Chronic Norepinephrine Elicits Desensitization by Uncoupling the $\beta$-Receptor

Dorothy E. Vatner, Stephen F. Vatner, Jun Nejima, Nobuhisa Uemura, Elena E. Susanni, Thomas H. Hintze,* and Charles J. Homcy

Departments of Medicine and Pediatrics, Harvard Medical School, and the Cardiac Unit and Children’s Service, Massachusetts General Hospital, Boston, Massachusetts 02114; Brigham and Women’s Hospital, Boston, Massachusetts 02115; and The New England Regional Primate Research Center, Southborough, Massachusetts 01772

Abstract

The goal of this study was to determine the mechanism of $\beta$-adrenergic receptor desensitization after chronic elevation of circulating NE levels. Osmotic minipumps containing either NE or saline were implanted subcutaneously in dogs for 3–4 wk. Physiologic desensitization to isoproterenol was confirmed in conscious dogs, i.e., left ventricular $dP/dt$ increased in response to isoproterenol (0.4 $\mu$g/kg per min) by 5,625±731 mmHg/s in control dogs with saline pumps, and significantly less, $P < 0.01$, by 2,093±263 mmHg/s in dogs with NE pumps. Myocardial $\beta$-adrenergic receptor density as determined with $^{125}$I-cyanopindolol binding was 49% higher ($P < 0.05$) in the NE pump group. However, $\beta$-adrenergic receptor agonist binding with isoproterenol demonstrated a significant shift into the low affinity state for the animals with NE pumps. Basal, GTP plus isoproterenol, 5-guanylylimidodiphosphate, sodium fluoride, and forskolin-stimulated adenylate cyclase activity in the NE pump group were significantly depressed ($P < 0.05$) by amounts ranging from 20 to 40%. The functional activity of the guanine nucleotide binding protein G, was also reduced ($P < 0.05$) in animals with NE pumps. Thus, the process of desensitization in response to chronic elevation of NE levels in intact, normal dogs does not involve a decrease in $\beta$-adrenergic receptor density. Rather, it is characterized by reduced adenylate cyclase activation and uncoupling of the $\beta$-adrenergic receptor in association with decreased activity of the GTP-coupling protein G,.

Introduction

Catecholamine desensitization is thought to be involved in the pathogenesis of heart failure, a state that is characterized by elevated circulating levels of NE. The primary goal of the present investigation was to determine whether cardiac $\beta$-adrenergic receptor desensitization occurs after chronic elevation of circulating NE levels in normal animals. Although the phenomenon of catecholamine desensitization has been recognized for some time (1, 2) and has been examined repeatedly in vitro (3), relatively few studies have examined this phenomenon in vivo in a chronic model. The second goal of the present investigation was to determine the mechanism of the desensitization, i.e., whether it involved downregulation of $\beta$-adrenergic receptor density or alterations at the level of receptor coupling and/or adenylate cyclase activation.

To study these questions, miniosmotic pumps containing saline or NE were implanted subcutaneously for 3–4 wk in normal dogs. Physiologic desensitization was examined by comparing responses to the $\beta$-adrenergic agonist, isoproterenol, in conscious dogs with either NE or saline pumps. The animals were then killed and cardiac sarcolemma was prepared and used to determine $\beta$-adrenergic receptor density and adenylate cyclase activity. Agonist competition curves were used to quantitate the percent of coupled receptors, i.e., those binding agonist with high affinity. Finally, using purified sarcolemma the functional level of $G_s$, the GTP-regulatory protein that couples the $\beta$-receptor to adenylate cyclase, was also measured.

Methods

26 adult mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg) and ventilated with a respirator (Harvard Apparatus Co. Inc., S. Natick, MA). Using sterile technique, and through an incision in the left fifth intercostal space, Tygon catheters (Norton Co., Akron, OH) were implanted in the descending thoracic aorta and a solid-state pressure gauge (P22; Konigsberg Instruments Inc., Pasadena, CA) was inserted into the left ventricle via an apical stab wound. The incision was closed in layers, the pneumothorax was reduced, and the animals were allowed to recover. After 3–4 wk of recovery and after control experiments were completed, anesthesia was induced with sodium thiamylal (6–8 mg/kg) and locally with lidocaine to implant a miniosmotic pump (Alza Corp., Palo Alto, CA) in the subcutaneous tissue. In 10 animals 2 ml saline was placed in the pump, while in 16 animals NE was placed in the pump to infuse at a rate of 0.5 $\mu$g/kg per min over a 2-wk period. A second pump was implanted 10 d to 2 wk after the first pump such that the duration of the chronically high NE levels was 3–4 wk (4). Plasma NE samples were drawn from the chronically implanted aortic catheter and levels were assayed weekly in animals with NE pumps to assure sustained high levels of NE.

Arterial pressure was measured using the implanted catheters and a P23 Db strain gauge manometer (Statham, Oxnard, CA). Left ventricular pressure was measured with the solid-state miniature pressure gauge and calibrated in vitro against a mercury manometer and in vivo against the arterial pressure measurement. In all 10 dogs with saline pumps and in 9 of the 16 dogs with NE pumps physiological desensitization was assessed in the conscious state by examining responses to isoproterenol challenges before and 3–4 wk after implantation of the pumps. The effects of acute challenges to isoproterenol, 0.1–0.4 $\mu$g/kg per min for 5 min, were examined on measurements of left ventricular pressure and the rate of change of left ventricular pressure, $dP/dt$, an index of the inotropic state. To avoid the complicating effects of $\beta_2$-adrenergic receptor-mediated hypotension on the interpretation of the rate of change of left ventricular pressure in these experiments, phenylephrine was infused to maintain arterial pressure constant in animals with NE and saline pumps. The dose of phenylephrine, ranging from
from 1.6 to 8.0 µg/kg per min was not different in the two groups of animals. The data were recorded on a multichannel tape recorder and played back on a direct writing oscillograph.

At 1–4 d after physiological verification of desensitization the animals were anesthetized with sodium pentobarbital (30 mg/kg) and their hearts excised and placed in iced saline. The remaining seven dogs were killed at 3–4 wk after implantation of the NE pumps. The left ventricle and septum were weighed, trimmed of fat and connective tissue, minced, and homogenized in 4 vol of buffer I (0.75 M NaCl and 10 mM histidine, pH 7.5) with a PT-20S poltron for 5 s at half speed. The homogenate was centrifuged at 14,000 g for 20 min. The pellet was resuspended in buffer I, homogenized for 5 s at half speed, and centrifuged at 14,000 g for 20 min. The pellet was homogenized and centrifuged as before. The pellet was resuspended in buffer II (10 mM NaHCO₃ and 5 mM histidine), homogenized for 30 s three times at half speed, and centrifuged at 14,000 g for 20 min. The pellet was filtered through one layer of Japanese silk screen, size 12, and saved as the crude membranes. The supernatant was centrifuged at 44,000 g for 30 min. The resulting pellet was resuspended in 10 ml cold deionized water. An equal volume of 2.0 M sucrose was then added. This was applied to a discontinuous sucrose density gradient and centrifuged at 170,000 g for 85 min. The layer between the 0.6- and 0.25-M sucrose interfaces was removed and diluted in deionized water. The suspension was centrifuged at 170,000 g for 30 min. The pellet was resuspended in 0.25 M sucrose and stored at −70°C as the purified sarclemma (5).

All studies were performed in triplicate in the presence of Tris buffer (100 mM Tris, 1 mM EGTA, and 5 mM MgCl₂, pH 7.2). β-Adrenergic receptor antagonist binding studies were performed using eight concentrations of 25 µl [125I]-cyanopindolol ([125I]-cyp) ranging from 0.02 to 1.0 nM, 25 µl isoproterenol (0.1 mM), or buffer and 100 µl of the crude membrane protein (10 µg/assay). The antagonist binding data were analyzed by the “Ligand” program (6). A linear regression was performed on the amount bound vs. bound/free ligand. An r² value of 0.7 was the criterion used for acceptability of the data. Both crude and purified membrane sarclemma preparations were used in this study because of the limited yield of the purified sarclemma. Studies were performed with the crude membranes except for the Gₛ labeling and reconstitution experiments, which require the enriched sarclemmal preparation for optimal results; agonist-binding experiments were performed using both crude and purified membrane preparations. These preparations were shown to yield similar results in terms of high and low affinity states.

Competitive inhibition agonist binding curves were performed using 100 µl of both the crude (10 µg/assay) and purified sarclemma (0.5 µg/assay), 25 µl [125I]-cyp (0.1 nM), 25 µl isoproterenol (0.1 mM-0.1 mM) with 21 concentrations of isoproterenol, and 25 µl 5′-guanylylimidodiphosphate (Gpp[NH]p, 0.1 mM) or buffer. The assays were performed using membranes from 7 dogs with saline pumps and 11 animals with NE pumps. Assays were incubated at 37°C for 30 min, terminated by rapid filtration on GF/C filters (Whatman Laboratory Products Inc. Clifton, NJ), and counted in a gamma counter (TM Analytic, Inc., Elkgrove Village, IL) for 1 min. Specific binding was > 80%. The binding data were analyzed by the Ligand computer program of Munson and Rodbard (6). In the computer analysis the F test was used to compare the best fit for the ligand binding competition data. The best fit, two-site vs. one-site, was determined by the P value for the F test.

ADP ribosylation of Gₛ by cholera toxin was determined in purified sarclemma as previously described (7). Purified sarclemma from nine dogs with saline pumps and nine dogs with NE pumps were also used for the reconstitution of Gₛ into S49 lymphoma cec- membranes as described by Sternweis and Gilman (8). Cec- membranes were prepared according to the method of Ross et al. (9). Reconstitution was performed over a wide range of solubilized sarclemmal concentra-

1. Abbreviations used in this paper: cyp, cyanopindolol; Gpp[NH]p, 5′-guanylylimidodiphosphate; Gₛ, GTP = coupling protein; NE, nor-epinephrine.

![Figure 1. Gₛ reconstitution data are shown over a wide range of sarclemma protein concentrations (50–2,500 ng) for one animal in the saline pump group (open circles) and one animal in the NE pump group (solid circles). With increasing amounts of purified sarclemma protein, Gₛ from the animal with the pump stimulated less cAMP production than that from the animal with the saline pump. The inset shows the linear portion of the curve that was used for calculation of Gₛ activity.](image-url)
Table I. Baseline Values

<table>
<thead>
<tr>
<th></th>
<th>Saline pumps</th>
<th>NE pumps</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mmHg)</td>
<td>123±3</td>
<td>120±4</td>
</tr>
<tr>
<td>Left ventricular dP/dt (mmHg/s)</td>
<td>2,946±160</td>
<td>3,182±118</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>96±3</td>
<td>98±5</td>
</tr>
<tr>
<td>Mean left atrial pressure (mmHg)</td>
<td>5±2</td>
<td>3±2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>94±4</td>
<td>92±5</td>
</tr>
</tbody>
</table>

* P < 0.05 difference between groups.

3–4-wk period. Left ventricular tissue NE levels were similar in
the saline and NE pump groups (502±60 vs. 492±76 pg/mg).

Morphology. Animals in the saline pump group weighed
22±1.3 kg, and animals in the NE pump group weighed
21±1.2 kg. The left ventricle to body weight ratios were not
significantly different between the two groups (5.0±0.2 g/kg
for the animals with saline pumps, and 5.4±0.2 g/kg for those
with NE pumps).

Hemodynamics (Tables I and II). As shown in Table I,
there were no significant differences in left ventricular pres-
sure, left ventricular dP/dt, mean arterial pressure, and left
atrial pressure between the two groups of dogs. Heart rate was
lower (P < 0.05) after chronically elevated NE levels when
compared with that in the dogs with saline pumps. Dose-re-
sponse relationships to isoproterenol were examined in the two
groups of animals before and after the pumps were implanted,
with arterial pressure controlled. Isoproterenol increased heart
rate less in the dogs with NE pumps than in the group of dogs
with saline pumps (Table II). Physiological desensitization of
left ventricular inotropy, i.e., left ventricular dP/dt, was ob-
served in response to all dose levels of isoproterenol in the
dogs with NE pumps (Fig. 2). For example, isoproterenol (0.4 μg/kg
per min) increased left ventricular dP/dt significantly less (P
< 0.01) in the animals with NE pumps (by 2,093±263
mmHg/s) than observed in the control dogs (5,625±731
mmHg/s).

β-Adrenergic receptors. In the 16 dogs with NE pumps β-
adrenergic receptor density in the left ventricle (94±9 fmol/mg)
increased significantly (P < 0.05) as compared with the
dogs with saline pumps (63±7 fmol/mg; Fig. 3). The Kd for
[125I]-cyp was not significantly different between the two groups
(0.14±0.02 nM in the control group and 0.17±0.02 nM in the
NE pump group).

In the seven animals studied with saline pumps the agonist
binding data were best fit to a two-site model with a prepon-
derence of receptors binding agonist with high affinity (Fig. 4).
The Kd for the high affinity site was 53±11 nM and the Kd for
the low affinity site was 543±109 nM. In four animals 0.1 mM
Gpp(NH)p was added to the assay and in all cases induced a
shift to a single low affinity site. In 9 of the 10 animals with NE
pumps in which competition curves were performed, the ago-
nist binding data in the absence of Gpp(NH)p were best fit to a
single low affinity site model with a Kd of 353±120 nM.
Gpp(NH)p did not induce any further shift in agonist affinity
in the sarcolemma from these animals. In 1 of the 10 animals
with NE pumps the agonist binding data in the absence of
Gpp(NH)p were best fit to a two-site fit. On average (n = 10)
the percent of β-adrenergic receptors binding agonist with high
affinity was reduced in animals with NE pumps (3±3%) com-
pared with animals with saline pumps (48±12%; Fig. 4). An
example of the shift in agonist binding using crude sarco-
lemma is shown in Fig. 5 and an example using purified sar-
crolemna in Fig. 6.

Figure 2. The effects of acute challenges to iso-
proterenol (0.1–0.4 μg/kg per min) on left
ventricular dP/dt were examined before (open
bars) and after (solid bars) implantation of
the miniosmotic pumps. Animals with
NE pumps are shown on the left, and animals with saline pumps are shown on the right.
There is a significant decrease in responsiveness (desensitization) to
isoproterenol in animals after implantation of the NE pumps. *P
< 0.05.

Figure 3. β-Adrenergic receptor density and Kd as determined by
Scatchard analysis using [125I]-cyp are shown for animals with saline
pumps (open bars) and animals with NE pumps (solid bars). β-Re-
ceptor density is significantly increased (P < 0.05) in the animals
with NE pumps as compared with animals with saline pumps. There
is no difference in Kd between the two groups. *P < 0.05.

Table II. Changes from Baseline in Response
to Isoproterenol (0.4 μg/kg per min)

<table>
<thead>
<tr>
<th></th>
<th>Saline pumps</th>
<th>NE pumps</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mmHg)</td>
<td>36±8</td>
<td>42±8</td>
</tr>
<tr>
<td>Left ventricular dP/dt (mmHg/s)</td>
<td>4,610±563</td>
<td>5,625±731</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>−4±4</td>
<td>−5±4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>93±12</td>
<td>103±8</td>
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* P < 0.05 difference between groups.

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GTP plus isoproterenol (40%), Gpp(NH)p (27%), sodium fluoride (20%), and forskolin (31%). After subtracting basal activity, stimulated adenylate cyclase activity was still reduced in the NE group in response to GTP plus isoproterenol (−43%, P < 0.001), Gpp(NH)p (−28%, P < 0.005), sodium fluoride (−18%, P < 0.14), and forskolin (−31%, P < 0.002).

*Sodium, potassium-ATPase.* As an index of the consistency of membrane preparation, the sarcolemmal marker, sodium, potassium-ATPase, was measured in the crude preparation and was not found to be significantly different in the two groups, i.e., 3.1 ± 0.3 μmol P_i/h per mg in the saline pump control group vs. 2.9 ± 0.3 μmol P_i/h per mg in the NE pump group. In the purified sarcolemma sodium, potassium-ATPase was 31.3 ± 3.5 μmol P_i/h per mg in the saline pump control group vs. 33.4 ± 1.1 in the NE pump group.

G


G


G


G protein levels, as determined by ADP ribosylation with cholera toxin, were significantly lower (P < 0.05) in the sarcolemma from dogs with NE pumps (5.4 ± 0.9 pmol/mg) than the corresponding values for dogs with saline pumps (10.3 ± 1.7 pmol/mg).

**Discussion**

The topic of desensitization has been studied extensively in isolated organ and cellular systems. These prior studies have demonstrated two primary mechanisms of desensitization, which have been referred to as homologous and heterologous (3, 14–16). Homologous desensitization is characterized by an attenuated responsiveness of adenylate cyclase only to the specific desensitizing hormone without affecting the enzyme's responsiveness to other hormones. In contrast, heterologous desensitization is characterized by diminished responsiveness of adenylate cyclase to a wide spectrum of activators, including other hormones as well as fluoride and guanine nucleotides.

Still unanswered are the questions of whether desensitization

**Table III. Adenylate Cyclase Activity**

<table>
<thead>
<tr>
<th>(Picomoles/Milligram per Minute)</th>
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<tr>
<td>Saline pump</td>
</tr>
<tr>
<td>Basal</td>
</tr>
<tr>
<td>GTP</td>
</tr>
<tr>
<td>GTP + isoproterenol</td>
</tr>
<tr>
<td>Gpp(NH)p</td>
</tr>
<tr>
<td>NaF</td>
</tr>
<tr>
<td>Forskolin</td>
</tr>
<tr>
<td>GTP – basal</td>
</tr>
<tr>
<td>GTP + isoproterenol – basal</td>
</tr>
<tr>
<td>Gpp(NH)p – basal</td>
</tr>
<tr>
<td>NaF – basal</td>
</tr>
<tr>
<td>Forskolin – basal</td>
</tr>
</tbody>
</table>

*Figure 4. β-Adrenergic receptor competitive inhibition agonist binding with isoproterenol and 125I-cyp was performed for animals with saline pumps (open bars) and animals with NE pumps (solid bars). There is a significant shift toward the low affinity state for the animals with NE infusions. *P < 0.05.*

*Figure 5. Isoproterenol agonist binding with the crude membrane preparation, and 0.1 nM 125I-cyp with increasing concentrations of isoproterenol is shown for the left ventricle from an animal with a saline pump (circles) and an animal with a NE pump (triangles). The NE pump curve is shifted to the right, indicating a shift toward a single, low affinity site.*

*Figure 6. Isoproterenol agonist binding with the purified membrane preparation and 0.1 nM 125I-cyp with increasing concentrations of isoproterenol is shown for the left ventricle from an animal with a saline pump (circles) and an animal with a NE pump (triangles). The NE pump curve is shifted to the right, indicating a shift toward a single, low affinity site.*

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to NE is observed in chronic models, and if so, which type of desensitization is observed. This topic is clinically relevant in that several disease states, including heart failure, are characterized by chronically elevated levels of catecholamines.

Most prior studies, however, have been conducted in vitro and the majority have used isoproterenol (3, 14–16) rather than the physiological neurotransmitter NE to induce desensitization. The unique features of the present investigation were that the neurotransmitter NE was used and that the study was performed in an intact, conscious, large animal model using chronic infusions of NE delivered from implanted miniosmotic pumps. The major findings were: (a) desensitization occurred as reflected by depressed inotropic responses to an isoproterenol challenge, and (b) the mechanism of desensitization did not involve a decrease in β-adrenergic receptor density, but did involve uncoupling of the β-adrenergic receptor as reflected by decreases in the number of receptor sites binding agonist with high affinity. Accompanying this decrease in coupled or high affinity receptors was a decrease in Gs activity as assessed by reconstitution as well as cholera toxin labeling. In addition, these changes were associated with decreases in basal and stimulated adenylate cyclase activation. Furthermore, adenylate cyclase activity was depressed in animals with NE pumps, whether stimulated through the β-adrenergic receptor or distally through the Gs protein, a pattern consistent with a complex mechanism for desensitization. In contrast, the majority of prior studies on myocardial tissue have shown a specific (i.e., homologous) desensitization with an associated decrease in β-adrenergic receptor density (3, 14–16) and a loss of isoproterenol-stimulated adenylate cyclase activity, although a generalized decrease in adenylate cyclase activity has also been observed (17).

The defect or defects that explain the findings observed in this chronic model of catecholamine desensitization could reside in a single component or a combination of the three components of the receptor-Gs-adenylate cyclase pathway. In terms of alterations at the level of the β-adrenergic receptor, a surprising finding in the present investigation was that with chronically elevated NE levels β-adrenergic receptor density actually rose. The finding of increased β-adrenergic receptor density in the animals with chronic NE infusion may be secondary to the fact that as a physiological neurotransmitter the reuptake mechanism at the sympathetic nerve terminal may be acting to maintain low levels of NE at the postjunctional β-adrenergic receptor. Arterial baroreflex buffering of the peripheral effects of NE could also help reduce the actual concentration of NE at the postjunctional receptor site. Thus, it is possible that chronic occupancy by agonist at the myocyte β-receptor is actually reduced in this model and that the chronic desensitization to NE occurs via a different pathway than the homologous form elucidated by the work of Lefkowitz and colleagues and others in recent years (3, 18, 19). Since isoproterenol has been used relatively acutely as the agonist in most prior studies of desensitization (3, 15, 16, 18, 19), it is conceivable that homologous desensitization may be more compatible with acute exposure to isoproterenol.

The decrease in high affinity agonist binding and adenylate cyclase activation might be explained by the depressed functional levels of Gs. When Gs activity was quantitated by reconstitution techniques its activity was found to be significantly lower over the entire range of sarcolemma protein in the left ventricle of NE-treated animals when compared with the saline pump group. Prior studies suggest that despite the high levels of Gs in myocardium (i.e., a molar excess over β-receptor) its activity may be rate limiting in terms of either formation of the agonist high affinity or coupled state of the β-receptor, as well as in achieving maximal adenylate cyclase activation (20, 21). Recently we attempted to quantitate more exactly the absolute amount of Gs by developing a standard curve for Gs activity using a purified Gs preparation whose specific activity was similar to that reported by Sternweis et al. (22). Based on such a standard curve, the levels of Gs in purified canine sarcolemma are in the range of 25–30 pmol/mg membrane protein (unpublished data). That these levels were greater than those detected by cholera toxin labeling was expected in view of the less than quantitative nature of the latter method. This concentration of Gs represents a 25-fold excess over β-receptor and is similar to the results reported by Ransnas and Insel in the S49 lymphoma cell membrane (23). Despite this molar excess we observed a loss in formation of the high affinity β-receptor agonist binding state with a decrement in Gs functional activity in this model of chronic desensitization as well as in a chronic heart failure model (20, 21). Parallel findings are observed in the membranes of patients with pseudohypoparathyroidism where Gs levels are decreased to a similar extent (24). Whether the apparent excess of Gs is associated with other membrane receptor/effecter systems and thus functionally unavailable to couple with the β-receptor, or whether Gs exists in discrete membrane domains and in effect is partitioned in the sarcolemma remains unanswered. Our present studies do not permit a determination as to whether the decrease in Gs functional activity is secondary to a decrease in absolute subunit concentration and/or to a depressed functional activity of available Gs.

Our studies have not specifically addressed the question of whether the functional activity of the β-receptor and cyclase catalytic unit are normal. The work of Lefkowitz and co-workers (18) indicates that with agonist occupancy the β-receptor becomes susceptible to phosphorylation by a relatively specific kinase that they termed β-adrenergic receptor kinase. Thereafter, receptor uncoupling followed by internalization occurs. However, as discussed earlier, the increase in receptor number in this model might indicate that an increased level of

Figure 7. Gs reconstitution data are summarized on the left and Gs labeling with cholera toxin ADP-ribosylation is summarized on the right for the animals with saline pumps (open bars) and animals with NE pumps (solid bars). These data demonstrate a significant reduction in the levels of Gs using both techniques. *P < 0.05.
occupancy by agonist is not occurring. Nevertheless, phosphorylation of the receptor leading to uncoupling might occur without a net loss of cell surface receptors. Evidence has been presented to suggest that the process of uncoupling and internalization are related but distinct (25–28).

As to the third member of the β-adrenergic receptor signaling pathway, the catalytic unit of adenylyl cyclase, our data indicate that all activators tested show a decreased stimulatory capacity. However, depression of cyclase activity in response to isoproterenol was most marked and in response to fluoride was least marked; i.e., after subtracting basal activity fluorided-stimulated cyclase was still depressed, but not significantly. Although forskolin has been shown to activate the catalytic unit directly (29), other evidence indicates that its stimulation is enhanced in the presence of G_{i} (30–35). Thus, the decreased levels of G_{i} in the sarcolemma in the NE pump group could explain the reduced adenylyl cyclase stimulation by forskolin. We attempted to assess the activity of the catalytic unit directly in two ways, but neither was definitive (unpublished data).

Both manganese and manganese plus forskolin were used in an attempt to assay the catalytic unit when uncoupled from the G protein. However, even in the presence of 20 mM manganese we continued to observe Gpp(NH)P-mediated stimulation of cyclase activity, indicating that complete uncoupling from G_{i} had not occurred.

Since NE, which stimulates both α- and β-adrenergic receptors, was used as the agonist for desensitization, it is conceivable that α_{1}-adrenergic receptor activation that leads to stimulation of protein kinase C may play a role in the desensitization observed in these studies. Yoshimasa and co-workers (36) have shown that protein kinase C can phosphorylate the catalytic unit of adenylyl cyclase in the frog erythrocyte. However, this led to an apparent enhancement of cyclase activity that would not explain the findings obtained in our study. Others have found, however, that phorbol ester–mediated stimulation of protein kinase C can lead to desensitization of β-receptor-mediated stimulation of adenylyl cyclase (37, 38). Thus, the question as to whether NE may ultimately exert an effect on the β-adrenergic receptor–adenylyl cyclase pathway by activation of protein kinase C via α_{1}-receptor stimulation remains a possibility. However, it must be kept in mind that the density of α_{1}-adrenergic receptors in the canine heart is relatively low (39). In view of NE’s role in stimulating baroreceptor reflex afferent and parasympathetic efferent pathways (40) it is also possible that muscarinic pathways may be involved in the mechanism of desensitization in response to chronic NE.

It is generally thought that catecholamine desensitization is an important mechanism involved in heart failure, a disease state characterized by chronic elevation in circulating catecholamine levels. In this connection there are several parallel features in a model of canine chronic left ventricular hypertrophy and heart failure (20, 21) and the present study. The model of heart failure is also characterized by a global depression in adenylyl cyclase (20) and a reduction in G_{i} (21). Moreover, in this canine model of heart failure, in contrast to studies in human heart tissue (41), β-adrenergic receptor density determined by antagonist binding is not reduced, although there is a marked reduction in β-adrenergic receptors that bind agonist with high affinity (20). The parallel findings in these two models, one of desensitization and the other of heart failure, provide support for the concept that the physiologic alterations might be attributed to the same underlying mechanism. However, the failing heart is associated with depletion of NE stores (20, 42), which is also observed in cardiac denervation (43). Under these conditions the reuptake mechanism and arterial baroreflexes might not be able to reduce the quantity of neurotransmitter at the postjunctional receptor site. In fact, in heart failure arterial pressure is generally reduced, which unloads the arterial baroreflexes and acts to increase sympathetic drive. In contrast, in the present investigation the dogs with NE pumps exhibited normal levels of myocardial catecholamines, and since arterial pressure was not elevated it is likely that arterial baroreflex buffering played a major role in the normalization of arterial pressure.

Another important parallel between the models of desensitization, hypertrophy, and heart failure is that chronic α-adrenergic stimulation may induce left ventricular hypertrophy (44), and in fact has been observed by others in animals with chronic NE pumps (45). However, left ventricular weight/body weight ratios were not increased significantly in the present investigation, perhaps due to the period of time NE stimulation (i.e., 3–4 wk). Accordingly, the present study does not support the possibility that the alterations in the β-adrenergic/adenylyl cyclase pathway are due to the genetic and metabolic perturbations associated with the hypertrophic process per se. By converse logic the similar alterations in β-adrenergic receptor–adenylyl cyclase activity and in the levels of G_{i} that were observed in our studies of animals with hypertrophy and heart failure (20, 21) may not be due to the genetic and molecular alterations intrinsic to the hypertrophic process, but to other complications of the heart failure state, e.g., elevated circulating catecholamines.

In conclusion, this study identifies processes occurring distal to the β-receptor that are important in regulating the activity of the β-adrenergic receptor–adenylyl cyclase pathway after chronic NE exposure. This process of desensitization involves a complex mechanism associated with a reduction in all aspects of stimulated adenylyl cyclase activity, and despite most intense decreases in isoproterenol-stimulated adenylyl cyclase activity there was no downregulation of β-adrenergic receptor density. Rather, uncoupling of the β-adrenergic receptor associated with decreased G_{i} activity was observed. Potential alterations in the catalytic unit of adenylyl cyclase and in the β-adrenergic receptor cannot be excluded. The failure to observe β-adrenergic receptor downregulation despite chronically elevated circulating NE levels may be unique to the intact, conscious animal model studied, in which arterial baroreflexes and neuronal uptake mechanisms act to reduce the concentration of NE at the postjunctional receptor site. This may result in noncoordinate regulation of G_{i} and β-adrenergic receptors, which has been reported in other physiological models (20, 21, 46). It is conceivable that in the absence of cardiac nerves or when an agent such as isoproterenol, which is not taken up by cardiac nerves, is used to elicit desensitization acutely, other mechanisms such as receptor downregulation may also play a role.

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