Myocardial Adrenergic Denervation Supersensitivity Depends on a Postreceptor Mechanism Not Linked With Increased cAMP Production

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Background. Two major hypotheses have been proposed to explain catecholamine supersensitivity after myocardial denervation, but neither sufficiently explains certain features of the phenomenon. In addition, a nonsurgical method for long-term myocardial adrenergic denervation is desirable but has not been accomplished or described with respect to catecholamine supersensitivity.

Methods and Results. We have accomplished chronic myocardial adrenergic denervation by using 6-hydroxydopamine (6-OHDA). Sixteen weeks after 6-OHDA administration to newborn pigs, we found substantial myocardial adrenergic denervation associated with β-adrenergic receptor (βAR) downregulation. Despite decreased βAR number, the dose of isoproterenol yielding 50% maximal heart rate change (ED₅₀) was decreased, and heart rates during exercise showed increased responsiveness despite decreased circulating catecholamines. Thus, stimulation of fewer receptors yielded an increased response, implying improved signal transduction efficiency. Competitive binding studies with isoproterenol showed an increased proportion of βAR with high-affinity binding in myocardial membranes from 6-OHDA pigs, suggesting that interaction between βAR and cardiac Gₛ may contribute to improved signal transduction efficiency. However, measures of adenylyl cyclase activity indicated marked reduction in βAR-dependent and Gₛ-dependent cAMP production in myocardial membranes from denervated animals despite a normal amount of cardiac Gₛ and decreased Gₛ.

Conclusions. We have demonstrated that substantial, long-term myocardial adrenergic denervation is possible using 6-OHDA. Denervation supersensitivity in this model does not depend on enhanced cAMP stimulation but rather depends on postreceptor elements in the βAR-responsive pathway that may be independent of Gₛ-activated adenylyl cyclase activity. In this model of adrenergic denervation supersensitivity, β-receptors, through Gₛ, may be linked to an alternative effector that drives heart rate responsiveness. (Circulation 1992;85:666–679)

Two hypotheses have been proposed to explain catecholamine supersensitivity after myocardial denervation. The presynaptic hypothesis proposes that supersensitivity is due to absence of uptake-1 clearance, which depends on intact adrenergic nerves.⁠¹⁵ Although this may account for supersensitivity to norepinephrine, it does not explain supersensitivity to isoproterenol, which does not depend on uptake-1 clearance. The postsynaptic hypothesis proposes that supersensitivity may involve changes in the β-adrenergic receptor (βAR)-responsive adenylyl cyclase pathway, such as βAR upregulation, but myocardium from transplanted (denervated) human hearts does not show βAR upregulation, and no studies have shown increased βAR-mediated adenylyl cyclase activation after myocardial denervation; therefore, this hypothesis, although appealing, has not been proven.

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Previous studies have used total cardiac denervation (sympathetic and parasympathetic) to study this phenomenon.\textsuperscript{1,2} We used 6-hydroxydopamine (6-OHDA) to induce selective, nonsurgical myocardial adrenergic denervation, avoiding the potentially confounding influences of thoracotomy and parasympathetic denervation. Because surgical myocardial denervation has limitations (i.e., requires thoracotomy and can result in high perioperative mortality rates, concomitant parasympathetic denervation, and nerve regrowth), a nonsurgical method of denervation is desirable.

A potential problem with 6-OHDA-induced denervation is nerve regrowth. For example, 2 hours after injecting adult mice with 6-OHDA, myocardial norepinephrine concentrations were decreased 95%; by 8 weeks, however, myocardial norepinephrine concentration returned to normal, suggesting regeneration of adrenergic nerve terminals.\textsuperscript{3} In contrast, when 6-OHDA was given repeatedly during the first 10 days of life to newborn rats, myocardial norepinephrine depletion was substantial (85%) 15 weeks later, suggesting that chronic myocardial adrenergic denervation is possible if 6-OHDA is given when adrenergic neural pathways are still forming.\textsuperscript{4} Thus, our study had two goals: to develop a model of long-term, nonsurgical myocardial adrenergic denervation, and to define the relations between myocardial \( \beta \)AR number, physiological responsiveness, and postreceptor elements resulting from myocardial adrenergic denervation.

To achieve these goals, we administered 6-OHDA to newborn pigs and studied the pigs 14–16 weeks later. We used pigs rather than smaller mammals because their larger hearts allow extensive biochemical analyses in multiple areas: the sinoatrial node region (SAN), right atrium (RA), and left ventricle (LV), thereby providing a means to correlate biochemical and physiological measures in individual animals. Our hypotheses were that denervation would result in decreased isoproterenol \( ED_{50} \) for heart rate change (increased postsynaptic responsiveness) and increased myocardial \( \beta \)AR number and cAMP production.

**Methods**

**Animals**

Experimental animals were 12 pigs (Sus scrofa), littermates of either sex. The neurotoxin 6-OHDA was dissolved in 0.001N HCl saturated with nitrogen gas and kept chilled and away from light until administration. Pigs were injected with 6-OHDA (70 mg/kg i.p.) on alternate days beginning the first day of life for a total of five injections in 10 days. Four pigs remained ill (vomiting, poor appetite) after the initial injection and died within the first 10 days of life; three others died several weeks later, unrelated to toxic effects of 6-OHDA. (Acute toxicity results from norepinephrine released from adrenergic nerves.) 6-OHDA is metabolized rapidly; it has been shown that 24 hours after intravenous injection of \( ^{3} \)H]-6-OHDA to rats, no \( ^{3} \)H-amines were found in heart or spleen\textsuperscript{5}; therefore, toxicity related to persistent drug is not likely. All pigs remained with their mother and were weaned at the usual time (8–10 weeks). Five pigs survived and underwent pharmacological and physiological testing at 14–15 weeks and were killed at 16 weeks. These pigs fed vigorously and were developmentally normal.

Controls were 10 weight-matched pigs (control, 14±3 kg; 6-OHDA, 13±3 kg; \( p=\text{NS} \)). Five were killed, and myocardial samples were used for biochemical studies; five underwent identical pharmacological and exercise tests as the experimental group. Experimental and control animals were housed in identical quarters and received identical amounts of human contact.

**Isoproterenol-Stimulated Chronotropic Response**

After placement of venous and arterial catheters (neck incision, aseptic) and full recovery from surgery (minimum 2 days), animals underwent pharmacological testing using (−)isoproterenol.\textsuperscript{6} Tests were conducted in early morning hours in a quiet, dark laboratory (21°C). Response to isoproterenol was assessed by graded bolus injections of (−)isoproterenol (10–500 \text{ \mu g/kg i.v.}) and recording heart rate until there was no further increase (usually 30 seconds). At low doses, the 12-second period showing the largest increase in heart rate was taken as the maximal response. This interval always included at least one full respiratory cycle. At higher doses, respiratory variation in heart rate was not present; therefore, the shortest RR interval was taken as the maximal response. Heart rate returned to baseline between each dose of isoproterenol. Injections were given until there was less than 5% increase in heart rate: The preceding dose was chosen as the one that caused a maximal response. The order of delivery of doses of isoproterenol does not affect the relation between dose and response by using this protocol. The relation between change in heart rate and the logarithm of the dose of isoproterenol (micrograms per kilogram) was examined by linear regression analysis. Two or three tests conducted on separate days were combined into a single linear regression in each animal. Correlation coefficients (\( r \)) ranged from 0.92 to 0.98 (mean, 0.95±0.02). The dose of (−)isoproterenol yielding \( ED_{50} \) was calculated from the linear regression equation.

In previous studies of mature pigs, we used atropine pretreatment to block reflex vagal withdrawal during isoproterenol testing\textsuperscript{7} because the intervention (exercise training) was expected to alter vagal tone. In the present study, we found that atropine (0.075 mg/kg) raised basal heart rates in these young animals to a degree that significantly limited the range at which the isoproterenol dose–response relation could be examined (171±15 beats per minute). In addition, we have found that after atropine administration, heart rate begins to return toward base-
line within 10 minutes and is completely normal within 30 minutes. Our tests last a minimum of 60 minutes; therefore, a single dose of atropine would have been unsuitable. Because of these problems, and because we did not expect altered vagal tone or differences between the two groups in vasodilating effects of isoproterenol administration, we elected to perform the studies without atropine. Nevertheless, to assess whether reflex withdrawal of vagal tone at the SAN (caused by isoproterenol-induced fall in mean blood pressure) or increased vagal tone at the SAN (caused by isoproterenol-induced increased pulse pressure) affected results, arterial blood pressure was monitored. The fall in mean arterial blood pressure at the ED₉₀ for isoproterenol was similar (control, −19±5 mm Hg; 6-OHDA, −17±3 mm Hg; p=NS), and the degree to which arterial pulse pressure was increased was also similar (control, 28±9 mm Hg; 6-OHDA, 23±13 mm Hg; p=NS). Because the afferent inputs of the reflex were unchanged, we made the assumption that reflex alterations of vagal tone at the SAN also were similar in both groups. Our approach is supported by data showing that the dose–response relation between bolus intravenous administration of isoproterenol and heart rate change is not influenced by pretreatment with atropine when the vagus is intact, and that preventing the fall in systemic blood pressure with angiotensin infusion during isoproterenol testing does not alter heart rate responsiveness.

**Treadmill Exercise Testing**

Animals were run to exhaustion on a motor-driven treadmill with the use of a graded protocol while heart rate was monitored. Animals were preconditioned to treadmill running and human handling in an identical manner. Two tests were performed in each animal at least 24 hours before or after pharmacological testing. Maximal effort was assessed by observing a plateau in the slope of the line relating heart rate and work rate. During each exercise protocol, animals ran at 66±5 m/min; grade was increased 5% every 2 minutes, and heart rate was recorded the last 20 seconds of each grade. Blood samples for plasma catecholamine measurements were obtained via indwelling venous catheters from each animal in the basal state and at peak exercise. Data analyzed included maximal work rate achieved (KPM per minute) and absolute heart rate at each stage of exercise.

**Terminal Surgery**

Forty-eight hours after all pharmacological and physiological tests were completed, animals were sedated with thiamylal sodium (10–15 mg/kg), intubated, and anesthetized with 1% halothane. Midline sternotomies were made, the heart was removed and placed in iced saline (4°C), and the RA, left atrium, and right ventricular free wall were removed. The LV and septum were weighed. A transmural sample of LV free wall was taken midway from base to apex near the midportion of the left anterior descending coronary artery. A transmural sample was taken from the midportion of the RA free wall. In addition, a sample that included the SAN was identified by anatomic landmarks: The junction between the superior vena cava and RA was located and a transmural sample above the crista terminalis including this junction but excluding venous tissue was taken (wet wt <300 mg). We have confirmed previously by histological inspection that samples obtained in this manner include the SAN. Furthermore, increased tissue norepinephrine content of SAN compared with RA in control animals (SAN, 7,117±927 pg/mg; RA, 4,294±621 pg/mg; p=0.0005) reflects denser adrenergic innervation, an expected finding in SAN tissue. Myocardial samples were rinsed free of blood and frozen (−70°C). Time from heart removal to placement in liquid nitrogen was 10–15 minutes. Adrenal glands were dissected, weighed, and frozen (−70°C).

**Plasma and Tissue Catecholamine Measurements**

Epinephrine and norepinephrine concentrations in plasma, myocardium, and adrenal glands were determined using a previously described sensitive radioenzymatic assay and are expressed as catecholamine per milligram of wet tissue, or per milliliter of plasma.

**Histological Examination**

LV samples were prepared for light microscopy and stained with hematoxylin and eosin for general histological assessment or with Masson’s trichrome to assess degree of fibrosis. Transmural sections of RA and LV underwent histochemical staining with glyoxylic acid to examine the distribution of residual norepinephrine after denervation. Slides were examined by fluorescent microscopy.

**Membrane Preparation**

Frozen (−70°C) transmural samples were powdered in a stainless steel mortar and pestle (also −70°C), placed in Tris buffer, and glass-glass homogenized, and contractile proteins were extracted (0.5 mM KCl for 20 minutes at 4°C). The pellet of a 45,000g centrifugation was resuspended in buffer, and radioligand binding experiments, adenyly cyclase studies, and reconstitution studies were performed.

**βAR Binding Studies**

β-Receptors were identified using the radioligand [¹²⁵I]iodocyanopindolol (ICYP) in saturation isotherm experiments described previously. Kᵣ for isoproterenol and proportion of β-receptors showing high-affinity binding were determined in competition binding experiments by incubating 100 μM ICYP with isoproterenol (10⁻¹⁰–10⁻⁴ mM) with and without 5'-guanylylimidodiphosphate (Gpp[NH]p, 100 μM). The proportion of β₁- and β₂-receptors was determined in competition experiments by using selective β₁-receptor antagonists (bisoprolol, betaxolol 10⁻¹⁰–10⁻⁴ M). Protein concentrations were determined by Bradford assay; a cardiac sarcolemmal marker, K⁺-
stimulated p-nitrophenyl-phosphatase (K+-pNPPase), was used to detect changes in sarcolemmal yield.\(^{15}\)

Experiments were conducted to establish that β-receptors were not lost to the supernatant. When LV membranes were prepared (as above), K+-pNPPase activity, a marker for sarcolemmal membrane, was negligible in the supernatant. We therefore concluded that receptors, if present in the supernatant, must be unassociated with sarcolemmal membrane fragments. To detect solubilized receptors, we dia

lyzed 10 ml supernatant from a 45,000 g centrifugation with 1.0 l potassium-deficient Tris buffer overnight at 4°C to reduce KCl concentration (to prevent interference with the receptor-radioligand interaction). Supernatants (0.3 mg/ml) then were used in saturation isotherm experiments (n=2; LV from control and 6-OHDA-treated animals). GF/C filter paper was pretreated with 2% polyethyleneimine to trap solubilized receptors. There was no specifically bound ICYP in the supernatant fraction of control or 6-OHDA-treated animals. Thus we believe that data obtained from saturation isotherms conducted on the membrane preparation used accounts for all of the β-receptors.

**Adenylyl Cyclase Assays**

Methods were modified from those of Salomon.\(^{17}\)

Adenylyl cyclase activity was determined in a final volume of 100 μl, and the assay mixture included final concentrations of 0.5 mM ATP, 5 mM creatine phosphate, 50 U/ml creatine phosphokinase, 0.1 mM cAMP, 10 mM HEPES buffer (pH 7.4), 2.5 mM MgCl₂, 0.25 mM EDTA, and 0.74 μM [α-32P]-ATP (800,000 cpm). The following agents were used to stimulate cAMP production (final concentrations are given): manganese (10 mM), Gpp[NH]p (100 μM), isoproterenol (10 μM), and NaF (10 mM). Appropriate concentrations of Gpp(NH)p and other stimulators were determined in preliminary studies. To clarify the efficiency of G₁-dependent stimulation of adenylyl cyclase, Gpp(NH)p was used in increasing doses and cAMP production was measured. To clarify the efficiency of βAR-dependent stimulation of adenylyl cyclase, (-)-isoproterenol was used in increasing doses (with 100 μM GTP) and cAMP production was measured.

Reactions were initiated by adding 120–140 μg of myocardial membrane homogenate to ice-cold reagents and incubating at 37°C for 15 minutes. We found that cAMP production under these conditions was linear with respect to time and protein concentration and that isobutyl methlyxanthine or adenosine deaminase (or both) had no effect on basal or maximally stimulated cAMP production. Reactions were terminated by placing tubes in an ice-cold bath and adding 100 μl stopping solution containing 40 mM ATP, 1.4 mM cAMP, [1H]-cAMP (7,000 cpm), and 50 mM Tris-HCl (pH 7.5); Na dodecyl SO₄ (2%) then was added to each tube, and tubes were heated to 100°C for 3 minutes. Volumes were then brought to 1 ml by adding 800 μl water, and cAMP was fractionated using Dowex-alumina sequential column chromatography. Recovery was 75–92% by these methods, and replicate variation was less than 10%.

Experiments were conducted to establish that adenylyl cyclase activity was not lost in the supernatant of a 45,000 g centrifugation. We found that forskolin-stimulated cAMP production was negligible in the supernatant of a 45,000 g centrifugation prepared from LV membranes (supernatant, 18 pmol/mg/min; pellet, 541 pmol/mg/min; n=2). NaF stimulation was used to assess the presence of G₁ and catalyst in the supernatant. Less than 2% of cAMP produced was found in the supernatant (control, 4±2 pmol/mg/min; 6-OHDA, 5±2 pmol/mg/min; p=NS; n=4 for both groups). These data exclude the possibility that reduced adenylyl cyclase activity was due to loss of adenylyl cyclase activity in the supernatant.

To determine the effects of endogenous catecholamines on adenylyl cyclase activity, we performed experiments on LV homogenates from seven control pigs, measuring the impact of 1.0 μM propranolol on basal, fluoride-stimulated, and Gpp(NH)p-stimulated cAMP production. Blocking the effects of endogenous catecholamines with propranolol did not alter basal (no propranolol, 57±12 pmol/mg/min; propranolol, 60±17 pmol/mg/min), fluoride-stimulated (no propranolol, 189±48 pmol/mg/min; propranolol, 181±61 pmol/mg/min), or Gpp(NH)p-stimulated adenylyl cyclase activity (no propranolol, 135±44 pmol/mg/min; propranolol, 134±41 pmol/mg/min). Thus, differences in adenylyl cyclase activity are not likely to result from differences in endogenous catecholamine concentrations.

**Quantification of G₁**

We modified a reconstitution assay for use with porcine myocardial membranes.\(^{18}\) The capacity of a cholate extract of myocardial membrane homogenate to reconstitute G₁-mediated production of cAMP in membranes of G₁-deficient (cyc⁻) murine S49 lymphoma cells served as a functional assay for G₁. Cyc⁻ cells (strain 94.15.1) were grown at 37°C in Dulbecco’s modified Eagle’s medium containing 25 mM Na HEPES (pH 7.4) and 10% heat-inactivated horse serum. Plasma membranes were prepared after the methods of Ross et al\(^{19}\) by using a nitrogen cavitation apparatus to lyse cells by rapid decompression after equilibration for 10 minutes with N₂ at 450 psi at 4°C. Cyc⁻ membranes were suspended (3 mg/ml) in buffer (20 mM Na HEPES, pH 8.0), 2 mM MgCl₂, and 1 mM EDTA. The preparation then was stored at -70°C.

Cardiac membrane homogenates (SAN and RA) were suspended (3 mg/ml) in Tris buffer and solubilized in 1% Na cholate for 1 hour at 4°C. The supernatant from a 45,000 g spin (at 4°C for 30 minutes) was heated at 30°C for 10 minutes to inactivate solubilized catalytic subunit of adenylyl cyclase. This extract was then diluted in 0.1% Lubrol PX (in the same buffer) to stabilize G₁, and 15 μl of extract (or serially diluted extract) was then added to
25 μl of lysed cyc⁻ membranes (75 μg of cyc⁻ membrane protein) and agitated for 30 minutes at 4°C. To maintain comparable concentrations of protein and detergent, diluted extract mixtures were supplemented with undiluted extract (heated at 100°C for 10 minutes to inactivate G_i). Preactivation was initiated by adding 10 μl of 1.0 mM GTP, 10 μl of 100 mM NaF, and 16.5 μl of reaction buffer and incubating for 20 minutes at 30°C. Adenyl cyclase activity was assayed by adding [α-³²P]-ATP (800,000 cpm) in 23.5 μl water to each sample tube and incubating for 10 minutes at 30°C. The reaction was then terminated, and cAMP production was measured as described above. Reconstitution assays were performed on undiluted extract and several serial dilutions, giving a wide range of protein content. We found that intrinsic adenylyl cyclase activity in extract and cyc⁻ membranes is negligible. In preliminary studies, we found cAMP production to be proportional to the amount of extract added, and that the rate of cAMP synthesis remains linear with time for 40 minutes. We performed assays on extracts from experimental animals side by side with appropriate control extracts, using the same batch of cyc⁻ membranes, to minimize the potential confounding influence of variation in catalytic subunit concentration in cyc⁻ membranes. Data are expressed as picomoles of cAMP produced per 10 minutes as a function of membrane protein used for the detergent extraction.

Assessment of G_i

We used pertussis toxin–dependent ADP ribosylation to assess G_i in membrane homogenates from SAN and LV. [³²P]-ADP ribose incorporation into G_i in the presence of pertussis toxin, using [³²P]-NAD substrate (specific activity, 40 Ci/mmol), was modified from the method of Bokoch et al. Ten microliters of sarcolemmal membrane protein (1 μg/ml) was incubated for 60 minutes at 30°C with 28 μl of a solution containing 10 mM DTT, 100 mM Tris, 10 mM thymidine, 100 μM GTP, 1.0 mM ATP, 2.5 mM MgCl₂, 0.25 mM EDTA, 0.5 mM β-NADP, 1.0 μM [³²P]-NADP, 1.0 μM [³²P]-NAD, and 0.2 μg pertussis toxin (pH 8.0). Preactivation of pertussis toxin was not necessary to achieve maximal ribosylation if toxin was added simultaneously with 10 mM DTT. Optimal incubation time and protein and NAD concentrations were determined in preliminary experiments.

Pretreatment of atrial membrane homogenates with pertussis toxin was used as a functional assay for cardiac G_i. Pertussis toxin was activated (25 mM DTT for 30 minutes at 32°C), added (10 μg/ml final concentration) to atrial membrane homogenates (3 mg/ml), and incubated for 90 minutes at 30°C. This concentration has been shown to inactivate pertussis toxin substrate in other membrane preparations. Preincubated homogenates then underwent adenylyl cyclase assays with the use of Gpp(NH)p as stimulant, thereby evaluating the extent to which G_i had been inhibiting G_i-dependent stimulation of adenylyl cyclase.

Data Analysis

Data are expressed as mean±1 SD. Comparisons of group means are made using t test for unpaired data. The null hypothesis was considered unlikely when p<0.10 and rejected when p<0.05 (two-tailed). The Pearson product–moment correlation coefficient (r) is reported as a measure of the strength of association between change in heart rate and logarithm of dose of isoproterenol determined by linear regression analyses. Saturation isotherm experiments underwent Scatchard analysis, and competition binding experiments were analyzed by nonlinear regression (Graphpad, Harvey Motulsky, UCSD). F ratios were used to test whether one- or two-component curves better fit the data, and when equivalent, the simpler model was selected.

Results

Tissue Catecholamines

Sixteen weeks after 6-OHDA treatment of neonatal pigs, histological analyses of sections of RA and LV stained with glyoxylic acid, which causes fluorescence of biogenic amines, showed that denervation was distributed evenly across the LV wall from endocardium to epicardium. Much of the histofluorescence remaining in RA of 6-OHDA-treated animals was associated with vascular structures (Figure 1). Radioenzymatic measurements of myocardial norepinephrine content (Figure 2) documented substantial denervation, with an 81% reduction in SAN norepinephrine content (control, 7,117±927 pg/mg; 6-OHDA, 1,329±303 pg/mg; p<0.001), a 79% reduction in RA norepinephrine (control, 4,294±621 pg/mg; 6-OHDA, 918±272 pg/mg; p<0.001), and an 83% reduction in LV norepinephrine (control, 1,471±574 pg/mg; 6-OHDA, 252±213 pg/mg; p<0.01). Myocardial epinephrine was unchanged by 6-OHDA treatment in SAN (control, 117±64 pg/mg; 6-OHDA, 95±44 pg/mg; p=NS), RA (control, 51±39 pg/mg; 6-OHDA, 88±139 pg/mg; p=NS), and LV (control, 52±25 pg/mg; 6-OHDA, 44±73 pg/mg; p=NS). Adrenal epinephrine (control, 335±145 ng/mg; 6-OHDA, 377±132 ng/mg; p=NS) and norepinephrine (control, 309±215 ng/mg; 6-OHDA, 365±154 ng/mg; p=NS) were unaltered by 6-OHDA treatment.

Isoproterenol-Stimulated Chronotropic Response

Figure 3 shows results of dose–response studies examining the ability of graded bolus doses of isoproterenol to increase heart rate. Basal heart rate was unchanged by 6-OHDA treatment (control, 117±14 beats per minute; 6-OHDA, 132±13
beats per minute; \( p = 0.17 \), but maximal isoproterenol-stimulated heart rate was decreased somewhat (control, 267±6 beats per minute; 6-OHDA, 245±12 beats per minute; \( p < 0.05 \)). Isoproterenol \( ED_{50} \) for heart rate change was decreased by 50% after denervation (control, 0.08±0.03 \( \mu \)g/kg; 6-OHDA, 0.04±0.01 \( \mu \)g/kg; \( p = 0.01 \); Figure 3). The threshold dose, the minimal dose of isoproterenol required for an effect on heart rate, was determined from the dose–response data (X intercept). This value was 50% reduced in animals treated with 6-OHDA (control, 21±5 ng/kg; 6-OHDA, 10±2 ng/kg; \( p = 0.04 \)), thus confirming, in a manner independent of maximal response, that heart rate response to isoproterenol stimulation was increased.

**Treadmill Exercise Testing**

Heart rate response to graded treadmill exercise is shown in Figure 4. Heart rates while standing on the treadmill, in anticipation of running, were similar (control, 148±22 beats per minute; 6-OHDA, 147±18 beats per minute; \( p = \text{NS} \)), as were maximal exercise heart rates (control, 306±18 beats per minute; 6-OHDA, 316±14 beats per minute; \( p = \text{NS} \)). Maximal work rates were similar (control, 218±34 KPM/min; 6-OHDA, 243±57 KPM/min; \( p = \text{NS} \)). However, heart rate responses during graded submaximal exercise were quite different. For example, during stage 1, at heart rates indicating complete parasympathetic withdrawal,22 heart rate was much higher in 6-OHDA-treated animals (control, 209±16 beats per minute; 6-OHDA, 257±26 beats per minute; \( p = 0.01 \); Figure 4). Increased heart rate response in 6-OHDA-treated animals was observed through the first several stages of exercise but became less apparent at higher work rates because maximal heart rate was unchanged.

**Plasma Catecholamine Measurements**

Basal plasma epinephrine (control, 143±75 pg/ml; 6-OHDA, 133±37 pg/ml) and norepinephrine (control, 285±55 pg/ml; 6-OHDA, 269±41 pg/ml) were unchanged 14–16 weeks after 6-OHDA treatment. However, the degree to which circulating catechol-

**Figure 3.** Plot showing isoproterenol-stimulated heart rate change as a function of dose: Typical data from one control and one 6-OHDA-treated pig. The entire curve was left-shifted following denervation, documenting increased heart rate responsiveness to isoproterenol stimulation. Inset: Data from all animals show that isoproterenol dose required for 50% maximal heart rate response (\( ED_{50} \)) was reduced in animals treated with 6-OHDA. Bars represent mean values (\( n = 5 \) for each group); error bars denote 1 SD.

**Figure 4.** Plot showing heart rate as a function of work rate during treadmill running. 6-OHDA-treated pigs had higher heart rates at lower work rates despite complete vagal withdrawal, but basal and maximal heart rates and maximal work rates were similar to control pigs. Points represent mean values from each stage of exercise (control, circles; 6-OHDA, triangles). Error bars represent 1 SEM. Inset: Results of radioenzymatic assays of plasma catecholamines obtained from animals at peak effort. Both epinephrine (EPI) and norepinephrine (NOREPI) were decreased at peak exercise in cardiac denervated animals (control, clear bars \( n = 5 \), 6-OHDA, hatched bars \( n = 4 \)). Bars represent mean values; error bars denote 1 SEM.
amines increased with exercise was blunted by 6-OHDA treatment; both epinephrine (control, 6,692±3,553 pg/ml; 6-OHDA, 2,617±1,363 pg/ml; p<0.01) and norepinephrine (control, 23,917±20,888 pg/ml; 6-OHDA, 9,510±3,776 pg/ml; p=0.06) were reduced at peak exercise (Figure 4, inset).

β-Adrenergic Receptor Binding Studies

Figure 5 shows the results of saturation isotherm experiments; βAR number was decreased by 6-OHDA treatment in SAN (control, 111±18 fmol/mg; 6-OHDA, 74±16 fmol/mg; p=0.02), RA (control, 91±12 fmol/mg; 6-OHDA, 67±15 fmol/mg; p<0.05), and LV (control, 92±7 fmol/mg; 6-OHDA, 73±9 fmol/mg; p<0.01). Data shown for RA and LV are the mean values of three saturation isotherms per tissue per animal, performed with triplicate points for each of eight concentrations of ICYP. Data for SAN are from a single experiment (triplicate points, eight concentrations of ICYP in each of five control and five 6-OHDA-treated animals). Kd for ICYP was invariant with denervation in both RA (control, 70±20 pM; 6-OHDA, 82±16 pM; p=NS) and LV (control, 70±12 pM; 6-OHDA, 65±17 pM; p=NS). Mean r² values for Scatchard analyses were 0.92±0.05.

Heart weight-to-body weight ratios were unaltered by 6-OHDA treatment (control, 3.5±0.3 g/kg; 6-OHDA, 3.3±0.6 g/kg), suggesting that myocardial hypertrophy did not occur. The possibility that membrane yield per milligram of crude membrane homogenate was altered by 6-OHDA was assessed using the specific activity of K⁺-PNNPase as a marker for sarcolemmal membrane.15 There was no significant difference in specific activities of this marker in membrane homogenates (control, 288±34 nmol/mg/hr; 6-OHDA, 278±28 nmol/mg/hr; p=NS). Histological analyses of myocardium from 6-OHDA-treated animals did not show inflammatory infiltrates or fibrosis.

The affinity of β-receptors for (-)-isoproterenol (with Gpp[NH]p) did not change with 6-OHDA treatment (control, Kᵢ=0.5±0.4 nM; 6-OHDA, Kᵢ=0.4±0.3 nM; p=NS). However, the proportion of high-affinity binding sites, determined by competitive binding with (-)-isoproterenol in the absence of guanosine triphosphate (GTP), was increased by 6-OHDA treatment. After adrenergic denervation, studies on both RA and LV membranes from 6-OHDA-treated animals showed a 2.5-fold increase in the proportion of β-receptors in the high-affinity state (Table 1).

Competitive binding studies with the selective β₁-antagonists betaxolol and bisoprolol demonstrated that βAR subtype proportions were changed by cardiac denervation (Table 2). Denervated animals showed a higher proportion of β₂-subtype receptors in LV membranes. The number of β₁- and β₂-receptors were calculated using the mean proportion of βAR subtypes obtained from the bisoprolol and betaxolol competition experiments. Downregulation occurred among β₁-receptors only (control, 77±13 fmol/mg; 6-OHDA, 37±9 fmol/mg; p<0.0001), with β₂-receptors actually increasing in number with 6-OHDA treatment (control, 21±13 fmol/mg; 6-OHDA, 35±11 fmol/mg; p<0.05).

Adenylyl Cyclase Assays

Basal (unstimulated) adenylyl cyclase activity was lower in denervated animals (control, 89±7 pmol/mg/min; 6-OHDA, 30±12 pmol/mg/min). Adenylyl cyclase activity is therefore reported as net increase in stimulation (basal subtracted) to adjust for differ-

![FIGURE 5. Bar graph showing results of saturation isotherms performed on myocardial tissue. Sixteen weeks after 6-OHDA administration, β-adrenergic receptor number was reduced in the sinoatrial node region (SAN), right atrium (RA), and left ventricle (LV). Data are expressed as fmol/mg protein. Bars represent mean values (n=5 for each group); error bars denote 1 SD. Kᵢ-stimulated p-nitrophenolphosphatase, a sarcolemmal membrane marker, was used to establish that sarcolemmal yield was similar between groups; histological analyses showed no fibrosis or inflammation in myocardial samples from denervated animals.](http://circ.ahajournals.org/content/60/6/678.full.pdf)
ences in basal activity. βAR-dependent and G₁-dependent stimulation of adenylyl cyclase were diminished markedly in SAN and RA membranes from denervated pigs (Figure 6). Mean reduction in cAMP production was 46% (range, 29–60%). Stimulation with Mn²⁺, a measure of catalytic subunit activity, was invariant with denervation, suggesting that the decrement in cAMP production was not due to decreased catalytic subunit in the denervated animals.

Gpp(NH)p-stimulated adenylyl cyclase activity was diminished markedly through a wide range of Gpp(NH)p concentrations (Figure 7). Similarly, isoproterenol-stimulated adenylyl cyclase activity was diminished markedly through a wide range of isoproterenol concentrations (Figure 8). In addition to marked diminution of G₁-dependent activation, the EC₅₀ for Gpp(NH)p-stimulated cAMP production was increased by denervation (control, 1.6±0.1 μM; 6-OHDA, 2.5±0.3 μM; p<0.001), suggesting G₁-catalyst uncoupling. In contrast, despite diminished βAR-dependent activity, the EC₅₀ for isoproterenol-stimulated cAMP production was preserved (control, 0.3±0.3 μM; 6-OHDA, 0.5±0.5 μM; p=NS), suggesting preserved receptor–G₁ coupling. These data strongly suggest decreased receptor-dependent and G₁-dependent adenylyl cyclase activation through a wide range of physiologically relevant concentrations of agonist.

Quantification of G₁

To see if diminution in adenylyl cyclase activity was the result of diminished G₁, we performed reconstitution assays by using cholate extracts (G₁ rich) from SAN (Figure 9) and RA membranes. Sodium fluoride stimulation (G₁ dependent) of sarcolemmal membrane extracts from SAN and RA was unaltered by 6-OHDA treatment, suggesting that G₁ activity was invariant with denervation. For example, cholate extracts from 65 μg SAN sarcolemmal protein, when reconstituted into ctc cells, yielded similar amounts of cAMP production when stimulated by sodium fluoride (control, 21.8±3.2 pmol/10 min; 6-OHDA, 22±4.3 pmol/10 min; p=NS). In similar experiments,

![Figure 6](image)

**Figure 6.** Bar graph showing β-adrenergic receptor-dependent and G₁-dependent stimulation of adenylyl cyclase. Right atrial crude membrane homogenates were stimulated with Gpp(NH)p (Gpp, 100 μM), sodium fluoride (NaF, 10 mM), isoproterenol (ISO, 10 μM), or manganese (Mn²⁺, 10 mM). Although catalytic subunit activity was unchanged (Mn²⁺), there were striking decreases in β-adrenergic receptor-dependent and G₁-dependent cAMP production associated with cardiac denervation (control, clear bars; 6-OHDA, hatched bars). Bars represent mean values (n=5 for each group); error bars denote 1 SD. Inset: β-adrenergic receptor-dependent and G₁-dependent stimulation of sinoatrial node tissue also demonstrated decreased cAMP production after denervation (control, clear bars; 6-OHDA, hatched bars). Bars represent mean values; error bars denote 1 SD.

![Figure 7](image)

**Figure 7.** Graph showing G₁-dependent adenylyl cyclase stimulation. Right atrial crude membrane homogenates were stimulated with increasing concentrations of Gpp(NH)p. Gpp(NH)p-stimulated adenylyl cyclase activity was diminished markedly through a wide range of concentrations in cardiac membranes from denervated pigs. Points represent mean net values (basal subtracted). Control, circles; 6-OHDA, triangles (n=5 for each group). Error bars represent 1 SEM. Values at each concentration of Gpp(NH)p are significantly reduced after denervation (p values range from 0.0002 to 0.001).
using a different batch of cyc− cells and extracting 90 µg RA sarcolemmal protein, we also found no difference in Gs-stimulated cAMP production (control, 93.0±11.1 pmol/10 min; 6-OHDA, 84.0±7.0 pmol/10 min; p=NS). Differences in absolute cAMP production between SAN and RA in reconstitution assays reflect differences in catalytic subunit content of cyc− cells that can vary 10- to 20-fold.18

Assessment of Gs
To see if diminution in adenylyl cyclase activity in denervated myocardium was the result of increased Gs, we performed pertussis toxin-mediated ADP ribosylation studies to assess Gi in sarcolemmal membranes from SAN (Figure 9, inset) and LV. We found that pertussis toxin-catalyzed incorporation of [32P] into sarcolemmal membranes was reduced nearly 50% after denervation in SAN (control, 2,310±579 fmol/mg; 6-OHDA, 1,204±354 fmol/mg; p<0.01) and in LV (control, 1,694±481 fmol/mg; 6-OHDA, 872±292 fmol/mg; p<0.02).

Pertussis toxin pretreatment of atrial tissue, performed to inactivate Gs, showed that 6-OHDA-treated animals still showed a 42% decrease in Gpp(NH)p-stimulated cAMP generation (p=0.02, n=4 for both groups). These data suggest that depressed cAMP production was not due to inhibition of adenylyl cyclase by Gi.

Discussion
There are four principal findings of this work. First, 6-OHDA, when given to newborn pigs, results in substantial myocardial adrenergic denervation that persists for at least 16 weeks. Second, myocardial adrenergic denervation is associated with noncoordinate changes in myocardial βAR number and physiological responsiveness. Thus, after denervation, the heart appears to have a more efficient signal transduction mechanism, resulting in an enhanced response to βAR stimulation of a reduced number of β-adrenergic receptors. Denervation supersensitivity, in this model, depends on postreceptor elements in the βAR responsive pathway. Third, a factor that may contribute to denervation supersensitivity is enhanced interaction of βAR and cardiac Gi, as reflected by increased high-affinity binding after adrenergic denervation. The implications of these findings are that receptor number and physiological responsiveness may not be coordinately regulated in the heart after adrenergic denervation and that postreceptor elements in the β-receptor responsive pathway may represent a key mechanism for maintaining effective physiological responsiveness. And finally, the finding with the most important implications is that increased βAR-dependent stimulation of heart rate occurs independently of adenylyl cyclase stimulation, suggesting that β-receptors, through Gi, may be linked with an alternative effector that drives heart rate responsiveness.

Increased physiological responsiveness to βAR stimulation after 6-OHDA treatment is supported by five observations. First, isoproterenol ED50 for heart rate was decreased by 50% (Figure 3). The reduction in ED50, 0.04 µg/kg, is substantial because this dose would be predicted to yield a 38-beat per minute
increase in heart rate when administered to a control animal. Second, the minimal dose of isoproterenol required for a heart rate response (threshold dose) was decreased by 50% in denervated animals. Third, despite myocardial adrenergic denervation and resultant unopposed vagal tone, basal heart rate tended to be higher in 6-OHDA-treated animals. Fourth, heart rate response to submaximal exercise was increased (Figure 4). Heart rates at submaximal work rates were in excess of 200 beats per minute and therefore imply complete withdrawal of vagal tone; variation in parasympathetic tone is, therefore, not a likely explanation for the differences observed. Fifth, maximal exercise heart rate was unchanged despite decreased plasma catecholamines. It may be argued that heart rate responses to exercise reflect deconditioning in 6-OHDA-treated animals, but 6-OHDA-treated pigs and control pigs were handled identically and achieved similar peak work rates; this would not occur if differences in aerobic capacity were present. Taken together, these five distinct measures of adrenergic responsiveness establish that denervation supersensitivity was present, an expected sequela of denervation.

We were surprised to find downregulation rather than upregulation of myocardial βAR after denervation with 6-OHDA, but studies reporting βAR upregulation have measured βAR number within 2–3 weeks of 6-OHDA administration. A recent study of the time sequence of altered βAR expression after 6-OHDA administration to rabbits reported a transient upregulation of myocardial βAR at 2 weeks, followed by a decrease in βAR number at 4 weeks. Myocardial samples from 6-OHDA-treated pigs did not show fibrosis or inflammation on histological inspection, and heart weight-to-body weight ratios, an indicator of myocardial hypertrophy, were unaltered. Finally, K+-PPase activity was unchanged, suggesting that sarcolemmal yield was not altered by 6-OHDA treatment. We feel that decreased βAR number is an actual decrease in myocyte βAR density and not the result of myocardial hypertrophy or altered nonmyocyte elements of the heart.

Vatner and colleagues, using total cardiac denervation (surgical) in dogs, found a 50% decrease in norepinephrine ED50 for heart rate, but unlike our study, they found a 50% increase rather than a decrease in myocardial βAR number. Differences between their study and ours may be due to a species difference, to the influence of parasympathetic denervation in their model (vagal fibers are unaffected by 6-OHDA), or to an effect of 6-OHDA on βAR development or regulation when administered to immature animals.

Myocardial βAR number does not change in hearts of human transplant recipients, despite complete cardiac denervation. Indeed, two recent reports show a tendency for βAR downregulation in the transplanted heart. We had speculated that failure of β-receptors to upregulate in cardiac transplantation may reflect the influence of combined parasympathetic and postganglionic sympathetic denervation. However, we have shown that denervation with 6-OHDA, which leaves parasympathetic nerves intact, is associated with myocardial βAR downregulation. Therefore, postganglionic parasympathetic innervation may not be an important determinant of βAR number after adrenergic denervation of the heart.

Isoproterenol, by interacting with β-receptors in the sinoatrial node, induces tachycardia. However, isoproterenol decreases arterial blood pressure and widens pulse pressure, and therefore can activate baroreceptor reflexes, which may, independently of direct interaction with SAN β-receptors, influence heart rate. Altered vagal tone at the SAN is an effector component in this reflex. Cardiac transplantation and complete surgical denervation interrupt preganglionic parasympathetic nerves, thereby introducing a potentially confounding element into data obtained from isoproterenol-stimulated heart rate studies, especially when compared with subjects with intact parasympathetic nerves. This may explain why the effects of complete cardiac denervation on heart rate responses to isoproterenol have been reported to increase or not change. Our study circumvents these problems: Parasympathetic innervation is not altered by 6-OHDA, the afferent components that determine baroreceptor-mediated reflex control of vagal tone (arterial blood pressure and pulse pressure) were not altered, and myocardial muscarinic cholinergic receptor has been reported to be unchanged. We believe that the demonstration of a 50% reduction in isoproterenol ED50 for heart rate change reflects enhanced SAN signal transduction independent of altered reflex modulation.

A study of human cardiac transplant patients (total cardiac denervation) showed increased heart rate responsiveness to epinephrine but not to isoproterenol. This report and the previous report by Vatner et al proposed that denervation supersensitivity results in part from decreased neural catecholamine uptake, thereby resulting in prolonged activation of βAR by exogenous norepinephrine or epinephrine. We found that sensitivity to isoproterenol and plasma catecholamines (exercise) were increased to similar degrees. Because isoproterenol clearance does not depend on neural uptake, the data suggest postsynaptic denervation supersensitivity.

The proportion of high-affinity binding sites, determined by competitive binding with (-)isoproterenol in the absence of GTP, was increased 2.5-fold in both RA and LV membranes after denervation (Table 1). A higher proportion of high-affinity binding sites implies tighter coupling of the βAR with G, in denervated myocardium; this may be important mechanistically in denervation supersensitivity.

Competitive radioligand binding studies using selective β1-receptor antagonists (Table 2) provided estimates of βAR subtypes in LV membranes. It has been suggested that β2-receptors measured in LV homogenates may be associated only with nonmyo-
cyte elements such as presynaptic adrenergic nerves. However, we found an increased number of LV β2-receptors with adrenergic denervation, suggesting that presynaptic β2-receptors comprise a very small proportion of total LV β2-receptors. Down-regulation of β-receptors was selective for β1-receptors. Our study does not address the mechanism for differential regulation of βAR subtypes, although we speculate that adrenergic nerves may play an important developmental role influencing β1-receptor number. Recent data suggest that cardiac β2-receptors are linked with Gi more tightly than β1-receptors. Our data indirectly support this observation. After 6-OHDA treatment, β1-receptor number increased, as did the proportion of β-receptors showing high-affinity binding. These data may reflect tighter coupling of β2-receptors to Gi, thereby contributing to denervation supersensitivity.

Whether the shift to high-affinity binding is due to increased coupling of β2- or β1-receptors (or both) to cardiac Gi, the pathway leading to increased physiological responsiveness does not depend on increased adenylyl cyclase activity. Thus, a key finding of this work is that alterations in these postsynaptic, pre-effector events may be important by virtue of triggering an effector other than adenylyl cyclase.

The findings of increased heart rate responsiveness, decreased βAR, and enhanced βAR-Gi coupling led us to expect that Gi-mediated stimulation of cAMP would be increased in cardiac membranes from denervated pigs. Instead, to our surprise, we found that βAR-dependent and Gi-dependent stimulation of cAMP production were diminished markedly in both SAN and RA membranes (Figure 6). These findings cannot be explained by altered membrane protein yields because K+-NPPase, a sarcolemmal membrane marker, was unchanged. Diminished cAMP production was not due to reduction in catalytic subunit because Mn2+-stimulated cAMP production, a measure of catalytic subunit activity, was similar in both groups. SAN (Figure 9) and RA Gi were unchanged by denervation, indicating that deficient cAMP production was not due to insufficient or functionally inactive Gi. The reduction in basal activity in denervated myocardium is of unknown significance. Manganese stimulation was unchanged by denervation; therefore, we doubt that an alteration of the catalytic subunit was a factor.

Our findings regarding adenylyl cyclase activity do not stand in isolation. For example, Denness et al found a 74% reduction in guanine nucleotide–stimulated (Gi-dependent) adenylyl cyclase activity in transplanted (denervated) human LV membranes (p<0.01). Horn et al90 found that cardiac denervation in baboons resulted in increased adrenergic responsiveness (isoproterenol-stimulated dT/dt in isolated left ventricular trabeculae) despite a 29% decrease (p<0.05) in Gi-dependent cAMP production in LV membranes. These studies, performed on different species and with the use of different methods for denervation, are very similar to our own and suggest that our findings may have broad application.

An obvious concern regarding the diminution of adenylyl cyclase activity in myocardial membranes from denervated pigs was whether increased Gi might be inhibiting full expression of cAMP production. However, we found a 50% decrease in pertussis toxin substrate in SAN and LV membranes after denervation and decreased Gi-dependent cAMP production after inactivation of Gi by pretreatment with pertussis toxin. A 50% decrease in adenylyl cyclase stimulation despite a 50% decrease of Gi underscores our contention that denervation supersensitivity, in this model, does not depend on stimulation of adenylyl cyclase. If Gi is activating an alternative effector that regulates heart rate response, decreased Gi would allow enhanced effector stimulation in the 6-OHDA-treated animals, thus contributing to denervation supersensitivity. Such speculation assumes that Gi as well as Gi can interact at effectors other than adenylyl cyclase.

Decreased pertussis toxin substrate may have important effects on muscarinic cholinergic receptor signal transduction, a pathway unexamined in the present work. Reduced cardiac Gi may have contributed to elevated basal heart rate and increased heart rate response early during exercise. However, if isoproterenol-stimulated heart rate response results partially from vagal withdrawal at the SAN, reduced cardiac Gi and less basal vagal tone would diminish heart rate responsiveness. That denervated animals had increased responsiveness to isoproterenol stimulation despite presumed decreased vagal tone underscores our findings, making the adrenergic denervation supersensitivity all the more impressive.

Although exact molecular mechanisms elude us, the findings of postsynaptic denervation supersensitivity in association with depressed adenylyl cyclase activity are, we believe, convincing. In addition, increased βAR–Gi coupling and preserved cardiac Gi function are provocative observations. It has been demonstrated in isolated cell preparations that Gi can stimulate voltage-gated calcium channels independently of adenylyl cyclase. Recent work has confirmed that myocardial cell calcium current can be stimulated through βAR activation of Gi independently of adenylyl cyclase and that Gi can be found closely associated with calcium channels.

On the basis of data from these recent studies, we believe the present data are best explained by a βAR-driven, Gi-mediated but adenylyl cyclase–independent control of heart rate. We speculate that Gi may be operating through stimulation of calcium channels in cells of the SAN. An alternative pathway for regulation of heart rate is a novel finding, with potentially broad implications. For example, human cardiac transplantation is associated with denervation supersensitivity despite unchanged myocardial βAR number. We postulate that absence of cardiac adrenergic innervation may permit an alternative Gi-mediated pathway to drive heart rate respon-
siveness in cardiac transplant recipients, as in the current model. Alternatively, our findings may reflect a developmental abnormality. For example, adrenergic nerves may be necessary for normal development of the βAR-responsive adenyl cyclase pathway in the heart. By denervation of neonatal animals, an alternative pathway (perhaps a Gβγ-activated calcium channel) may have become dominant.

Recently, we found that when myocardial membrane homogenates are subjected to 45,000g centrifugation, Gβγ activity can be measured in the supernatant. It is possible that denervation supersensitivity, with attendant increased receptor stimulation, may have yielded an increased proportion of αi in the supernatant of membranes prepared from 6-OHDA-treated animals. If so, we would have underestimated cardiac Gβγ in denervated animals. Because we postulate that cardiac Gβγ is an important component for adrenergic responsiveness in denervated animals, albeit through an effector other than adenyl cyclase, a theoretical underestimation of cardiac Gβγ would underscore our findings.

These data are limited somewhat because analyses were not conducted sequentially; whether significant reinnervation has occurred or whether denervation was simply incomplete cannot be determined. We believe that persistent catecholamines probably represent 6-OHDA-resistant nerves in vascular structures, which are less susceptible to denervation with 6-OHDA compared with those innervating myocardium. Indeed, qualitatively, the majority of residual histofluorescence in RA tissue was associated with vessels rather than with myocardium (Figure 1). Because we did not assess myocardial muscarinic cholinergic receptor number or responsiveness, we cannot exclude the possibility that alterations in this pathway may have affected some of the physiological data.

Conclusions

We have demonstrated that long-term myocardial adrenergic denervation is possible with the use of 6-OHDA. Denervation is associated with myocardial βAR downregulation but increased responsiveness to catecholamine stimulation, indicating enhanced responsiveness of fewer receptors. Improved signal transduction efficiency was associated with an increased proportion of βAR showing high-affinity binding, suggesting that enhanced signal transduction may, in part, be due to altered interaction between myocardial βAR and cardiac Gβγ. We have found that denervation supersensitivity does not depend on enhanced cAMP stimulation but rather depends on postreceptor elements in the βAR responsive pathway that may be independent of Gβγ-activated adenyl cyclase activity. We speculate that Gβγ may be activating a calcium channel, thereby influencing heart rate responsiveness independently of cAMP production.

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