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Myocardial β-adrenoceptor down-regulation by norepinephrine is linked to reduced norepinephrine uptake activity

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Abstract

Chronic administration of norepinephrine for 8 weeks has been shown to reduce neuronal norepinephrine uptake activity and increase interstitial norepinephrine concentration in the heart. To determine whether the changes could lead to myocardial β-adrenoceptor down-regulation or β-adrenergic subsensitivity, we measured left ventricular contractile responses to dobutamine, myocardial β-adrenoceptor density, β subtype distribution, competitive inhibition agonist binding, and adenylyl cyclase activity activation by isoproterenol, 5′-guanylylimidodiphosphate, and forskolin in dogs after a norepinephrine or saline infusion for 8 weeks. We found that norepinephrine infusion reduced myocardial β-adrenoceptor density, β_1-adrenoceptor subtype density, and high-affinity site for isoproterenol. Left ventricular contractile responses to dobutamine were reduced in the norepinephrine-infused animals. In addition, norepinephrine infusion decreased the basal adenylyl cyclase activity and the adenylyl cyclase responses to isoproterenol, 5′-guanylylimidodiphosphate, and forskolin. The findings indicate that a decrease in cardiac norepinephrine uptake predisposes the heart to norepinephrine-induced myocardial β-adrenoceptor down-regulation, and that norepinephrine, when present in a sufficient amount over a long period as it is in chronic heart failure, can reduce myocardial β-adrenergic responsiveness by both homologous and heterologous desensitization.

Keywords: Myocardium; β-Adrenoceptor; Norepinephrine; Adenylyl cyclase; Heart failure

1. Introduction

Congestive heart failure is characterized by myocardial β-adrenergic subsensitivity and β-adrenoceptor down-regulation (Bristow et al., 1986). This reduction of myocardial β-adrenoceptors was thought to be caused by chronically elevated circulating norepinephrine (NE) known to occur in patients with congestive heart failure (Thomas and Marks, 1978). Agonist-induced desensitization may occur because of either reduction of β-adrenoceptor down-regulation (homologous desensitization) (Lohse, 1992), reduction of adenylyl cyclase activation by a variety of agonists such as fluoride and guanine nucleotides (heterologous desensitization) (Hausdorff et al., 1990; Brodde, 1991), or both. Studies have shown that there is a statistically significant negative correlation between interstitial norepinephrine concentration and myocardial β-adrenoceptor density (Delehanty et al., 1994). However, intravenous administrations of norepinephrine over 3–4 weeks induced only heterologous desensitization; myocardial β-adrenoceptor density actually increased (Vatner et al., 1989). These findings suggest that plasma norepinephrine probably does not accurately reflect the interstitial norepinephrine at the post-synaptic β-adrenoceptor site.

Synaptic norepinephrine is influenced not only by the circulating norepinephrine, but also by the amount of neuronally released norepinephrine, local metabolizing enzymes, and adequacy of norepinephrine uptake activity at the presynaptic nerve endings. Neuronal reuptake of norepinephrine is considered the most effective mechanism of removing the effects of norepinephrine on β-adrenoceptors (Goldstein et al., 1988). Failure of norepinephrine infusion to reduce myocardial β-adrenoceptor density in the prior study of 3–4 weeks of infusion (Vatner et al., 1989), probably is caused, at least in part, by the effective nor-

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epinephrine uptake mechanism in normal hearts that kept the interstitial norepinephrine at relatively low concentrations surrounding the β-adrenoceptors.

However, in animals with congestive heart failure or after 8 weeks of norepinephrine infusion, there is a defect in the myocardial norepinephrine uptake mechanism (Himura et al., 1993). This defect is expected to impair the ability of the heart to remove norepinephrine and cause an increase of norepinephrine in the synaptic cleft in the presence of norepinephrine infusion (Lai et al., 1996a). To determine whether norepinephrine causes myocardial β-adrenoceptor down-regulation in dogs with reduced cardiac norepinephrine uptake, we carried out the present study in dogs with 8 weeks of norepinephrine infusion. We measured cardiac norepinephrine uptake activity, myocardial β-adrenoceptor density, and distribution of β_1- and β_2-adrenoceptor subtypes in dogs after 8 weeks of administration of either norepinephrine or saline vehicle. We also measured left ventricular contractile responsiveness to dobutamine, competitive inhibition agonist binding, and responses of adenylate cyclase activity to isoproterenol, forskolin, and 5′-guanylylimidodiphosphate (Gpp(NH)p). Our results indicate that unlike the short-term infusion of norepinephrine, norepinephrine infusion given over 8 weeks caused myocardial β-adrenoceptor down-regulation. Both homologous and heterologous mechanisms of desensitization are present in the animals treated with chronic norepinephrine infusion.

2. Materials and methods

The present studies were approved by the University of Rochester Committee on Animal Resources and conformed to the guiding principles approved by the Council of the American Physiological Society and the *National Institutes of Health Guide* on the humane care and use of laboratory animals. Aseptic thoracotomies were performed in animals after intravenous sodium pentobarbital (25 mg/kg) anesthesia and under mechanical ventilation.

2.1. Animal preparation

Adult mongrel dogs, weighing 19.6 to 27.5 kg, were anesthetized and instrumented with chronic indwelling catheters in the left atrium, main pulmonary artery, and descending aorta, and a Konigsberg transducer (Konigsberg Instrument, Houston, TX, USA) into the left ventricle. One week later, an Alzet model 2ML4 osmotic minipump (Alza, Palo Alto, CA, USA) was implanted subcutaneously at the nape of the neck under local anesthesia with xylocaine. Dogs were then assigned to receive either normal saline or norepinephrine at a rate of 0.5 μg/kg/min. Because each minipump delivered this amount only for 4 weeks, a second minipump was implanted 4 weeks later to ensure constant norepinephrine infusion for 8 weeks. Dogs were studied at the end of 8 weeks of infusion.

2.2. Hemodynamic measurements

Dogs were trained to lie quietly in a lateral decubitus position for the hemodynamic studies. The previously implanted catheters were attached to Spectramed P23XL (Spectramed, Oxnard, CA, USA) transducers and an 8-channel Brush model 480 recorder (Gould, Instruments System Division, Cleveland, OH, USA) for measuring heart rate, and left atrial and aortic pressures. The Konigsberg transducer was connected to the Brush recorder for measuring left ventricular pressure. The rate of rise of left ventricular pressure (dP/dt) was derived using an electronic differentiater. The ratio of left ventricular dP/dt at a developed pressure of 50 mm Hg during isovolumic systole and developed pressure ($dP/dt/P$), which has been shown to be relatively independent of ventricular afterload (Davidson et al., 1974), was calculated as an index of left ventricular contractility. Cardiac output was measured by the indocyanine green (Cardio-Green; Hynson, Westcott, & Dunning, Baltimore, MD, USA) dye dilution technique, using a Gilford model 140 cardiac output system (Gilford Instrument Laboratories, Oberlin, OH, USA).

Resting systemic hemodynamic measurements were obtained in triplicate. Aortic blood was taken for norepinephrine determinations. Animals were then administered intravenous dobutamine at 3 increasing doses (4, 8, 16 μg/kg/min), each for 15 min. Triplicate measurements were obtained at 10–15 min of each dose of infusion. The triplicate measurements obtained at each stage were averaged and used for statistical analyses.

After the hemodynamic studies, the animal was given a lethal dose (> 100 mg/kg) of sodium pentobarbital. The hearts were removed and weighed. The ventricles were separated from the septum and rinsed in an ice-cold oxygenated normal saline. The left ventricular weight included both the septum and left ventricular free wall; the right ventricular weight included only the free wall. Ventricular muscle blocks were removed from ventricular free walls and stored for measuring β-adrenoceptor density and adenylyl cyclase activity assay. Fresh muscle samples were also taken for measuring myocardial norepinephrine uptake activity.

2.3. Myocardial norepinephrine uptake activity

Fresh tissue slices (20 to 30 mg) were incubated in quadruplicate in 50 nmol/l l-[7-^{3}H(N)]norepinephrine (13.8 Ci/mmol; New England Nuclear, Boston, MA, USA) at 37°C and 4°C for 15 min. The difference in radioactivity between tissue slices incubated in a [^{3}H]norepinephrine-containing solution at 37°C and those at 4°C is considered

to approximate tissue norepinephrine uptake activity (Liang et al., 1989).

2.4. Cardiac membrane preparation

Frozen myocardium (0.5 g) was trimmed, minced and homogenized in an ice-cold 50 mM Tris buffer containing 120 mM NaCl and 5 mM KCl. The homogenate was centrifuged at 40,000 g for 15 min at 4°C. The pellets were filtered through a nylon membrane and resuspended in an appropriate buffer for the following studies. The protein content was determined using BCA protein assay reagent (Pierce, Rockford, IL, USA) with bovine serum albumin as a standard.

2.5. Myocardial β-adrenoceptor assay

Myocardial β-adrenoceptor density was measured by specific binding of the highly specific [^{125}I]iodocyanopindolol (2,200 Ci/mmol; New England Nuclear) (Lai et al., 1996b). Approximately 20 μg membrane protein was suspended in a 0.2 ml of Tris–HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. Eight concentrations of [^{125}I]iodocyanopindolol (10 to 150 pM) were utilized. Nonspecific binding was determined by parallel incubation of samples containing 0.1 mM (−)-isoproterenol. Incubations were performed in triplicate at 37°C for 60 min in a final volume of 0.25 ml. The reaction was terminated by addition of ice-cold Tris buffer. The membranes were rapidly washed and filtered through Whatman GF/B filters (Whatman Chemical Separation, Clifton, NJ, USA) on a Brandel cell harvester (Biomedical Research and Development Laboratories, Gaithersburg, MD, USA). The filters were processed and counted for radioactivity utilizing a Packard liquid scintillation spectrometer (Packard Instrument, Downer's Grove, IL, USA). The difference between binding in the absence and presence of (−)-isoproterenol was taken as specific binding. The maximum number of receptor binding sites and the dissociation constant were calculated, using the EBDA computer software program (Elsevier Science Publisher, Cambridge, UK) developed by McPherson (1985).

2.6. Myocardial β-adrenoceptor subtypes

Myocardial β_1- and β_2-adrenoceptor subtypes were identified by analysis of displacement curves utilizing the highly selective β_1-adrenoceptor antagonist CGP 20712A (Ciba-Geigy Pharmaceuticals, Basel, Switzerland) (Dooley et al., 1986). Membrane tissue (20 μg) was incubated in a 50 mM Tris buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 50 pM [^{125}I]iodocyanopindolol, and 18 different concentrations of CGP 20712A (ranging from 0.1 pM to 0.1 mM). The incubation procedure was identical to that described for total β-adrenoceptor density. The displacement curves were analyzed for β_1 and β_2 receptor density, and their respective dissociation constants (K_{D1} and K_{D2}) using the GraphPad Prism two-site competition program (GraphPad Software, San Diego, CA, USA).

2.7. Myocardial β-adrenoceptor agonist binding

Myocardial β-adrenoceptor agonist binding curve was obtained using (−)-isoproterenol as previously described (Kent et al., 1980). Myocardial membrane protein (20 μg) was suspended in 250 μl of a 75 mM Tris–HCl buffer (pH 7.4), containing 2.5 mM $MgCl_2$, 2 mM EDTA, 50 pM [^{125}I]iodocyanopindolol and 14 increasing concentrations of (−)-isoproterenol (0.1 nM to 0.1 mM). Incubation were performed in triplicate at 37°C for 60 min. Nonspecific binding was determined by the difference between the presence and absence of 0.1 mM propranolol. The dissociation constants (K_H, K_L) and fractions of β-adrenoceptor high and low affinity sites for (−)-isoproterenol were calculated with the GraphPad Prism program (GraphPad Software).

2.8. Adenylyl cyclase activity

Adenylyl cyclase activity was assayed in duplicate as described previously (Fan et al., 1987) with a mixture containing 50 mM Tris–HCl (pH 7.4), 0.4 mM EGTA, 0.5 mM 2-isobutyl-1-methylxanthine, 2 mM $MgCl_2$, 5 mM phosphocreatine, 15 units creatine phosphokinase, and approximately 50 μg membrane protein in a final volume of 0.45 ml at 37°C for 5 min in the presence and absence of isoproterenol (0.1 mM with 0.1 mM GTP), Gpp(NH)p (0.1 mM) or foskolin (0.1 mM). Isoproterenol and Gpp(NH)p were dissolved in distilled deionized water; while forskolin required 50% dimethylsulfoxide to dissolve completely. The agonist concentrations were chosen to produce maximal adenylyl cyclase stimulation as demonstrated in pilot

Table 1
Resting hemodynamics
Values are mean ± S.E.M.; LV = left ventricular.

	Saline infusion	Norepinephrine infusion
No. of animals	10	9
Plasma norepinephrine (ng/ml)	0.30 ± 0.04	4.59 ± 0.49[a]
Body weight (kg)	22.6 ± 0.8	24.4 ± 0.8
Heart rate (beats/min)	105 ± 5	83 ± 4[b]
Mean aortic pressure (mm Hg)	115 ± 3	121 ± 4
Cardiac output (l/min)	4.7 ± 0.3	4.0 ± 0.5
LV dP/dt (mm Hg/s)	3358 ± 130	3805 ± 238
LV $dP/dt/P$ (s^{-1})	44 ± 1	45 ± 1
Left atrial pressure (mm Hg)	8.5 ± 0.5	8.3 ± 0.9
LV weight (g)	108 ± 5	118 ± 4

[a] $P < 0.001$ compared with saline-infused dogs.
[b] $P < 0.01$ compared with saline-infused dogs.

studies. The samples were assayed for cyclic AMP levels by the competitive protein-binding technique (Tovey et al., 1974) using a cyclic AMP assay system (Amersham Life Science, Little Chalfont, Buckinghamshire, England).

2.9. Statistical analyses

Results are given in mean ± S.E.M. The data were analyzed with RS/1 Research System (Bolt, Beranek and Newman Software Products, Cambridge, MA, USA) The statistical significance of differences between the saline and norepinephrine infusion was determined by Student's *t*-test for unpaired data. The statistical association between myocardial norepinephrine uptake activity and β-adrenoceptor density was determined by correlation analysis. Two-way analysis of variance was used to determine the statistical significance of differences between two different dose–response curves. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Resting hemodynamics and cardiac contractile response to dobutamine

Table 1 shows the resting hemodynamics in dogs 8 weeks after saline and norepinephrine infusion. Plasma norepinephrine was markedly elevated in the norepinephrine infusion group, but the two groups did not differ in body weight, mean aortic pressure, cardiac output, left atrial pressure, left ventricular dP/dt or $dP/dt/P$, or left ventricular weight. There was, however, a significant decrease in heart rate in the norepinephrine-infused dogs. In addition, norepinephrine infusion attenuated the responses of left ventricular dP/dt and $dP/dt/P$ to dobutamine (Fig. 1). Heart rate did not increase with the two low doses of dobutamine. It increased only slightly with the highest dose.

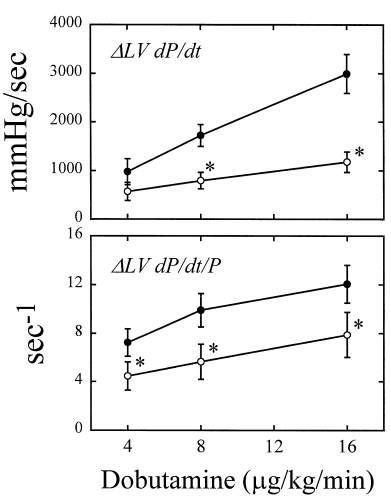

Fig. 1. Increases in left ventricular dP/dt (upper panel) and $dP/dt/P$ (bottom panel) produced by serially increasing doses of dobutamine infusion in the saline-infused and norepinephrine-infused animals. Two-way analysis of variance indicates that dobutamine infusion produced dose-dependent increases of dP/dt in both groups of animals ($F = 24.02$, $P < 0.0001$), and that the difference in the dP/dt responses between the two groups was statistically significant ($F = 40.66$, $P < 0.0001$). Similarly, there was a dose-dependent increase of left ventricular $dP/dt/P$ by dobutamine ($F = 9.37$, $P < 0.0001$) and a reduced response of left ventricular $dP/dt/P$ in the norepinephrine-infused animals compared to the saline-infused animals ($F = 29.48$, $P < 0.0001$). $^*P < 0.05$, compared to the corresponding value in the saline group.

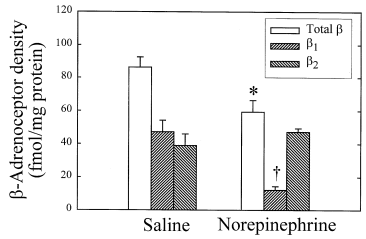

Fig. 2. Differences in myocardial total β-adrenoceptor density, β_1 adrenoceptor subtype density, and β_2 adrenoceptor subtype density between the saline-infused and norepinephrine-infused animals. $^*P < 0.05$, and $\dagger P < 0.01$, compared to the saline group.

3.2. Myocardial β-adrenoceptor and subtype density

Fig. 2 shows the differences in myocardial β-adrenoceptors between the saline- and norepinephrine-infusion groups. Total β-adrenoceptor density was reduced in norepinephrine-infused dogs compared with that in saline-in-

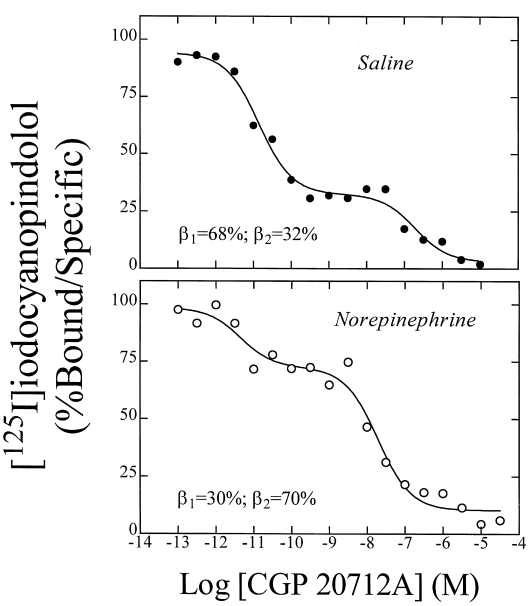

Fig. 3. Representative β-adrenoceptor competition assays utilizing [^{125}I]iodocyanopindolol and the β_1-adrenoceptor-specific antagonist CGP 20712A in the myocardial membrane preparation of a saline-infused animal (upper panel) and a norepinephrine-infused animal (bottom panel).

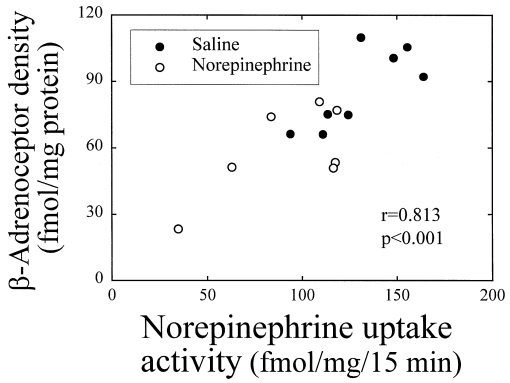

Fig. 4. Correlation between myocardial β-adrenoceptor density and norepinephrine uptake activity in both saline-infused and norepinephrine-infused animals. r = Correlation coefficient.

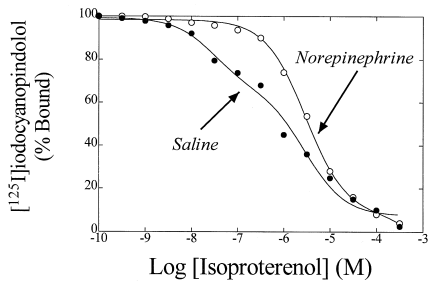

Fig. 5. Representative competitive inhibition agonist binding assays utilizing [^{125}I]iodocyanopindolol and isoproterenol in the myocardial membrane preparation of a saline-infused animal and a norepinephrine-infused animal.

fused dogs. There was, however, no difference in dissociation constant between the two groups (37 ± 4 vs. 31 ± 2 pM). The figure also shows that the decrease in total β-adrenoceptors in the norepinephrine infusion group was caused exclusively by a change in β_1-adrenoceptor subtype. There was no difference in β_2 receptor subtype density between the saline- and norepinephrine-infusion groups. The relative proportions of β_1- and β_2-subtypes were determined by [^{125}I]iodocyanopindolol displacement curves in the presence of increasing doses of CGP 20712A (Fig. 3). The competition curve was fit into a two-site binding model (GraphPad Prism). The ratio of the K_{D1} and K_{D2} for the two binding sites was in the order of 10^3–10^4, indicating that CGP20712A is a highly specific antagonist for the β_1-adrenoceptor. Norepinephrine infusion had no significant effect on either K_{D1} (11 ± 4.1 nM) or K_{D2} (29 ± 8 μM), compared to the corresponding values in the saline control group (4.4 ± 2.5 nM and 12 ± 6 μM).

3.3. Myocardial norepinephrine uptake activity and correlation with myocardial β-adrenoceptor density

Myocardial norepinephrine uptake activity was reduced in norepinephrine-infused animals (80 ± 16 fmol/mg/15 min), compared to saline-infused animals (132 ± 7 fmol/mg/15 min). Fig. 4 shows a statistically significant correlation between myocardial norepinephrine uptake activity and myocardial β-adrenoceptor density.

3.4. Myocardial β-adrenoceptor agonist binding

Fig. 5 shows that myocardial [^{125}I]iodocyanopindolol binding curve was biphasic in the presence of isoproterenol in a saline-infused animal. The competitive-inhibition agonist binding curve was fit into a two-site binding model. The high- and low-affinity sites exhibited inhibition constants of 24.8 ± 2.2 and 481 ± 46 nM, respectively. The high-affinity sites made up $36 \pm 3\%$ of the total binding sites in saline-infused animals. However, in the norepinephrine-infused animals, the isoproterenol competitive-inhibition curve shifted to the right, indicating loss of high-affinity sites. Only the low affinity sites with inhibitor constants of 464 ± 90 nM could be demonstrated in the norepinephrine-infused dogs.

3.5. Myocardial adenylyl cyclase activity

Myocardial tissue basal adenylyl cyclase activity was reduced in the norepinephrine-infused animals (40 ± 2 pmol/mg protein/min) compared to the saline-infused animals (94 ± 8 pmol/mg protein/min, $P < 0.001$). Norepinephrine infusion also reduced the activation of myocardial adenylyl cyclase in response to isoproterenol, Gpp(NH)p and forskolin (Fig. 6).

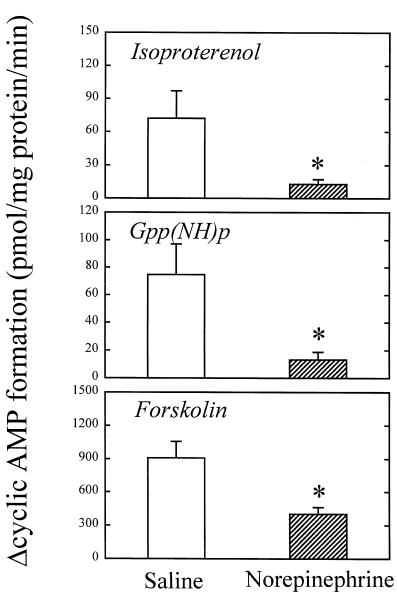

Fig. 6. Net myocardial production of cyclic AMP by isoproterenol, Gpp(NH)p, and forskolin in the saline-infused and norepinephrine-infused animals. * $P < 0.05$, compared to the saline infusion group.

4. Discussion

The dose of norepinephrine chosen for the present study is identical to those given in several prior experiments (King et al.,1987; Patel et al., 1989, 1991; Vatner et al., 1989; Lai et al., 1996a). It is subhypertensive and causes no myocardial fibrosis (Patel et al., 1991) or death. In our study, norepinephrine infusion produced no obvious changes in cardiac output or cardiac contractile function. However, it reduced myocardial β-adrenoceptor density and left ventricular contractile responsiveness to β-agonist stimulation. The decrease in myocardial β-adrenoceptors was selective, involving only the β_1-adrenoceptor subtype. In addition, norepinephrine infusion caused reduced adenylyl cyclase activation by isoproterenol, Gpp(NH)p and forskolin, and uncoupling of the β-adrenoceptors to the G-protein-adenylyl cyclase system as measured by isoproterenol competitive inhibition. In this study, we did not measure the effect of norepinephrine infusion on adenylyl cyclase activation by manganese which directly stimulates the catalytic unit of the adenylyl cyclase system (Bender and Neer, 1983). We have shown previously that the adenylyl cyclase response to manganese chloride is well preserved in the myocardial membrane preparation of heart failure dogs (Fan et al., 1987), suggesting that the catalytic unit of adenylyl cyclase is unaffected in failing heart.

The uncoupling of the β-adrenoceptors and post-adrenoceptor changes of the adenylyl cyclase activity have been reported previously in dogs after 3–4 weeks of norepinephrine infusion (Vatner et al., 1989). However, unlike our present study, the prior study showed an increase in myocardial β-adrenoceptor density. These two studies utilized the same doses of norepinephrine and animal species. However, since we did not study the effect of shorter terms of infusion in the same experimental setting, we could not attribute the difference of effects of norepinephrine on myocardial β-adrenoceptor density between the two studies to the difference in duration of norepinephrine infusion alone. In a recent study by Zhao et al. (1996), norepinephrine was infused to rats for one week. The dose of norepinephrine (200 μg/kg/h) employed in that study was much larger than that we used in this study, and was sufficient to cause necrosis in 10% of heart tissue. Nevertheless, as in dogs of short-term infusion (Vatner et al., 1989), myocardial β_1-adrenoceptor density also increased in the rats infused with norepinephrine. In contrast, implantation of pheochromocytoma in rats for 5–12 weeks has been shown to induce β-adrenoceptor, predominantly the β_1-subtype, down-regulation in many organs, including the heart (Snavely et al., 1983; Tsujimoto et al., 1984). The reason for the differences among the studies is not known. However, it is possible that β-adrenoceptor down-regulation would occur in the heart only after a long term of exposure to exogenous norepinephrine when cardiac norepinephrine uptake activity is reduced as observed in our present study. The close correlation between myocardial norepinephrine uptake activity and myocardial β-adrenoceptor density (Fig. 4) suggests that alterations in cardiac norepinephrine uptake mechanism may account for as much as 66% (r^2) of the changes in myocardial β-adrenoceptor density.

Myocardial β-adrenoceptor down-regulation is a salient feature in end-stage human cardiomyopathy. The reduction of myocardial β-adrenoceptors probably is caused by excessive sympathetic stimulation, as it can be reversed by β-adrenoceptor blockers (Liang et al., 1991). Like after norepinephrine infusion, the reduction of β-adrenoceptor density in failing heart is selective to the β_1-adrenoceptor subtype (Bristow et al., 1986; Lai et al., 1996b). The correlation between myocardial β-adrenoceptor density and interstitial norepinephrine concentrations in a rapid pacing-induced heart failure is consistent with a role of elevated interstitial norepinephrine (Delehanty et al., 1994).

Recently, in a rabbit heart failure model using rapid ventricular pacing, we found that development of adrenergic nerve terminal abnormalities and reduction of norepinephrine uptake sites occurs before the onset of myocardial β-adrenoceptor down-regulation (unpublished data). The fact that reduction of cardiac norepinephrine uptake activity precedes the development of myocardial β-adrenoceptor down-regulation supports the cause-and-effect relationship of the parameters in heart failure.

Unlike humans and animals with heart failure, where the β_1-adrenoceptor is selectively down-regulated in the heart, animal studies have shown that the myocardial β_2-adrenoceptor is more likely to show rapid desensitivity and down-regulation than the β_1-subtype following administration of isoproterenol (Nanoff et al., 1989; Molenaar et al., 1990; Kompa et al., 1994; Brodde et al., 1995). Qualitatively similar differential changes also have been shown with norepinephrine infusion in rats (Kompa et al., 1992; Zhao et al, 1996). The underlying mechanism responsible for the differential regulation of β-adrenoceptor subtypes in response to various β-adrenergic agonists in normal and heart failure state remains to be elucidated. It has been suggested that the mechanism may be related to differences in the molecular structure between the different β-adrenoceptor subtypes and/or potential differences in the molecular regulation between the two β-adrenoceptor subtypes (Zhao and Muntz, 1993; Muntz et al., 1994).

β-Adrenoceptors are known to exist in two affinity states for the agonist isoproterenol (Kent et al., 1980). The high affinity form of the β-adrenoceptor is functionally coupled to the G-protein. Our present study showed that norepinephrine infusion abolished the high affinity sites, and reduced the coupling of the β-adrenoceptors to the regulatory G-protein. Furthermore, there was a defect in the post-receptor function of adenylyl cyclase system in the norepinephrine-infused dogs. The amount of cyclic AMP formation produced by isoproterenol, Gpp(NH)p and forskolin was reduced in the norepinephrine infusion group. These changes are probably caused by norepinephrine

which has been shown to increase myocardial Gi level in cultured neonatal rat muscle cells (Reithmann et al., 1989) and intact rats (Urasawa et al., 1992).

In summary, chronic administration of norepinephrine over an 8-week period causes significant adrenergic subsensitivity in dogs. The close correlation between myocardial β-adrenoceptor density and norepinephrine uptake activity suggests that the reduction of norepinephrine uptake activity plays an important role in the homologous regulation. The findings further suggest that chronic elevation of circulating norepinephrine in chronic advanced heart failure is responsible for the β-adrenergic subsensitivity in heart failure.

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