine. After hemodynamic measurements were made, the heart was stopped by rapid injection of potassium chloride. The left and right ventricular free walls were isolated and weighed. Portions of the left and right ventricular myocardium were frozen with liquid nitrogen and stored at −80°C for assay of myocardial norepinephrine content by high performance liquid chromatography (11).

Membrane preparation. Membrane fractions were prepared as described by Feldman et al. (13). Briefly, the myocardium was soaked in ice-cold isotonic sucrose buffer

(0.25 mol/liter sucrose, 1 mmol/liter potassium bicarbonate, 1 mmol/liter magnesium chloride), and the connective tissue, endocardium, epicardium and vessels were removed as much as possible. The myocardium was minced with scissors and homogenized with tissue disrupter (Physcotron, Niti-On, Tokyo, Japan) at a maximal speed of 10 s. For beta-adrenoceptor antagonist binding studies, the homogenate of myocardium was added to an equal volume of 0.5 mol/liter potassium chloride and stirred for 15 min at 4°C for extraction of contractile proteins. After centrifugation at 50,000*g* for 15 min at 4°C, the resulting pellet was resuspended in 75 mmol/liter of Tris hydrochloride (pH 7.5) and 10 mmol/liter of magnesium chloride buffer and recentrifuged. The final membrane pellet was resuspended in 50 mmol/liter of Tris hydrochloride (pH 7.5) containing 250 mmol/liter of sucrose and 1 mmol/liter of ethylene glycol-bis(beta-aminoethyl ether)-*N,N,N'N'*-tetraacetic acid (EGTA) and stored at −80°C. For adenylyl cyclase assay, the homogenate of myocardium was centrifuged at 1,085*g* for 20 min at 4°C, and the resulting pellet was washed twice more by the same procedure and stored at −80°C until assay.

Assay of beta-adrenoceptors and beta-adrenoceptor subtypes. The protein concentration of membrane samples was determined as previously described (14) and then adjusted to 0.3 mg/ml. The density of myocardial beta-adrenoceptors was determined by a ligand binding assay with iodine-125 (^{125}I) iodocyanopindolol (Amersham, Tokyo, Japan). Twenty-five microliters of 10 different concentrations of ^{125}I iodocyanopindolol (30 to 800 pmol/liter), together with 25 μl of 1.6×10^{-6} mol/liter of propranolol or Tris ascorbic acid buffer, was added to 100 μl of membrane sample. After incubation at 30°C for 120 min, the reaction was terminated by addition of 750 μl of ice-cold Tris buffer. The reaction mixture was filtered through Whatman GF/C glass fiber filters presoaked in 0.5% polyethylenimine solution, and then washed twice with 5 ml of Tris buffer. Filter-bound radioactivity was counted using a gamma counter (model ARC-600, Aloka Inc., Tokyo, Japan) with an efficiency rate of 76.5%. Specific binding was determined by subtracting the amount of nonspecific binding measured in the presence of propranolol from the total binding. The maximal numbers of binding sites and the dissociation constant were calculated by Scatchard's analysis (15).

Competition curve experiments were carried out using the selective $beta_1$-antagonist CGP27012A (Ciba-Geigy, Basel, Switzerland) as a competing ligand with 16 points in the range of 10^{-10} mol/liter to 10^{-3} mol/liter. Adopted concentration of ^{125}I iodocyanopindolol was 200 pmol/liter. Ratios of high and low affinities were determined using nonlinear regression analysis by a GraphPad Prism program (Intuitive Software for Science Inc.) based on Munson and Rodbard's method (16). High affinity sites were estimated as $beta_1$-adrenoceptors and low affinity sites as $beta_2$-adrenoceptors (3,17).

Assay of adenylyl cyclase activities. Adenylyl cyclase activities were determined by radioimmunoassay using the particulate fraction of the ventricular myocardium prepared as described above (18). Twenty-five microliters of membrane preparations, adjusted to 1 mg/ml of protein concentration, was added to 225 μl of reaction medium (50 mmol/liter of Tris hydrochloride, 2.5 mmol/liter of disodium adenosine 5′-triphosphate, 5 mmol/liter of magnesium chloride, 1 mmol/liter of EGTA, 20 mmol/liter of creatine phosphate, 50 U/ml of creatinine phosphokinase and 0.8 mmol/liter of isobutylmethylxanthine). The reaction mixture was incubated at 25°C for 10 min, and the reaction was stopped by boiling the tubes at 100°C for 2 min. The samples were centrifuged at 6,000*g* for 20 min, and the supernatants were stored at −80°C until the assay. The reaction mixture without membrane sample or adenosine 5′-triphosphate was used as a blank. The cyclic 3′,5′-adenosine monophosphate (cAMP) level was measured according to the method of Steiner et al. (19) using an assay kit (Yamasa, Chiba, Japan). Adenylyl cyclase activities were expressed as cAMP produced per minute per milligram of protein. Adenylyl cyclase activities were determined under basal conditions and after various stimulants, including 10^{-5} mol/liter of isoproterenol plus 10^{-5} mol/liter of 5′-guanylylimidodiphosphate, 10^{-5} mol/liter of 5′-guanylylimidodiphosphate, 8×10^{-3} mol/liter of sodium fluoride or 2×10^{-5} mol/liter of forskolin.

Data analysis. Total forward stroke and regurgitation volumes were calculated by digitization of the positive and negative components of aortic flow with the use of a digitizer (type KD 4300, Graphtec Corp.). Stroke volume was calculated by subtraction of regurgitant volume from total forward stroke volume. The regurgitant fraction was calculated as (regurgitant volume/total forward stroke volume) × 100.

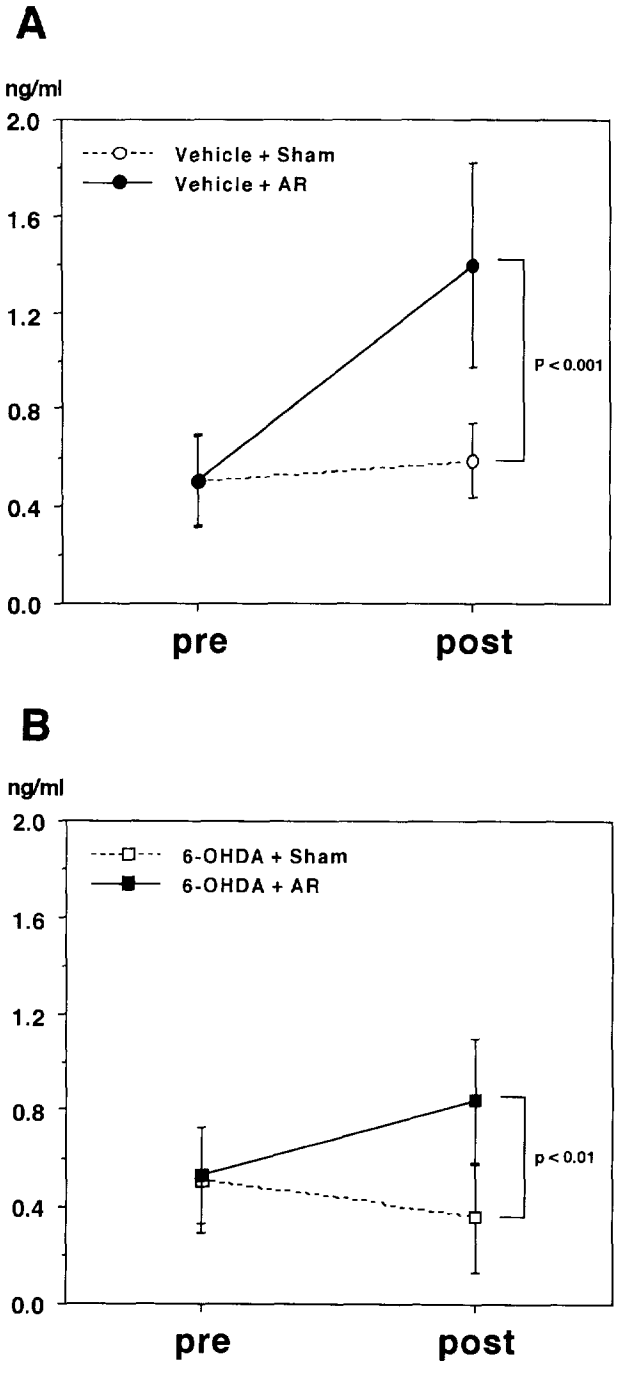
Statistical analysis. Data were expressed as mean values ± SD. Changes in plasma norepinephrine content in each group were assessed by repeated measures analysis of variance (ANOVA). Comparison between two groups was performed with an unpaired *t* test. Comparison among three or more different groups was performed by one-way ANOVA and the Fisher protected least significant difference test. Statistical significance was set at $p < 0.05$.

Results

Plasma norepinephrine. The plasma norepinephrine concentration before the 6-hydroxydopamine or vehicle treatment was similar among the four groups (Fig. 1). Increases in the plasma norepinephrine concentration 1 week after the operation were greater in the vehicle-pretreated aortic regurgitation group than in the vehicle-pretreated sham group ($p < 0.001$). In the 6-hydroxydopamine–pretreated sham group, the plasma norepinephrine concentration tended to be lower after the operation; however, in the 6-hydroxydopamine–pretreated aortic regurgitation group, it was higher after the operation. Changes in the plasma norepinephrine concentration were significantly different between the 6-hydroxydopamine–pretreated groups ($p < 0.01$).

Body weight and left ventricular mass. Body weights were lower and left ventricular mass per body weight was higher in the aortic regurgitation groups than in the sham groups. There

Figure 1. A, Plot of the plasma norepinephrine concentration of control animals before (pre) and 1 week after (post) the operation for aortic regurgitation (vehicle + AR, **solid circles, solid line**) or a sham operation (vehicle + sham, **open circles, dashed line**). Increases in the plasma norepinephrine concentration in the vehicle + AR group are greater than those in the vehicle + sham group (repeated measures analysis of variance). **B,** Plot of the plasma norepinephrine concentration of 6-hydroxydopamine (6-OHDA)–treated animals before (pre) and 1 week after (post) the operation for aortic regurgitation (6-OHDA + AR, **solid squares, solid line**) or a sham operation (6-OHDA + sham, **open squares, dashed line**). In the 6-OHDA + sham group, the plasma norepinephrine concentration tended to be decreased after the operation, but was increased after the operation in the 6-OHDA + AR group. Changes in the plasma norepinephrine concentration are significantly different between the 6-OHDA–pretreated groups (repeated measures analysis of variance).



were no differences in right ventricular mass between the aortic regurgitation groups and the sham groups (Table 1).

Hemodynamic data. Table 1 also shows the hemodynamic data recorded before excising the heart 1 week after induction of aortic regurgitation or sham operation. Between the 6-hydroxydopamine–treated groups, aortic systolic pressures in the aortic regurgitation group were lower than those in the sham group. Aortic diastolic pressures were lower in the aortic regurgitation groups than those in the sham groups, although there were no differences between the two groups with aortic regurgitation. The decrease in aortic diastolic pressure immediately after production of aortic regurgitation was also comparable between the two groups (16.6 ± 2.7 vs. 16.3 ± 2.6 mm Hg). The regurgitant fraction was similar between the vehicle-pretreated and the 6-hydroxydopamine–pretreated aortic regurgitation groups (40.5 ± 5.7% vs. 41.4 ± 6.6%). Cardiac output was lower and left ventricular diameters were larger in both aortic regurgitation groups than in the sham groups. End-diastolic pressure in the aortic regurgitation groups was higher in the left ventricle, but not in the right ventricle, than that recorded in each sham group.

Myocardial norepinephrine. Myocardial norepinephrine concentration was lower in the vehicle-pretreated aortic regurgitation group than in the vehicle-pretreated sham group in the left ventricle ($p < 0.05$), but not in the right ventricle (Table 2). Myocardial norepinephrine concentration in the 6-hydroxydopamine–pretreated sham group decreased in the left ventricle by 80% ($p < 0.0005$) and by 77% in the right ventricle ($p < 0.0005$), compared with those concentrations in the vehicle-pretreated sham group. Between the 6-hydroxydopamine–pretreated groups, there were no significant differences in both the ventricles.

Beta-adrenoceptor binding studies. Maximal binding sites of total beta-adrenoceptors were lower in the vehicle-pretreated aortic regurgitation group than in the vehicle-pretreated sham group in the left ventricle but not in the right ventricle. The total beta-adrenoceptor densities were higher in the 6-hydroxydopamine–pretreated sham group than in the vehicle-pretreated sham group in both the left and right ventricles. In contrast, these values were markedly lower in the 6-hydroxydopamine–pretreated aortic regurgitation group than in the 6-hydroxydopamine–pretreated sham group in both the ventricles (Table 2). The dissociation constants for iodocyanopindolol were similar among the four groups (left ventricle: vehicle plus sham 55 ± 42, vehicle plus aortic regurgitation 71 ± 33, 6-hydroxydopamine plus sham 67 ± 35, 6-hydroxydopamine plus aortic regurgitation 47 ± 30 pmol/liter; right ventricle: vehicle plus sham 61 ± 20, vehicle plus aortic regurgitation 52 ± 25, 6-hydroxydopamine plus sham 65 ± 22, 6-hydroxydopamine plus aortic regurgitation 72 ± 62 pmol/liter).

Subtypes of beta-adrenoceptors. In the vehicle-pretreated aortic regurgitation group, $beta_1$-adrenoceptor densities were lower than in the vehicle-pretreated sham group in the left ventricle but not in the right ventricle. $Beta_1$-adrenoceptor was higher in the 6-hydroxydopamine–

Table 1. Left Ventricular Mass and Hemodynamic Characteristics

	Vehicle + Sham (n = 8)	Vehicle + AR (n = 8)	6-OHDA + Sham (n = 8)	6-OHDA + AR (n = 8)
BW (kg)	2.75 ± 0.21	2.41 ± 0.25*	2.55 ± 0.33	2.19 ± 0.26†
LV weight/BW (g/kg)	0.62 ± 0.07	0.90 ± 0.11*	0.63 ± 0.08	1.00 ± 0.15†
RV weight/BW (g/kg)	0.26 ± 0.05	0.25 ± 0.04	0.24 ± 0.03	0.26 ± 0.04
Heart rate (beats/min)	231 ± 16	215 ± 22	229 ± 29	205 ± 36
AoSP (mm Hg)	102 ± 14	87 ± 11	101 ± 14	81 ± 19†
AoDP (mm Hg)	80 ± 16	47 ± 15*	77 ± 16	43 ± 13†
LVEDP (mm Hg)	3.5 ± 3.1	12.4 ± 5.3*	4.5 ± 2.8	14.4 ± 6.3†
RVEDP (mm Hg)	2.6 ± 1.8	3.5 ± 1.5	2.9 ± 1.5	3.4 ± 1.5
CO (ml/min per kg)	97 ± 33	61 ± 15*	116 ± 35	60 ± 24†
LVEDD (mm)	21.4 ± 1.5	24.5 ± 1.9*	20.8 ± 1.7	23.6 ± 2.4†
LVESD (mm)	19.1 ± 1.6	21.8 ± 2.4*	19.0 ± 2.1	21.5 ± 2.1†

Data are presented as mean value ± SD. *$p < 0.05$, vehicle + AR versus vehicle + sham; †$p < 0.05$, 6-OHDA + AR versus 6-OHDA + sham (analysis of variance). AoDP = aortic diastolic pressure; AoSP = aortic systolic pressure; AR = aortic regurgitation; BW = body weight; CO = cardiac output; EDD = end-diastolic diameter; EDP = end-diastolic pressure; ESD = end-systolic diameter; LV = left ventricle; 6-OHDA = 6-hydroxydopamine; RV = right ventricle.

pretreated sham group than in the vehicle-pretreated sham group in both the ventricles. Comparing the 6-hydroxydopamine–pretreated groups, $beta_1$-adrenoceptor densities in the aortic regurgitation group were lower than those in the sham group in both the left and right ventricles. There were no differences in $beta_2$-adrenoceptor in these four groups (Table 2). An average dissociation constant (k_i) value for $beta_1$-receptor was 4.1 ± 1.6 nmol/liter and for $beta_2$-receptor it was 1.5 ± 0.7 μmol/liter. There was no significant difference in the k_i value among the four groups or between the left and right ventricles.

Adenylyl cyclase assays. Figure 2 shows the results of the adenylyl cyclase activity assay. Basal adenylyl cyclase activities in both ventricles were similar among the four groups. Isoproterenol plus 5′-guanylylimidodiphosphate– ($p < 0.05$), 5′-guanylylimidodiphosphate– ($p < 0.005$) and sodium fluoride–stimulated ($p < 0.01$) adenylyl cyclase activities (basal subtracted) were lower in the vehicle-pretreated aortic regurgitation group than in the vehicle-pretreated sham group in the left ventricle but not in the right ventricle. In the 6-hydroxydopamine–pretreated sham group, the 5′-guanylylimidodiphosphate–(left ventricle, $p < 0.001$; right ventricle, $p < 0.05$) and sodium fluoride–stimulated (left ventricle, $p < 0.05$; right ventricle, $p < 0.01$) adenylyl cyclase activities were lower than in the vehicle-pretreated sham group in both ventricles, although there were no differences in isoproterenol plus 5′-guanylylimidodiphosphate–stimulated adenylyl cyclase activities between the two groups in both ventricles. Comparing the 6-hydroxydopamine–pretreated groups, the isoproterenol plus 5′-guanylylimidodiphosphate–(left ventricle, $p < 0.01$; right ventricle, $p < 0.05$), 5′-guanylylimidodiphosphate–(left ventricle, $p < 0.05$; right ventricle, $p < 0.05$) and sodium fluoride–stimulated (left ventricle, $p < 0.01$; right ventricle, $p < 0.05$) adenylyl cyclase activities were lower in the aortic regurgitation group than in the sham group in both ventricles. Finally, the forskolin-stimulated adenylyl cyclase activities in both ventricles were similar among the four groups.

Table 2. Myocardial Norepinephrine and Beta-Adrenoceptor

	Vehicle + Sham (n = 8)	Vehicle + AR (n = 8)	6-OHDA + Sham (n = 8)	6-OHDA + AR (n = 8)
LV				
MNE (ng/g tissue)	1933 ± 451	1263 ± 459*	385 ± 247†	205 ± 108
$Beta_1$-AR (fmol/mg protein)	28.8 ± 4.7	20.3 ± 3.9*	43.5 ± 10.2†	17.6 ± 4.0‡
$Beta_2$-AR (fmol/mg protein)	4.3 ± 0.8	4.0 ± 1.0	4.1 ± 2.0	4.3 ± 2.1
Total beta-AR (fmol/mg protein)	33.1 ± 4.7	24.3 ± 3.8*	47.6 ± 10.6†	21.9 ± 3.9‡
RV				
MNE (ng/g tissue)	2034 ± 631	2250 ± 863	458 ± 236†	374 ± 187
$Beta_1$-AR (fmol/mg protein)	24.8 ± 5.7	21.0 ± 3.7	36.9 ± 9.8†	13.2 ± 5.1‡
$Beta_2$-AR (fmol/mg protein)	3.7 ± 0.6	3.1 ± 1.2	3.2 ± 1.5	3.1 ± 1.6
Total beta-AR (fmol/mg protein)	28.5 ± 6.0	24.1 ± 4.4	40.1 ± 9.7†	16.3 ± 6.0‡

Data presented are mean value ± SD. *$p < 0.05$, vehicle + AR versus vehicle + sham; †$p < 0.05$, 6-OHDA + sham versus vehicle + sham; ‡$p < 0.05$, 6-OHDA + AR versus 6-OHDA + sham (analysis of variance). Beta-AR = beta-adrenoceptor; MNE = myocardial norepinephrine. Other abbreviations as in Table 1.

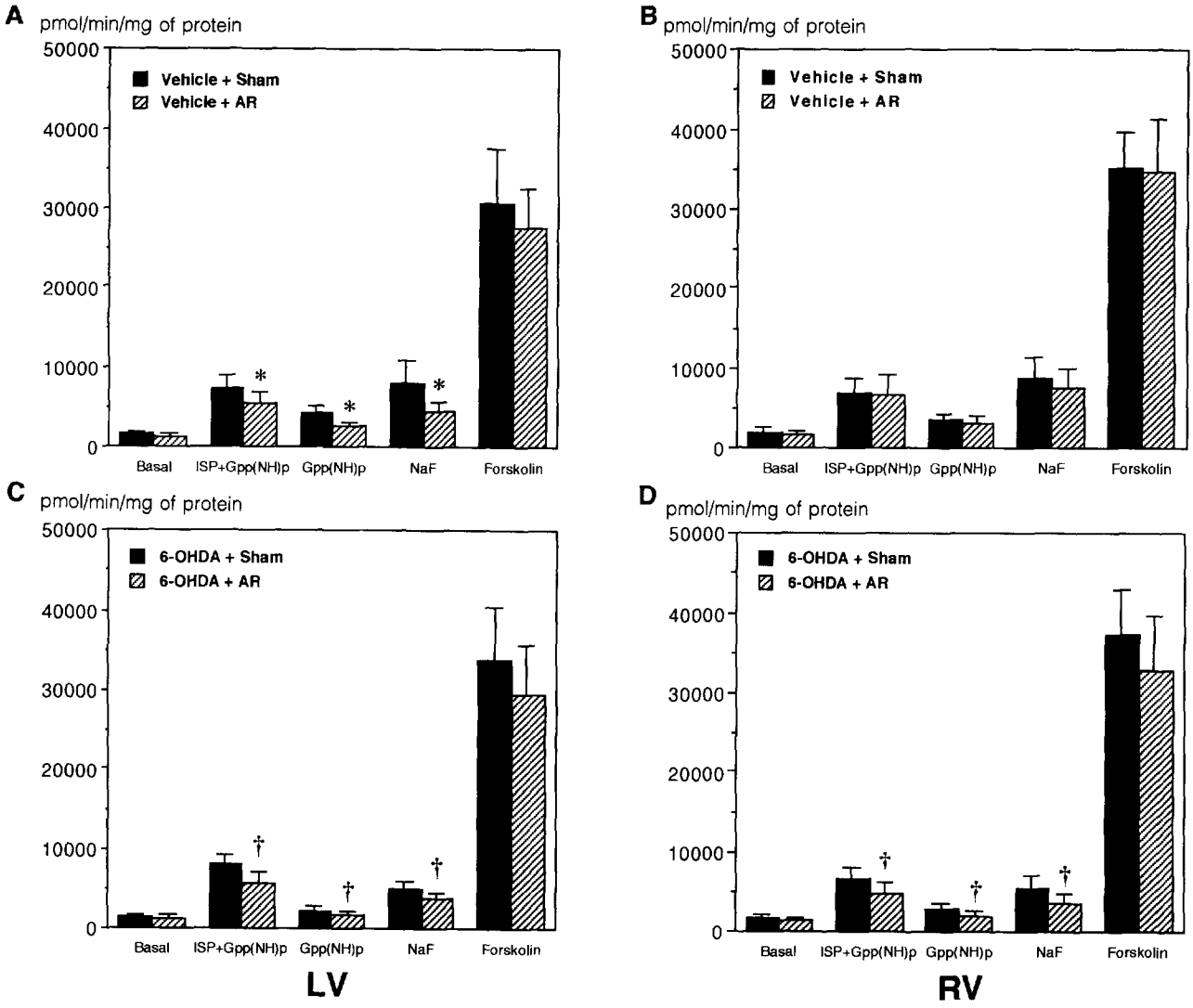


Figure 2. Bar graphs of adenylyl cyclase activities (pmol/min per mg of protein) from the left (LV) and right ventricle (RV) of vehicle or 6-hydroxydopamine–treated animals that underwent a sham (vehicle + sham, 6-OHDA + sham) or operative procedure for aortic regurgitation (vehicle + AR, 6-OHDA + AR). Data represent cyclic AMP produced in pmol/mg per min ± SD and are net values (basal subtracted) under stimulation with 10^{-5} mol/liter isoproterenol + 10^{-5} mol/liter 5′-guanylylimidodiphosphate (ISP + Gpp(NH)p); 10^{-5} mol/liter Gpp(NH)p; 8×10^{-3} mol/liter sodium fluoride (NaF); and 2×10^{-5} mol/liter of forskolin. **A,** Adenylyl cyclase activity in the left ventricle significantly differs (*$p < 0.05$) between the vehicle + sham and vehicle + AR groups after stimulation with isoproterenol + Gpp(NH)p, Gpp(NH)p and NaF. **B,** Adenylyl cyclase activity in the right ventricle does not differ under these conditions. **C, D,** Adenylyl cyclase activity in both the left and right ventricles significantly differs ($p < 0.05$) between the 6-OHDA + sham and 6-OHDA + AR groups after stimulation with isoproterenol + Gpp(NH)p, Gpp(NH)p and NaF.

Discussion

$Beta_1$-adrenoceptor downregulation and a decrease in G protein–mediated adenylyl cyclase activity occurred specifically in the failing left ventricle, but not in the nonfailing right ventricle, after induction of aortic regurgitation. Chemical denervation by 6-hydroxydopamine pretreatment did not prevent the alteration of beta-adrenergic signaling in the failing myocardium. In contrast, chemical denervation by 6-hydroxydopamine caused a marked decrease in beta-adrenoceptor densities and G protein–mediated adenylyl cyclase activity in the nonfailing right ventricle as well as in the failing left ventricle. The chamber-specific alteration of beta-adrenergic signaling after induction of acute left ventricular failure was abolished by sympathetic denervation.

Features of chemical denervation using 6-hydroxydopamine. Chemical denervation using 6-hydroxydopamine can produce selective sympathetic denervation, whereas surgical denervation or heart transplantation cannot avoid parasympathetic denervation (9). Histochemical examination shows marked depletion of catecholamine-containing nerve terminals after 6-hydroxydopamine treatment (20). Himura et al. (6) have shown that a similar loss of noradrenergic nerve terminals occurred in failing myocardium, as evidenced by the reduction of catecholaminergic histofluorescence– and tyrosine hydroxylase–immunostained profiles. Daly and Sole (21) hy-

pothesized that the production of 6-hydroxydopamine–like toxic metabolites may destroy the sympathetic nerve terminals in the failing myocardium, based on their following findings. Incessant hyperactivity in sympathetic nerve terminals leads to a decrease in norepinephrine and an increase in dopamine (22). High levels of neuronal dopamine found in such circumstances are present in the extravesicular space and are thus relatively unprotected (23). Such stores may be susceptible to oxidation, leading to the production of 6-hydroxydopamine, a potent neurotoxin that causes autodestruction of the nerve terminal.

Beta-adrenergic signaling in denervated myocardium. Sympathetic denervation causes a supersensitivity to exogenous catecholamines. Alterations in the postreceptor mechanisms of denervated myocardium remain to be determined. Vatner et al. (24) observed that isoproterenol plus 5′-guanylylimidodiphosphate– and sodium fluoride–stimulated maximal adenylyl cyclase activities tended to be lower in surgically denervated dogs than in control dogs, although l-[^{3}H]dihydroalprenolol binding sites were higher, and median effective concentration (EC_{50}) for isoproterenol-stimulated adenylyl cyclase activity and muscarinic receptor density was lower in the denervated dogs. Studies on cardiac denervation after transplantation also supported these findings (25–27). Recently, Loh et al. (28) reported that guanine nucleotide–stimulated adenylyl cyclase activity was decreased in parallel with decreased levels of Gs_α protein and its messenger RNA after orthotopic cardiac transplantation.

In experimental studies using chemical denervation, Pik and Wollemann (29) observed beta-adrenoceptor upregulation and an increase in norepinephrine- and isoproterenol-stimulated adenylyl cyclase activity in denervated rat heart 2 h after the administration of 6-hydroxydopamine. Chiu also found that norepinephrine-, isoproterenol-, sodium fluoride– and 5′-guanylylimidodiphosphate–stimulated adenylyl cyclase activities were enhanced in the 6-hydroxydopamine–treated rat myocardium, and suggested that the enhancement of the signal transduction pathway was related to the denervation supersensitivity, although beta-adrenoceptor binding assays were not performed (30). However, recent findings are unexpectedly in contrast to these classic findings. Hammond et al. (31) reported that 6-hydroxydopamine administration during the neonatal phase induced downregulation of beta-adrenoceptors and a decrease in isoproterenol-, 5′-guanylylimidodiphosphate– and sodium fluoride– but not manganese-stimulated adenylyl cyclase activities in the sinoatrial node and right atrium, despite an increased chronotropic response to isoproterenol and treadmill exercise testing. These discrepant results may be related to species differences, age differences or differences in the protocols for sympathetic denervation. 6-Hydroxydopamine also has an acute toxic effect owing to the release of norepinephrine, in addition to its substantial effect of sympathetic denervation. The change in beta-adrenergic signaling immediately after 6-hydroxydopamine injection may therefore be different from that observed in the long-term phase of 6-hydroxydopamine treatment.

Preliminary finding from our laboratory revealed that inotropic responsiveness to dobutamine, as determined by measurement of peak positive left ventricular dP/dt, was increased in rabbits with 6-hydroxydopamine treatment. However, isoproterenol plus 5′-guanylylimidodiphosphate–stimulated adenylyl cyclase activities were not increased, possibly because of the combined effects of upregulation of beta-adrenoceptor and the decrease in G protein–mediated adenylyl cyclase activities, suggesting that catecholamine supersensitivity was related to a postreceptor mechanism not linked to cAMP production. These findings are consistent with recent findings that showed G-protein–mediated adenylyl cyclase activity was attenuated in denervated myocardium (24,25,27,31). It is possible that sympathetic innervation may regulate G-protein levels independently from beta-adrenoceptor density, in a manner similar to the relation between sympathetic innervation and expression of calcium channels densities (32).

Alteration of beta-adrenergic signaling in failing myocardium. In the present study, a decrease in beta-adrenoceptor densities and G protein–mediated adenylyl cyclase activities was observed specifically in the failing ventricle. However, forskolin-stimulated adenylyl cyclase activity was not reduced in the failing ventricle, which suggested that a catalytic unit of adenylyl cyclase was preserved. This finding is in contrast to the previous study in patients with primary pulmonary hypertension, in which forskolin- and manganese-stimulated adenylyl cyclase activities, as well as 5′-guanylylimidodiphosphate-stimulated adenylyl cyclase activities, were decreased in the failing right ventricle. The discrepancies may be related to the severity of heart failure, the period of morbidity or the therapeutic interventions. In the clinical studies, failing hearts were obtained from patients who underwent cardiac transplantation after they had suffered from protracted heart failure. The morbidity period of the animal model used in the present study was shorter than that found with the human failing heart. In the animal model for chronic right ventricular failure produced by progressive pulmonary artery constriction and tricuspid avulsion, forskolin- as well as isoproterenol- and 5′-guanylylimidodiphosphate–stimulated adenylyl cyclase activities were significantly decreased, although manganese-stimulated activities were preserved (33). It is possible that the beta-adrenergic signal transduction pathway is more profoundly affected in chronic heart failure than in acute left ventricular failure.

The attenuation of both 5′-guanylylimidodiphosphate– and sodium fluoride–stimulated adenylyl cyclase activities indicates that a change in the amount or function of Gs_α occurred, although we do not have any direct evidence of the change in Gs_α. Although it is generally agreed that Gs_α is not changed in human heart failure, animal experiments showed that either the amount or function or messenger RNA levels for Gs was decreased, as stated in a recent review (34). Our findings are consistent with previous studies in animal models.

Relation between sympathetic innervation and chamber-specific alteration of beta-adrenergic signaling in heart failure. Diverse interstitial norepinephrine concentration is presumed to be the most plausible mechanism leading to the

chamber-specific downregulation of beta-adrenoceptor. Norepinephrine concentration in local sympathetic cleft is determined by three factors: 1) circulating levels of norepinephrine that can diffuse into the interstitium, 2) the rate of release of norepinephrine into the interstitium from cardiac sympathetic neurons, and 3) the rate of reuptake of norepinephrine by the neuronal terminals (4). The circulating level of norepinephrine that is required for beta-adrenoceptor downregulation in the intact heart in vivo is much higher than that seen in heart failure. Vatner et al. (35) failed to demonstrate beta-adrenoceptor downregulation in chronic norepinephrine infusion models in which the plasma norepinephrine level exceeded the control level by more than 15 times.

If the cause of chamber specificity is a local increase of norepinephrine release, the administration of 6- hydroxydopamine would inhibit the downregulation of beta-adrenoceptors. Alternatively, if the local decrease of norepinephrine reuptake is responsible for chamber specificity, 6-hydroxydopamine administration could facilitate beta-adrenoceptor downregulation. In the present study, myocardial denervation and induction of aortic regurgitation facilitated the downregulation of beta-adrenoceptor in the nonfailing right ventricle as well as in the failing left ventricle. These results suggest that a defect of neuronal reuptake in the failing ventricle may be responsible for the chamber-specific regulation of beta-adrenergic signaling in heart failure.

Chamber-specific abnormalities of the neuronal norepinephrine reuptake mechanism have been previously described using an experimental model for right ventricular failure (5,6). These investigators reported that local neuronal norepinephrine uptake activity and tissue norepinephrine content were decreased. In addition, noradrenergic nerve terminals themselves were lost from the failing ventricle, as evidenced by tyrosine hydroxylase immunocytochemical staining. The reuptake mechanism at the synaptic nerve terminals maintains a low level of norepinephrine at the postjunctional beta-adrenoceptor. When the reuptake mechanism is disturbed, the beta-adrenoceptors might be easily downregulated by the local increase in norepinephrine concentration. Intact sympathetic innervation in nonfailing myocardium may exert a protective effect against the downregulation of beta-adrenoceptor.

Conclusions. Sympathetic denervation using 6-hydroxydopamine abolished the chamber-specific alterations in beta-adrenergic signaling in rabbits with heart failure. Local loss of sympathetic nerve endings, especially the defect of neuronal norepinephrine uptake, is likely to be responsible for the chamber-specific downregulation of beta-adrenoceptor. Such local denervation may also affect postreceptor, G-protein–mediated signaling.

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