Regulation of Cardiac $\beta$-Adrenergic Receptors by Captopril
Implications for Congestive Heart Failure

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The interaction of the renin-angiotensin system and the sympathetic nervous system in patients with congestive heart failure is not well understood. We tested the hypothesis that angiotensin-converting enzyme inhibitors can resensitize the $\beta$-adrenergic receptor system. Guinea pigs were given captopril, isoproterenol, or both for 2 weeks. At death, cardiac sarcolemmal and light vesicle fractions and intact mononuclear leukocytes were prepared. Captopril treatment led to an up-regulation of cardiac $\beta_1$- but not mononuclear leukocyte $\beta_2$-adrenergic receptors and an increase in isoproterenol-stimulated adenylate cyclase activity in the heart. Animals treated with isoproterenol developed cardiac hypertrophy, had increased plasma norepinephrine levels, and had a decreased number and responsiveness of both cardiac and mononuclear leukocyte $\beta$-adrenergic receptors. Concomitant treatment with captopril attenuated alterations of heart weight, plasma norepinephrine levels, and cardiac $\beta$-receptor density and function. In contrast to its cardiac effects, captopril treatment did not diminish the down-regulation of mononuclear leukocyte $\beta_2$-adrenergic receptors by isoproterenol. Our data suggest that captopril may resensitize the cardiac but not the mononuclear leukocyte $\beta$-adrenergic receptor–adenylate cyclase system after long-term catecholamine exposure. (Circulation 1989;80:669–675)

Activation of the sympathetic nervous system is involved in the pathophysiology of congestive heart failure.1–3 While heightened adrenergic activity helps to maintain perfusion pressure for variable periods of time in the face of a decreased effective plasma volume, it may ultimately contribute to the deterioration of cardiac function.4 It has been proposed that a reduced myocardial $\beta$-adrenergic receptor density and responsiveness due to increased norepinephrine release might be involved in this deterioration.5,6

The renin-angiotensin system is also activated in heart failure and seems to play an important role in the pathophysiology of this disease complex.7,8 Accordingly, converting enzyme inhibitors like captopril have hemodynamic and clinical benefits in many of these patients.9 This may be due to a number of different mechanisms including reduction of vasopressin release from the central nervous system, restoration of a reduced baroreflex sensitivity, and vasodilation via unopposed prostaglandin action.8 Another important mechanism may be related to the facilitatory action of angiotensin II on norepinephrine release.10 Thus, increased angiotensin II levels might contribute to the down-regulation of cardiac $\beta$-adrenergic receptors by enhancing norepinephrine release. Therefore, the possibility exists that the sustained effects of captopril in the treatment of congestive heart failure may in part be due to a resensitization of the cardiac $\beta$-adrenergic receptor adenylate cyclase pathway.

To study the basic mechanisms of interaction between $\beta$-adrenergic agonists and converting enzyme inhibitors in the regulation of $\beta$-adrenergic receptors, we infused guinea pigs with isoproterenol, captopril, or a combination and measured the density and responsiveness of cardiac $\beta$-adrenergic receptors. $\beta$-Adrenergic receptors on circulating mononuclear leukocytes were assessed in comparison, because mononuclear leukocytes are not innervated and thus might help to dissect effects related to altered synaptic catecholamine release from those independent of release.
Methods

Drug Infusions

Under light anesthesia (ketamine 100 mg/kg i.m.), osmotic minipumps (Alzet, Palo Alto, California) were implanted subcutaneously in male Hartley guinea pigs (400–500 g, 4–6 weeks, Simonsen Co., Palo Alto, California). These pumps contained captopril (1.25 mg/kg/day), isoproterenol (3.5 mg/kg/day in 10 mM HCl and 1% ascorbic acid), or vehicle (10 mM HCl plus 1% ascorbic acid). Some animals received two minipumps (captopril and isoproterenol). Drug- and vehicle-treated animals were always studied simultaneously. Guinea pigs treated with vehicle for 1 and 2 weeks did not differ for any parameter studied; thus, their data were pooled for further analysis.

Assay of Cardiac β-Adrenergic Receptors

Animals were killed after 1 or 2 weeks by injection of ketamine (80 mg/kg i.m.) and xylazine (8 mg/kg i.m.). Blood was obtained via direct left ventricular puncture, mixed with EDTA (5 mM final), and placed on ice. The heart was removed and perfused with ice-cold 50 mM Tris-HCl (pH 7.4), and the left ventricle was trimmed, weighed, and frozen at −70°C. Two fractions were prepared from excised left ventricles: a partially purified sarcolemmal fraction in which Na/K-ATPase, β-adrenergic receptors, and adenylate cyclase activity are enriched and in which the receptors are responsive to agonist; the second fraction contained membranes of lighter density that have little adenylate cyclase, 5′-nucleotidase, or Na/K-ATPase activities; it is not enriched in markers for lysosomes or mitochondria; and has β-adrenergic receptors that are functionally uncoupled from the stimulatory GTP-binding protein, Gs. Details of the purification have been described previously.11,12 Briefly, after extraction of contractile proteins with 750 mM NaCl, the samples were vigorously homogenized. The light vesicle fraction was obtained by centrifuging a 45,000g supernatant at 137,000g for 90 minutes. Sarcolemmal membranes were obtained by serial washings of the 45,000g pellet followed by centrifuging at 137,000g. The resulting rim above the pellet was considered the sarcolemma fraction. Protein was determined by the method of Lowry using bovine serum albumin as the protein standard.13

Binding of [125I]iodocyanopindolol (ICYP) to β-adrenergic receptors was measured by incubating membranes in triplicate with varying concentrations of radioligand (10–500 pM) at 37°C for 1 hour. Binding was terminated by diluting samples to 10 ml with buffer, filtering over Whatman GF/B filters that had been presoaked in 2% polyethyleneimine (to trap small membrane fragments14), and washing the filters with 10 ml ice-cold buffer. Radioactivity retained on the filters was determined using a gamma counter at 80% efficiency. Nonspecific binding for both sarcolemmal and light vesicle fractions was determined as [125I]ICYP binding in the presence of 1 μM (−)-propranolol (Ayerst, New York) and routinely was 10–20% of total binding for both membrane fractions.

Assay of Mononuclear Leukocyte β-Adrenergic Receptors

Techniques for preparation of guinea pig mononuclear leukocytes and radioligand binding to β-adrenergic receptors have been previously described.15 Briefly, blood from two or three animals was placed into tubes containing EDTA (5 mM final), diluted with an equal volume of ice-cold phosphate-buffered saline, and centrifuged at 500g for 10 minutes. The supernatant containing the platelet-rich plasma was removed, an equal volume of phosphate-buffered saline was added, and the tube was centrifuged at 500g again. After discarding the supernatant, phosphate-buffered saline was added to bring the volume to twice that of the starting blood volume. Ten milliliters of Ficoll-Hypaque were subfused slowly, and samples were then centrifuged at 1,400 rpm for 15 minutes. Mononuclear leukocytes were collected from the interface, washed three times in phosphate-buffered saline, and then resuspended in Dulbecco’s modified Eagle’s medium with 20 mM HEPES (pH 7.4) and 1 mg/ml bovine serum albumin. More than 90% of cells were able to exclude trypan blue. Mononuclear leukocytes (2–3 × 10⁷) were incubated in a total volume of 0.5 ml Dulbecco’s modified Eagle’s medium containing varying concentrations of (−)[125I]iodopindolol (IPIN) (10–120 pM) and either buffer, 1 μM (−)-propranolol, or 1 μM CGP-12177 (kindly provided by Dr. M. Staehelin, CIBA-Geigy, Basel, Switzerland) at 4°C for 40 hours, at which time the cells were filtered over Whatman GF/C filters and washed with 20 ml phosphate-buffered saline at room temperature. The difference between total binding and binding in the presence of propranolol represents specific binding. Cell surface receptors were determined as specific binding sites that could be competed for by the hydrophilic antagonist CGP-12177.16,17 The number of sequestered or redistributed β-adrenergic receptors was defined as the number of specific binding sites minus the number of surface receptors.

Adenylyl Cyclase Assay

Adenylyl cyclase was assayed by the method of Salomon et al18 in a buffer containing 50 mM Tris-HCL, 10 mM MgCl₂, 0.3 mM cyclic AMP, 1 mM ATP, 100 μM GTP, 20 mM creatine phosphate, 50 units/ml creatinine phosphokinase, 800,000 counts/min [3H]ATP, and, in some tubes, 10 μM isoproterenol. Incubations lasted 20 minutes at 30°C.

Cyclic AMP Assay

Cells were added to the final reaction mixture containing 100 μM isobutylmethylxanthine, 100 μM Ro20-1724, 10 μg/ml superoxide dismutase and
catalase, and, in some tubes, 10 μM isoproterenol. The reactions were terminated after 2 minutes, and cells were assayed for cyclic AMP (cAMP) by the competitive binding protein method of Gilman.21

Plasma norepinephrine and isoproterenol levels were determined by the method of Durrett and Ziegler.20 Plasma samples were incubated with 3H-S-adenosylmethionine and catechol-O-methyltransferase. The products of the reaction, 3H-normetanephrine and 3H-O-methylisoproterenol, were solvent extracted and spotted on a silica gel chromatography plate. Plates were developed in a 3:6:2 mixture of methylvamine, tert-amyl alcohol, and benzene. In this system, the Rf of normetanephrine is 0.18, the Rf of O-methylisoproterenol is 0.39, and the cross-over of O-methylisoproterenol into the normetanephrine band is only 0.002. The appropriate sections of the thin-layer chromatographic plate were localized under ultraviolet light and scraped into counting vials. The samples were peridated to vanillin with NaIO₄, extracted into toluene, and quantitated by liquid scintillation spectroscopy. Interference of isoproterenol into the norepinephrine band was corrected mathematically, but this interference was small relative to the high plasma levels of norepinephrine.

Data Analysis

Radioligand binding data were analyzed by iterative nonlinear regression analysis to fit the specific binding to an equation describing binding of ligands to a single class of homogenous binding sites. All experiments were performed with triplicate data points. Unless stated otherwise, the data are presented as mean±SEM. To determine statistical significance of differences between the treatment groups, we calculated unpaired two-tailed t tests. One-way analysis of variance (ANOVA) was used to determine whether changes with various treatments were significantly different. We used the Bonferroni-corrected t tests to correct for multiple comparisons for treatment groups of interest.

Results

Interaction of Captopril With Cardiac β₁-Adrenergic Receptors

Two weeks of captopril treatment increased the number of cardiac β₁-adrenergic receptors in both the sarcolemmal (control, 134±12 fmol/mg; captopril treatment, 180±14 fmol/mg; p≤0.05) and the light vesicle fractions (control, 104±10 fmol/mg; captopril treatment, 130±12 fmol/mg; p<0.01; Figure 1), without affecting the affinity of the receptor for ICYP (98±21 pM in sarcolemma and 92±25 pM in the light vesicle fraction under control conditions and 93±18 and 85±25 pM in the sarcolemma and light vesicle fractions, respectively, after captopril treatment). Simultaneously, isoproterenol-stimulated adenylate cyclase activity was increased (Figure 2).

Animals treated with constant infusion of isoproterenol developed cardiac hypertrophy (left ventricle/body weight×100=2.43±0.012 in the isoproterenol group vs. 2.0±0.08 in controls; p<0.01) along with a marked down-regulation of β₁-adrenergic receptors (sarcolemma: control, 134±12 fmol/mg vs. isoproterenol, 50±10 fmol/mg, p<0.01; light vesicle: control, 104±10 fmol/mg vs. isoproterenol, 30±6 fmol/mg, p≤0.01; Figures 1 and 3).

With concomitant captopril treatment, increases in heart weight were lessened (left ventricle/body weight×100: isoproterenol, 2.43±0.01; isoproterenol plus captopril, 2.2±0.06; p<0.01), the isoproterenol-induced down-regulation was mitigated (sarcolemmal β₁-receptors: control, 134±12 fmol/mg; isoproterenol treatment, 50±10 fmol/mg; isoproterenol plus captopril treatment, 108±8 fmol/mg;
Results

There were similar findings in experiments performed with guinea pig lymphocytes, with 76±3% in the captopril-treated group (p=0.01, Figure 6). This sequestration was associated with a decreased cAMP accumulation in response to isoproterenol. In control animals, isoproterenol-stimulated cAMP accumulation (stimulated minus basal) was 73±17 pmol/10^7 cells (n=5). After captopril treatment, isoproterenol-stimulated cAMP accumulation decreased to 28±7 pmol/10^7 cells (n=6, p=0.025). To determine whether mononuclear leukocyte β-adrenergic receptor sequestration was due to a direct action of captopril, blocking of angiotensin II formation, or a secondary effect, we incubated whole blood with captopril and angiotensin II, alone or with the addition of isoproterenol (Figure 7). Neither captopril or angiotensin II caused in vitro β-adrenergic receptor sequestration, and neither drug mitigated the sequestration induced by isoproterenol.

Interaction of Captopril With Mononuclear Leukocyte β-Adrenergic Receptors

A 2-week captopril infusion did not affect the total number of mononuclear leukocyte β-adrenergic receptors (control, 760±49 sites/cell; captopril, 730±70 sites/cell). Isoproterenol treatment markedly down-regulated mononuclear leukocyte β-adrenergic receptors (221±50 sites/cell) that was not mitigated by concomitant treatment with captopril (176±41 sites/cell; Figure 5). The K_d for IPIN was similar in all treatment groups: 19±5 pM in control animals (n=10), 22±7 pM in captopril-treated animals (n=10), 12±2 pM in isoproterenol-treated animals (n=6), and 18±4 pM when both drugs were infused (n=6).

Although captopril infusion did not alter the total mononuclear leukocyte β-adrenergic receptor number, it was accompanied by β-adrenergic receptor sequestration and desensitization. In control animals, 88±1% of the β-adrenergic receptors were on the cell surface compared with 76±3% in the captopril-treated group (p=0.01, Figure 6). This sequestration was associated with a decreased cAMP accumulation in response to isoproterenol. In control animals, isoproterenol-stimulated cAMP accumulation (stimulated minus basal) was 73±17 pmol/10^7 cells (n=5). After captopril treatment, isoproterenol-stimulated cAMP accumulation decreased to 28±7 pmol/10^7 cells (n=6, p=0.025). To determine whether mononuclear leukocyte β-adrenergic receptor sequestration was due to a direct action of captopril, blocking of angiotensin II formation, or a secondary effect, we incubated whole blood with captopril and angiotensin II, alone or with the addition of isoproterenol (Figure 7). Neither captopril or angiotensin II caused in vitro β-adrenergic receptor sequestration, and neither drug mitigated the sequestration induced by isoproterenol.

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Accordingly, increased plasma norepinephrine levels (as an indicator of sympathetic function) have been shown to be a negative prognostic indicator in patients with congestive heart failure.4

The renin-angiotensin system is also involved in the pathophysiology of congestive heart failure.8 Angiotensin-converting enzyme inhibition improves long-term hemodynamics9 and prolongs survival12 in these patients. A preliminary study has indicated that after anterior myocardial infarction, captopril may attenuate ventricular dilatation.24 These beneficial effects might be due to the influence of the renin angiotensin system on sympathetic activity. Angiotensin II facilitates norepinephrine release by an action at a presynaptic site,10,25 blocks neuronal uptake of norepinephrine,26 and enhances norepinephrine biosynthesis.26 It is not surprising, therefore, that angiotensin-converting enzyme inhibition decreases neurohumeral overstimulation in some patients with heart failure.27

In the present study, we infused guinea pigs with the β-adrenergic agonist isoproterenol. Although this induced cardiac hypertrophy, it cannot necessarily be regarded as a model of human heart failure. However, this treatment decreased cardiac β-adrenergic receptor density and responsiveness in a way similar to that observed in failing human hearts.5,6 In addition to its direct effect, isoproterenol down-regulates cardiac β-adrenergic receptors, probably also by isoproterenol-enhanced norepinephrine release from sympathetic nerve terminals via presynaptic β-adrenergic receptors and via baroreflex inactivation after peripheral vasodilation. Such an increase in norepinephrine release was confirmed in the present study by our finding of elevated plasma norepinephrine levels after isoproterenol treatment. Indirect β-adrenergic receptor down-regulation via enhanced norepinephrine release should not occur in noninnervated cells like the circulating mononuclear leukocytes.

Treatment with the angiotensin-converting enzyme inhibitor captopril slightly, but not significantly, decreased plasma norepinephrine levels under basal conditions; the high plasma norepinephrine levels after isoproterenol treatment were markedly reduced. As the vasodilator captopril should enhance, if anything, the activation of the baroreflex by isoproterenol, the decrease in plasma norepinephrine can most likely be attributed to abolished presynaptic actions of angiotensin II. However, as circulating plasma norepinephrine levels are a complex function of release, uptake, and metabolism and because only a small portion of the neuronally released norepinephrine reaches the circulation, this cannot be proven from the present data.

Additionally, captopril treatment increased the density and responsiveness of cardiac β-adrenergic receptors under basal conditions and at least partially prevented the down-regulation after isoproterenol treatment. Concomitantly, captopril treat-
ment increased isoproterenol-stimulated adenylate cyclase activity in cardiac membranes under basal conditions and prevented the reduction after in vivo isoproterenol treatment. A direct effect of captopril on cardiac β-adrenergic receptors appears to be unlikely because captopril did not affect ICYP saturation binding in vitro and did not alter the affinity for ICYP in vivo. These data are compatible with our hypothesis that captopril treatment might affect cardiac β-adrenergic receptors by reducing the stimulatory effect of angiotensin II on norepinephrine release. This effect is only modest under basal conditions and is more pronounced after isoproterenol treatment when norepinephrine release is enhanced.

No such β-adrenergic receptor up-regulation was observed in circulating mononuclear leukocytes, which in contrast showed a slight sequestration. This was accompanied by a decreased cAMP accumulation in response to isoproterenol. The reason for the sequestration of mononuclear leukocyte β-adrenergic receptors by captopril treatment in vivo is not clear. One explanation for the desensitization of mononuclear leukocyte β-adrenergic receptors might be an increased release of epinephrine (which has the same high affinity for β1- and β2-adrenergic receptors) from the adrenal medulla in response to captopril-induced vasodilation. A direct effect of captopril or angiotensin II on mononuclear leukocyte β-adrenergic receptors was excluded by in vitro experiments. In vivo captopril did not prevent the isoproterenol-induced down-regulation of mononuclear leukocyte β-adrenergic receptors. This is in contrast to its action on the heart and gives further support to the idea that captopril prevents isoproterenol-induced β-adrenergic receptor down-regulation not by a direct interaction with the β-adrenergic receptor but rather by attenuating norepinephrine release.

We conclude that captopril may increase the number and responsiveness of postsynaptic cardiac β-adrenergic receptors, most likely by preventing down-regulation through norepinephrine release. Captopril does not improve the function of extrasynaptic mononuclear leukocyte β-adrenergic receptors. Because down-regulation of cardiac β-adrenergic receptors by norepinephrine seems to play a role in the deterioration of cardiac function in congestive heart failure, and captopril can reduce plasma norepinephrine levels in such patients, we suggest that the beneficial effects of captopril might at least partly be due to this mechanism. An indirect improvement of cardiac function via prevention of sympathetic overstimulation might also explain the absence of tachyphylaxis after long-term converting enzyme inhibition in heart failure patients. It should be noted that another approach of preventing sympathetic overstimulation, treatment with β-adrenergic antagonists, also up-regulates cardiac β-adrenergic receptors and might prolong survival in some patients with congestive heart failure. Our data also show that mononuclear leukocyte β-adrenergic receptors, which have similar in vitro properties to cardiac β-adrenergic receptors and frequently underlie a similar regulation in vivo, are not always a suitable model to monitor cardiac β-adrenergic receptor function.

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References

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