Desensitization of Myocardial β-Adrenergic Receptors During Cardiopulmonary Bypass
Evidence for Early Uncoupling and Late Downregulation

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Background. Cardiopulmonary bypass (CPB), a process routinely used during cardiac surgery, is a potent stimulant to the release of endogenous catecholamines. Hence, we tested the hypothesis that CPB results in myocardial β-adrenergic receptor (BAR) desensitization.

Methods and Results. We obtained canine transmyocardial left ventricular biopsies before, during (155 minutes), and after CPB (pre-CPB, CPB, and post-CPB, respectively) and determined BAR density, proportion of β,AR to β,AR, and β,AR coupling capacity to adenylyl cyclase. BAR density was stable at 112±14 fmol/mg (pre-CPB) and 103±9 fmol/mg (CPB) but decreased post-CPB to 84±7 fmol/mg. The ratio of β,AR to β,AR (determined by two-site fit for [125I]-iodocyanopindolol competition binding with the β,AR selective antagonist ICI189,406) remained constant throughout (60±3:40±3 pre-CPB, 55±3:44±3 CPB, and 61±2:39±2 post-CPB), revealing that both β,AR and β,AR subtypes were downregulated. A different pattern was noted in the functional properties of these receptors during CPB. Decreased maximal isoproterenol-stimulated adenylyl cyclase activity (252±14 to 216±12 pmol/30 min/mg), submaximal isoproterenol-stimulated adenylyl cyclase activity (183±10 to 157±11 pmol/30 min/mg), and zinterol-stimulated adenylyl cyclase activity (187±11 to 159±11 pmol/30 min/mg), a measure of β,AR subtype activation) were noted during CPB, at the time when weaning from CPB takes place. However, this desensitized pattern was found to be completely reversed by 30 minutes post-CPB, with adenylyl cyclase activities returning to pre-CPB levels or slightly higher. Control dogs that did not receive CPB showed no change in BAR density or adenylyl cyclase activity.

Conclusions. These data suggest that myocardial BAR desensitization does occur during CPB in healthy, nonischemic canine myocardium and that this pattern is reversed 30 minutes after discontinuation of CPB. In addition, a slower process of BAR downregulation persists after discontinuation of CPB. Because successful weaning from CPB is a critical process during myocardial surgery, these findings have potentially important implications in the management of such patients. (Circulation 1991;84:2559-2567)

Cardiopulmonary bypass (CPB), a process routinely used during cardiac surgery, is one of the most potent stimulants to the release of endogenous catecholamines known. Systemic elevations of ninefold to 15-fold of plasma epinephrine and twofold to fivefold of plasma nor-

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epinephrine (NE) have been repeatedly documented.1-7 The highest catecholamine levels tend to occur late in the course of CPB, when the heart and lungs are excluded from the circulation and while the patient is being rewarmed1-4; circulating catecholamines return toward baseline after CPB.1,2,4,8 During CPB (specifically aortic crossclamping), the myocardium is cooled, arrested, and deprived of its native blood flow. Under similar conditions, release of NE has been demonstrated from anoxic, isolated hearts,9-12 and cooling maintains elevated catecholamines in ischemic myocardium.13,14 In addition, elevated coronary sinus NE and epinephrine are observed during reperfusion.15 Hence, upon termination of CPB, increased catecholamine levels may have been experienced locally within myocardial
tissue, and the rewarmed uncrossclamped heart is exposed to blood containing markedly elevated circulating catecholamines.

Discontinuation of CPB is an extremely critical event during cardiac surgery, and depressed myocardial performance during this time period may result in an inability to be weaned from CPB. Because continuous exposure to catecholamines has been shown to desensitize β-adrenergic receptors (βARs),16,17 we hypothesized that myocardial βAR desensitization might occur during CPB. This may be important clinically because βAR agonists may need to be infused in those patients in whom adequate cardiac output cannot be achieved upon termination of CPB (secondary to a relative lack of βAR-mediated myocardial inotropic responses).18-21 To test the hypothesis that βAR desensitization occurs during CPB, we obtained canine transmyocardial biopsies before, during, and after CPB and determined βAR density, proportion of β3-AR versus β2-AR, and βAR functional coupling to adenyl cyclase.

Methods
Experimental Protocol

All experiments involving animals conform to the guiding principles of the American Physiological Society. After institutional approval, 12 dogs weighing 22-26 kg were anesthetized with thiopental (15-25 mg/kg i.v.) and placed supine. Normocarbic ventilation was established using 100% oxygen through an endotracheal tube, and halothane anesthesia was briefly used for placement of monitors and sternotomy. Arterial blood gas analysis, arterial plasma catecholamine levels, and arterial blood pressure were monitored with a pressure-transducer-tipped catheter inserted into the femoral artery and advanced to the thoracic aorta; plasma catecholamine determinations were performed using high performance liquid chromatography as previously described.22 After sternotomy, halothane was discontinued, and an anesthetic typically used for cardiac surgery in humans was instituted (boluses of fentanyl [5-10 μg/kg] and midazolam [50 μg/kg], followed by continuous infusions [fentanyl 0.1-0.2 μg/kg/min and midazolam 1-2 μg/kg/min]) for the rest of the experiment.

The timing of myocardial biopsies is illustrated in Figure 1. CPB was designed to mimic typical bypass occurring during cardiac surgical procedures in humans. The biopsies were transmyocardial, including both epicardium and endocardium. They were obtained in the anatomically identical plane in the apex of the left ventricle (LV) with a 7F Tru-cut biopsy needle. At each time point, four biopsies (each weighing 15-20 mg, total 60-80 mg wet weight) were obtained, immediately frozen in liquid nitrogen, and stored at −70°C. Studies in our laboratory showed that these small biopsies do not induce altered ventricular function. In order to ensure a stable baseline preparation after surgical manipulations, 90 minutes passed before CPB. Baseline myocardial biopsies (designated “pre-CPB”) were obtained before initiation of CPB. After heparin administration (300 units/kg), nonpulsatile CPB was instituted by draining the venous blood from the right atrium and returning the arterialized blood into the proximal left subclavian artery; flow rate was set at 1.7 l/min/m², and mean arterial blood pressure was held constant at 55–65 mm Hg. PaCO₂ was maintained at 30–40 mm Hg, Pao₂ at 100–200 mm Hg, and pH at 7.35–7.45. After the dog was stabilized at a body temperature of 28–32°C for 90 minutes, the aorta was crossclamped and the heart arrested by an initial dose (350 ml) of cold (4°C) cardioplegia solution (Na=120 meq/l, K=16 meq/l, Ca=2.4 meq/l, Mg=32 meq/l, Cl=160 meq/l, HCO₃⁻=20 meq/l, heparin=1,000 units/l, procainamide=150 mg/l, osmolality=300 mosm/l, pH=7.80 at 4°C) infused into the aortic root. The heart was packed in crushed ice, and myocardial septal temperature was maintained between 14 and 18°C. After 20 minutes, a second dose of cardioplegia solution (250 ml) was infused. The aortic crossclamp was released after 40 minutes, and rewarming was begun. After 25 minutes of rewarming (155 minutes after initiation of CPB), a stable body temperature of 36±1°C was achieved, and myocardial biopsies (designated “CPB”) were obtained as described above. The dogs were then weaned from CPB, and the right atrium was decannulated and stabilized at an LV end-diastolic pressure of 5–8 mm Hg with volume (no inotropic agents were used). Thirty minutes after CPB, myocardial biopsies (designated “post-CPB”) were obtained as described above.

![Figure 1. Graph showing timing of canine left ventricular (apical) transmyocardial needle biopsies. Pre-cardiopulmonary bypass (pre-CPB) biopsies were obtained after resting 90 minutes, at normothermia, before beginning of CPB. Cardiopulmonary biopsies (CPB) were obtained after 155 minutes on bypass (including cooling, aortic crossclamp, cardioplegia infused twice, and rewarming; see text) at normothermia just before termination of CPB. Post-cardiopulmonary bypass biopsies (post-CPB) were obtained at normothermia 30 minutes after termination of CPB. (See text for details of biopsy methods.)](http://circ.ahajournals.org/doi/abs/10.1161/01.CIR.84.6.2560?journalCode=circ)
Five additional dogs served as a control group. An identical protocol was followed (including transmural myocardial apical biopsies) with the exception of CPB. In addition, an extra myocardial apical biopsy was performed in these dogs immediately after sternotomy. Hence, these dogs served as controls for time and anesthesia.

Sample Preparation

Frozen canine myocardial biopsy samples were thawed in ice-cold lysis buffer (10 mM Tris, 5 mM EDTA; pH 7.40 at 4°C) and homogenized in 1 ml lysis buffer with a Brinkman polytron at 50% maximal speed for 8 seconds. The lysis buffer, as well as all subsequent buffers described for ligand binding or adenyl cyclase assays, contained the protease inhibitors benzamidine (10 μg/ml), leupeptin (5 μg/ml), and soybean trypsin inhibitor (10 μg/ml). After homogenization, the particulate suspension was diluted 15-fold in lysis buffer and centrifuged at 30,000g for 20 minutes at 4°C. The pellet was resuspended in 75 mM Tris, 5 mM MgCl₂, and 2 mM EDTA (pH 7.40 at 37°C) buffer and filtered through one layer of 100-μm nylon mesh to remove occasional clumps.

Radioligand Binding

βAR radioligand binding was performed with [125I]-iodocyanopindolol (ICYP) by methods similar to those we have previously described for skeletal muscle,23 lung,24 and cultured cells.25 Briefly, for saturation binding studies, membranes (~30 μg) were incubated with various concentrations of ICYP (5–300 pM) in the absence (total binding) or presence (nonspecific binding) of 100 μM isoproterenol for 2 hours at 25°C. For this and other ligand binding studies, GTP (100 μM) was included in the incubations to eliminate any retained agonist binding. Reactions were terminated by rapid vacuum filtration over glass fiber filters (GF/C), which were subsequently washed three times with ice-cold 10 mM Tris buffer. Filters were counted in a gamma counter at 70% efficiency. To assess the proportion of β₂AR versus β₁AR present in canine ventricle, competition assays were performed in duplicate with 15 concentrations (10⁻⁴–10⁻¹¹ M) of the relatively β₁AR-selective antagonist ICl89.406 in the presence of 60 pM ICYP. Reaction conditions were as above.

Adenyl Cyclase Activities

Canine ventricular membrane adenyl cyclase activities were assessed by the method of Salomon26 as modified and described previously.25,27–29 Samples (~60 μg) were incubated in triplicate with various agents in a reaction mixture consisting of 30 mM Tris, 2 mM MgCl₂, 0.8 mM EDTA, 0.8 mM ascorbic acid (for catecholamine oxidation protection), 0.12 mM ATP, 0.06 mM GTP, 2.8 mM phosphoenolpyruvate, 50 μg/ml myokinase, 0.1 mM cAMP, and 5 μCi of [32P]ATP in a final volume of 50 μl for 30 minutes at 37°C. The final free Mg²⁺ concentration in these reactions was ~1 mM. Reactions were performed in the presence of water (basal), various concentrations of isoproterenol, or the selective β₂AR agonist zinterol. [32P]cAMP was isolated by sequential chromatography over 1.0 ml Dowex and alumina columns. Individual column recovery of [32P]cAMP was normalized on the basis of recovery of a known amount of [3H]cAMP added to the stop buffer; routine recovery was approximately 75–80%. Samples were eluted off the alumina columns with 0.1 M imidazole into 15 ml of scintillation cocktail and counted with a dual-channel liquid scintillation counter. Under these conditions, accumulation of [32P]cAMP was linear with time, temperature, and protein. No significant depletion of ATP substrate was present, and phosphodiesterase activity was totally suppressed by the unlabeled cAMP. Preliminary studies (see below) using full isoproterenol dose–response curves revealed half-maximal adenyl cyclase stimulation at a concentration of ~500 nM and maximal stimulation at 50–100 μM. Therefore, because of the paucity of membrane material available, triplicate determinations of basal, submaximal (500 nM isoproterenol to assess any shift in potency), and maximal (100 μM) isoproterenol-stimulated adenyl cyclase activities were performed in samples from study dogs. Maximal zinterol-stimulated adenyl cyclase activities were significantly less (~50%) than those for maximal isoproterenol, and a submaximal stimulation was often difficult to detect, so only the maximal response to zinterol (100 μM) was determined.

Data Analysis

Data from radioligand binding assays and adenyl cyclase dose–response studies were analyzed by non-linear iterative least-squares techniques.30,31 For ICl89.406 competition curves, data fit poorly to a one-site model as expected, with Hill coefficients significantly less than 1.0. Data were therefore fit to a two-site model, as given by the following equation32:

$$B_{\text{maxT}} = \frac{B_{\text{max1}}}{L+k_{d1}(1+1/k_{i1})} + \frac{B_{\text{max2}}}{L+k_{d2}(1+1/k_{i2})}$$

where $B_{\text{maxT}}$ is the total amount of radioligand bound, $B_{\text{max1}}$ and $B_{\text{max2}}$ are the densities of the β₁AR and β₂AR, L is the concentration of ICYP used, I is the concentration of competing ligand (such as ICl89.406), $k_{d1}$ and $k_{d2}$ are the dissociation constants for ICYP binding to β₁AR and β₂AR, and $k_{i1}$ and $k_{i2}$ are the dissociation constants for the inhibition of ICYP binding to β₁AR and β₂AR by the competing ligand. The twofold greater selectivity of ICYP for the β₁AR as compared to the β₂AR was taken into account.32 The “goodness of fit” was assessed by the relative distance method, and $R^2$ was greater than 0.950 for all curves. Both receptor densities and adenyl cyclase activities were normalized to protein, which was measured by the copper–bicinchoninic acid method.33 Comparisons were made with paired two-tailed Student’s $t$ tests as indicated. Data are presented as mean±SEM.
TABLE 1. Pharmacological Properties of β-Adrenergic Receptors from Canine Left Ventricular Myocardial Biopsies

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Binding Properties</th>
<th>Adenyl cyclase activity</th>
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<tbody>
<tr>
<td>ICYP</td>
<td>B_{max} (fmol/mg)</td>
<td>113±6</td>
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<tr>
<td></td>
<td>k_d (pM)</td>
<td>61±14</td>
</tr>
<tr>
<td>ICI89.406/ICYP competition</td>
<td>p_k (β_2AR) (-log)</td>
<td>8.42±0.10</td>
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<tr>
<td></td>
<td>p_k (β_13AR) (-log)</td>
<td>5.35±0.04</td>
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<tr>
<td></td>
<td>β_2AR (%)</td>
<td>55.5±2</td>
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<tr>
<td></td>
<td>β_13AR (%)</td>
<td>44.4±2</td>
</tr>
</tbody>
</table>

Adenyl cyclase activity

- Basal (pmol/30 min/mg): 130±5
- ISO maximal (pmol/30 min/mg): 275±36
- Zinterol maximal (pmol/30 min/mg): 201±20
- EC_{50} (ISO, nM): 508±96

Ligand binding properties and adenyl cyclase activities of canine left ventricular (apical) biopsies obtained from three to six individual dogs during preliminary investigations. Conditions were identical to those described for pre-CPB (see text). ICYP is a β_2AR-selective antagonist and zinterol is a β_2AR-selective agonist. ICYP, [125I]-iodocyanopindolol: B_{max}, receptor density; k_d, dissociation constant for ICYP binding; p_k, dissociation constant for inhibition of ICYP binding; AR, adrenergic receptor; ISO, isoproterenol.

Materials

Analysis of ligand binding and adenyl cyclase data was performed as described above using the computer programs LIGAND and ALLFIT (gifts from P.J. Munson, NIH) and INPLOT (Graphpad, San Diego, Calif.). The following were kindly provided as gifts: ICI89.406 and ICI118.551 (Imperial Chemical Industries), betaxolol (Searle), prenalterol (Ciba-Geigy), and zinterol (Mead-Johnson). [125I]cyanopindolol, α-[32P]ATP, and [3H]cAMP were from New England Nuclear. Sources for all other compounds were as previously listed.

Results

Characterization of Canine Myocardial βAR

Table 1 summarizes the pharmacological properties of βAR from canine LV biopsies. The receptor density found (113±6 fmol/mg) is similar to that reported for human and guinea pig LV, lower than that reported for lamb LV (~300 fmol/mg), and higher than that generally reported for rat LV (~25 fmol/mg). It is also somewhat higher than the ~60 fmol/mg that has been previously reported for canine LV membranes. The k_d for ICYP is typical of that reported by others as listed above. In preliminary studies, we found a ratio of 56:44 for β_2AR:β_13AR in canine LV, using ICYP competition binding studies with the selective β_2AR antagonist ICI89.406 (Table 1, Figure 2). The ~1,000-fold greater affinity of ICI89.406 for the β_2AR versus the β_13AR (Table 1) is characteristic. The β_2AR:β_13AR ratio differs somewhat from the 80:20 ratio reported in human LV and the 75:25 ratio reported in transmural sections of canine LV studied by autoradiographic techniques. To confirm the unexpectedly high proportion of β_2AR that we found in canine LV, we performed preliminary studies with two additional selective antagonists, ICI118.551 (β_2AR selective) and betaxolol (β_13AR selective). These studies also revealed similar proportions of β_2AR:β_13AR (data not shown). The approximately 2.5-fold maximal stimulation over basal of adenyl cyclase activity by isoproterenol, with an EC_{50} of ~500 nM, is typical of myocardial membranes from a number of species. We also considered several methods by which to assess the relative contributions of β_2AR versus β_13AR to agonist-stimulated adenyl cyclase activities. Prenalterol, a relatively specific β_2AR partial agonist, however, stimulated canine LV membrane adenyl cyclase activities minimally (~10%), and we therefore did not consider this an appropriate agent to assess the relative subtype contribution. Zinterol, on the other hand, a relatively selective β_2AR agonist, provided a 50% increase in adenyl cyclase activity over basal in these membranes (Table 1). In U937 cell membranes, which we have previously shown to have exclusively β_2AR, zinterol demonstrated partial agonist properties, providing ~40% of the stimulation obtained by the full agonist isoproterenol. In addition, in canine LV membranes, zinterol stimulation was not blocked by 10^{-7} M ICI89.406 in this mixed-subtype preparation. Thus, as also recently concluded by Bristow et al., the use of zinterol appears to provide an accurate assessment of the relative contribution of β_2AR agonist-stimulated adenyl cyclase activities in a mixed population of β_2AR and β_13AR. Interestingly, the stimulation of canine LV adenyl cyclase by zinterol amounts to ~50% of the maximal activity achieved by isoproterenol (Table 1). Noting that β_2AR represents less than one half of the receptors and that zinterol is only a partial agonist, it thus appears that the β_2AR is more strongly coupled to...
adényl cyclase in this system, a situation similar to that recently reported in human LV.35

Effects of CPB on Plasma Catecholamines

Figure 3 shows arterial plasma catecholamine levels (epinephrine and NE) from a representative dog during the entire study protocol. A transient increase in catecholamine levels associated with surgical manipulations occurs at the beginning of the protocol, quickly followed by a return to basal levels until CPB. During CPB, significant increases in plasma epinephrine levels (310±119 to 2,214±713 pg/ml, p<0.05) and NE levels (254±19 to 1,835±379 pg/ml, p<0.01) were seen over time. Although trending downward, plasma catecholamines did not decrease significantly by 30 minutes post-CPB. Of note, in control dogs where CPB was not initiated, catecholamine levels did not change from basal over the same time period.

Effects of CPB on Cardiac βARs

In control dogs not exposed to CPB, canine LV βAR density did not change at time points corresponding to pre-CPB (93±5 fmol/mg), CPB (101±7 fmol/mg), and post-CPB (100±5 fmol/mg). These values for canine LV βAR density in control dogs were the same as an additional biopsy done in control dogs immediately upon opening of the chest at the beginning of the protocol. In addition to a lack of change in canine LV βAR density in control dogs, functional properties of these receptors (as demonstrated by agonist-stimulated adényl cyclase activity) also did not change. In control dogs, maximal isoproterenol (100 μM)-stimulated activities were not significantly different at any time point including immediately upon opening the chest (223±62 pmol/30 min/mg) and at matched time points corresponding to pre-CPB (264±26 pmol/30 min/mg), CPB (322±130 pmol/30 min/mg), and post-CPB (201±45 pmol/30 min/mg). Similar results (no change from baseline) were also noted in submaximal isoproterenol (500 nM)-stimulated adényl cyclase activities in control dogs.

In dogs exposed to CPB as shown in Figure 4, the density of βARs on canine LV membranes decreased at the post-CPB time point from 112±14 to 84±7 fmol/mg, p<0.05. The proportion of B1AR/B2AR was unchanged (60±3:40±3 to 61±2:39±2). When the individual receptor densities of B1AR and B2AR are calculated on the basis of the proportions and the total Bmax, it appears that both B1AR (66±9 to 52±5 fmol/mg) and B2AR (45±6 to 32±3 fmol/mg) densities are decreased at this time point. The receptor densities during CPB, while showing a downward trend (112±14 to 103±9 fmol/mg total, 66±9 to 58±6 fmol/mg B1AR, and 45±6 to 46±5 fmol/mg B2AR) did not reach statistical significance (Figure 4), and no changes in the B1AR:B2AR ratio (60±3:40±3 to 55±3:44±3) were noted.

An entirely different pattern was noted in the functional properties of these receptors during CPB. As shown in Figure 5, a decrease in agonist-stimulated adényl cyclase activities was observed during CPB. Maximal isoproterenol-stimulated activities were decreased from 252±19 to 216±12 pmol/30
min/mg (p<0.05). Submaximal isoproterenol-stimulated adenylyl cyclase activities were also depressed during CPB, decreasing from 183±10 to 157±11 pmol/30 min/mg (p<0.05). In a similar fashion, zinterol-stimulated adenylyl cyclase activities were decreased from 188±11 to 159±11 pmol/30 min/mg (p<0.05). This time point represents the period when depressed myocardial inotropic responses are found clinically during attempts to wean from CPB (Figure 1). Notably, however, this desensitized pattern was found to be completely reversed at the final time point (post-CPB), with all adenylyl cyclase activities returning to pre-CPB levels or slightly higher (maximal isoproterenol 268±22 pmol/30 min/mg, submaximal isoproterenol 207±20 pmol/30 min/mg, and maximal zinterol 223±18 pmol/30 min/mg; p=NS compared to pre-CPB).

Discussion

Adrenergic receptors are subject to dynamic regulation by a variety of mechanisms. One of the most intensively studied of these mechanisms is desensitization, also referred to as tachyphylaxis or refractoriness. Desensitization is a common phenomenon observed in a diverse group of signal transduction systems and refers to dampening of a biologic response during continuous exposure to a stimulus. At the cellular level in the βAR receptor system, this is observed as a decrease in agonist-stimulated intracellular content of the second messenger cAMP or in membrane assays as a decrease in the agonist-stimulated activity of the enzyme that catalyzes cAMP formation, adenylyl cyclase. Desensitization has been observed during chronic βAR agonist therapy and with some pathologic conditions. Three associated processes have been described to occur during agonist-promoted βAR desensitization. The earliest process is a rapid (seconds to minutes) uncoupling of the receptor from the stimulatory guanine nucleotide protein (G), which then leads to dampened adenylyl cyclase activity. The hallmarks of this uncoupling are phosphorylation of the receptor by either the cAMP−dependent protein kinase or the βAR kinase. During more prolonged exposure to agonist (hours), the total cellular βAR complement decreases, a phenomenon referred to as downregulation. It is not uncommon to find receptor density reduced by ~80% after 24 hours of agonist exposure in some systems; agonist-stimulated adenylyl cyclase activities are also often profoundly depressed. Another process, whose role in agonist-induced desensitization is not yet defined with certainty, is the redistribution of receptors from the cell surface to a compartment (presumably intracellular) not accessible to agonists. This is referred to as sequestration or internalization, and it occurs during brief (minutes) exposure to agonist. In whole cell assays, this can be assessed using hydrophilic and hydrophobic ligands. In membrane preparations, sequestered receptors may be lost in the supernatants of the centrifugation process, with the resulting receptor density of plasma membranes found to be decreased. It should be emphasized that desensitization is a functional term as defined above, and may not specifically require the presence of any one of the three above-mentioned processes. Likewise, the existence of one process, such as downregulation, may not necessarily result in desensitization.

We hypothesized that alterations in cardiac βARs might occur during CPB, a process known to be associated with high catecholamines. Because CPB has previously been shown to be associated with depressed myocardial inotropic responses and therapeutic adrenergic agonists are not frequently used during discontinuation of CPB, desensitization of myocardial βAR may be important clinically. Because it is impossible to examine functional responsiveness to exogenously administered catecholamines and concurrently measure myocardial βAR desensitization caused solely by CPB, we chose to concentrate on a detailed analysis of changes in myocardial βARs during CPB at the cellular level. This study was designed to evaluate several possible alterations in βARs known to be associated with desensitization. One alteration that could be expected during the relatively short period of time between pre-CPB and CPB biopsies is uncoupling of the receptor, which we assessed by agonist-stimulated adenylyl cyclase activity. Desensitization of this
type can be expressed in different forms in these assays, the two most common being either a change in the maximal isoproterenol-stimulated activity or a shift in isoproterenol dose–response curve to the right with or without a change in the maximal.\textsuperscript{16,17} When a small amount of sample is available, a single submaximal agonist concentration suffices for the detection of such a shift.\textsuperscript{45} We found that CPB induces a decrease in both 100 µM and 500 nM isoproterenol-stimulated adenyl cyclase activities, revealing both a decrease in the maximal agonist stimulatory capacity and a shift in the dose–response curve. These were completely reversed 30 minutes post-CPB, a time course typical of that found during resensitization. It should be noted that the timing of the CPB sample was actually after 25 minutes of rewarming (just before discontinuation of CPB); desensitization may be even more profound if assessed immediately before rewarming. The CPB time point chosen is appropriate clinically, however, because it is during discontinuation of CPB (a time point of maximal stress on the myocardium) that functional βARs are required. We also found that zimetroz (a relatively selective β2-AR agonist) -stimulated adenyl cyclase activities were depressed during CPB. This implies that of the βAR subtypes, β2 responses are specifically depressed. However, given that zimetroz is not totally selective for β2ARs and that it is only a partial β2-AR agonist, we cannot conclude that only the β2-AR subtype undergoes this desensitization during CPB.

The βAR desensitization noted during CPB did not appear to be related to a loss of myocardial βAR. At the CPB time point, the apparent decrease in βAR density was not statistically significant. Notably, however, at the post-CPB time point, a clear decrease in βAR density was found. Because no alterations in agonist-stimulated adenyl cyclase activities were found at this time point, it appears that this loss of receptor density may be a second mechanism only beginning to be invoked at this time point. This is consistent with the usual time course of receptor downregulation, with ~3–6 hours of agonist exposure being typical for significant changes, and may reflect continued elevation of plasma catecholamine levels after CPB. That a mild decrease in receptor number causes no alteration in agonist-stimulated adenyl cyclase is not altogether unexpected. This implies that some “spare receptors” are present and that their loss does not significantly affect the overall response. It is also possible that functional desensitization of myocardial βARs may be more related to elevated catecholamines locally than systemically. As described in the introduction, during CPB (specifically aortic cross-clamping) the myocardium is cooled, arrested, and deprived of its native blood flow. Under similar conditions, release of NE has been demonstrated from anoxic, isolated hearts,\textsuperscript{9–12} and cooling maintains elevated catecholamines in ischemic myocardium.\textsuperscript{13,14}

We also assessed whether any changes in βAR subtype densities occur as a result of CPB. That βARs and β2ARs may have different downregulatory patterns has been suggested.\textsuperscript{46} We found no change, however, in the relative proportion of β1AR:β2AR. When individual β1AR and β2AR densities were determined (see “Results” and Figure 4), both subtypes appeared to be decreased at the post-CPB time point. These decreases were small and did not result in a significant decrease at the post-CPB time point (Figure 4). The total receptor density, however, representing the sum of both β1AR and β2AR, was significantly decreased at this time point. These data provide evidence for early downregulation of the βARs, but (as noted above) this may represent only a loss of cell surface βARs and not a decrease in the entire cellular complement of βAR. In addition, β1AR and β2AR subtypes decreased to the same degree. The overall effect of either sequestration or downregulation, however, is presumably the same—a decrease in the number of available βARs to bind agonist and to couple to Gs. Our methods, of course, do not allow for assessment of the cellular distribution of βAR subtypes. It should be noted, however, that autoradiographic studies of transmuralized canine LV have shown that myocytes possess both β1 and β2ARs, whereas small arterioles contain almost exclusively β2ARs.\textsuperscript{41}

Figure 6 summarizes our working model of the effects of CPB on cardiac βARs based on the current study. Desensitization of βARs occurs during CPB, as evidenced by a decrease in agonist-stimulated adenyl cyclase in membranes from the pre-CPB biopsy samples to the post-CPB biopsy samples. The latter time point represents the critical period just before weaning from CPB. Recovery occurs by at least 30 minutes after CPB is discontinued, the time of the post-CPB biopsy. The processes involved in downregulation of βAR number, known to require several hours, are initiated from the onset of CPB. Downregulation, which was minimal at the CPB time point, is clearly present at the post-CPB time point.
Our findings of desensitization of LV βARs during CPB have important clinical relevance. Discontinuation of CPB is a critical event during cardiac surgery. It is during this time period that βAR-mediated myocardial inotropic and chronotropic dysfunction (which is often relatively refractory to endogenous or infused catecholamines) is found, potentially leading to difficulty in weaning from CPB after completion of cardiac surgery. Although 15% βAR desensitization upon termination of CPB represents a relatively small percentage of overall myocardial βAR function, it is important to recognize that these experiments were performed in healthy dogs with normal hearts. Patients who require cardiac surgery usually have ischemic myocardial disease or valvular heart disease, and many of these patients have depressed myocardial function. A 15% decrease in βAR function in these patients upon termination of CPB may have important and significant implications regarding their ability to be weaned from CPB. We also found a decrease in βAR density in post-CPB biopsies. Although this was not associated with desensitization, it suggests that receptor downregulation in response to prolonged exposure to catecholamines experienced during the entire period is under way. Whether this continues into the immediate postoperative period is unknown.

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