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Acute Depression of Myocardial β-Adrenergic Receptor Signaling during Cardiopulmonary Bypass

Impairment of the Adenylyl Cyclase Moiety

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Background: **Previously the authors showed that myocardial β-adrenergic (βAR) function is reduced after cardiopulmonary bypass (CPB) in a canine model. Whether CPB results in similar effects on βAR function in adult humans is not known. Therefore the current study tested two hypotheses: (1) That myocardial βAR signaling is reduced in adult humans after CPB, and (2) that administration of long-term preoperative βAR antagonists prevents this process.**

Methods: **After they gave informed consent, 52 patients undergoing aortocoronary surgery were enrolled. Atrial biopsies were obtained before CPB and immediately before discontinuation of CPB. Plasma catecholamine concentrations, myocardial βAR density, and functional responsiveness (basal, isoproterenol, zinterol, sodium fluoride, and manganese-stimulated adenylyl cyclase activity) were assessed.**

Results: **Catecholamine levels increased significantly during CPB ($P < 0.005$). Myocardial βAR adenylyl cyclase coupling decreased during CPB, as evidenced by a 21% decrease in isoproterenol-stimulated adenylyl cyclase activity (750 [430] pmol cyclic adenosine monophosphate per milligram total protein 15 min before CPB compared with 540 [390] at the end of CPB, $P = 0.0062$, medians [interquartile range]) despite constant βAR density. Differential activation along the βAR signal transduction cascade localized the defect to the adenylyl cyclase moiety. Administration of long-term preoperative βAR antagonists did not prevent acute CPB-induced myocardial βAR dysfunction.**

Conclusions: **These data indicate that the myocardial adenylyl cyclase response to βAR agonists decreases acutely in adults during aortocoronary surgery requiring CPB, regardless of whether long-term preoperative βAR antagonists are administered. The mechanism underlying acute βAR dysfunction appears to be direct impairment of the adenylyl cyclase moiety. Similar increases in manganese-stimulated activity before and at the end of CPB show preserved adenylyl cyclase catalytic activity, suggesting that other mechanisms (such as decreased protein levels or altered isoform expression or function) may be responsible for decreased adenylyl cyclase function. (Key words: Cardiac surgery; catecholamines; myocardium.)**

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MYOCARDIAL dysfunction is a significant problem that can occur after cardiac surgery.[1] Possible causes of transient myocardial dysfunction after cardiopulmonary bypass (CPB) include incomplete myocardial protection, myocardial stunning, suboptimal revascularization, on-

going ischemia, and acute myocardial β-adrenergic receptor (βAR) desensitization. Desensitization is an adaptive mechanism in biological systems that dampens receptor responsiveness to agonist exposure and thereby reduces second messenger formation and biological effect.[2] Myocardial βAR desensitization occurs in chronic diseases such as congestive heart failure, where sympathetic stimulation results in twofold increases in plasma norepinephrine levels.[3] However, CPB represents the only clinical situation in which acute myocardial βAR desensitization occurs.[4]

Previously we found significant decreases in myocardial βAR coupling to adenylyl cyclase after CPB in transmural ventricular myocardial biopsies obtained during a canine model of CPB.[4] In this model, ventricular βAR density did not change significantly during CPB but declined after CPB, suggesting initial acute uncoupling of the βAR from its signal transduction cascade during CPB followed by βAR downregulation (receptor internalization and destruction).[4] Despite these data, direct evidence for myocardial βAR dysfunction in adult humans during CPB remains controversial. Indirect evidence for reduced responsiveness of βARs after heart surgery has been demonstrated using a lymphocyte model, although these studies did not control for the use of perioperative inotropic drugs.[5,6] Furthermore, lymphocytes express only β_2ARs, whereas human myocytes express both β_1- and β_2ARs.[7] Therefore, in this human study, we tested two prospective hypotheses. The first hypothesis is that myocardial βAR function (measured as βAR coupling to adenylyl cyclase in the right atrium) is reduced in adults after undergoing CPB. The second prospective hypothesis is that administration of long-term preoperative βAR antagonist therapy prevents intraoperative decreases in βAR function during CPB.

Methods

Clinical Protocol

Patients undergoing elective aortocoronary surgery were enrolled after we received institutional review board approval and informed patient consent. Patient characteristics were recorded, including demographics, history of chronic disease, and preoperative medications. Preoperative cardiac medications were continued until the time of surgery; of note, patients taking long-term βAR antagonists were given their usual dose on the morning of surgery. Patients received oral premedication consisting of 5–10 mg methadone and 5–10 mg diazepam 1.5 h before surgery. Hemodynamic monitors were placed immediately before anesthesia and included radial and pulmonary artery cannulation. Anesthesia was induced with fentanyl (5–15 μg/kg) and midazolam (1–5 mg) and maintained with continuous drug infusions (0.05 to 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ fentanyl and 0.5 to 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ midazolam). Pancuronium was used for muscle relaxation and then the trachea was intubated. Ventilation was controlled to maintain normocarbia. A small piece of right atrial appendage (30–100 mg wet weight) was obtained immediately before CPB at the time of atrial cannulation. During CPB, patients were cooled to 32°C before administration of cardioplegia (crystalloid cardioplegia: 25 g/l dextrose, 100 mEq/l NaCl, 15 mEq/l KCl, 2 mEq/l $CaCl_2$, 2 mEq/l $MgCl_2$, titrated to *p*H 7.5 at 4°C with Tris buffer; 362 mOsm/l) and aortic cross-clamping. Just before CPB was terminated (approximately 20–30 min after release of the aortic cross-clamp), once the patient's core body temperature had returned to 36°C, a second similar atrial biopsy (10–30 mg wet weight) was obtained proximal to the atrial cannulation suture line (from the blood-filled atrium side not excluded by the suture, and therefore nonischemic tissue). No inotropic drugs were administered until after the second biopsy was obtained, and the study was discontinued in any patient who required intraoperative βAR antagonists for acute myocardial ischemia. Blood was obtained at the time of atrial biopsies to determine plasma catecholamine concentrations.

Atrial Tissue Samples

Atrial tissue samples were immediately placed in liquid nitrogen and then stored at −70°C until analysis. Samples were weighed, then homogenized (polytron PT3000, Brinkman Instruments, Westbury, NY at maximum speed for 30 s in 2 ml ice-cold lysis buffer (5 mM Tris, 2 mM EDTA) with protease inhibitors (10 μg/ml soybean trypsin inhibitor, 10 μg/ml benzamidine, and 5 μg/ml leupeptin). The solution was filtered through a 210-μm mesh filter (Spectra-mesh, VWR, Boston, MA) to remove any nonhomogenized particulate tissue. Further lysis buffer (10 ml) was added and the homogenate centrifuged at 36,000*g* for 15 min at 4°C; this step was repeated twice to remove retained βAR antagonist (a fact documented in pilot experiments using extremely high concentrations [10^{-4} M] of several clinically used βAR antagonists). The final pellet was suspended in assay buffer (75 mM Tris, 12.5 mM $MgCl_2$, 2 mM EDTA, *p*H 7.4

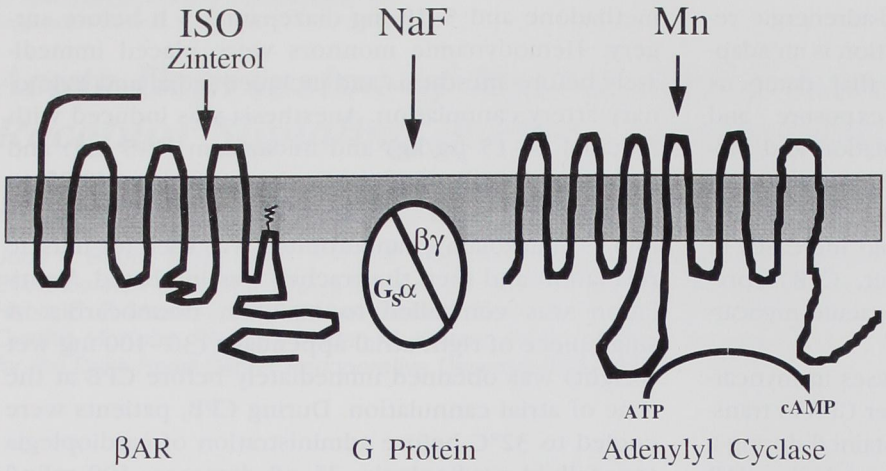

Fig. 1. A schematic of the β-adrenergic receptor (βAR) signal transduction cascade highlighting the site of action for each drug used in adenylyl cyclase assays. ISO, isoproterenol (nonselective βAR agonist); zinterol (selective β_2AR agonist); Gsα, α subunit of stimulatory G protein; βγ, stimulatory G protein βγ subunit; NaF, sodium fluoride (stimulates Gs); AC, adenylyl cyclase; Mn, manganese (stimulates adenylyl cyclase moiety directly); ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate.

with protease inhibitors at 4°C), 20 μl/mg tissue wet weight. The protein concentration was determined using the bicinchoninic acid method (Pierce, Rockford, IL) with bovine serum albumin as the standard.

Adenylyl Cyclase Assays

Atrial membrane adenylyl cyclase activities were assessed using the method of Salomon,[8] as modified and described previously.[4] Briefly, 20 μl atrial membranes were incubated in triplicate with either water (basal), 100 μM isoproterenol (ISO-MAX), 500 nM isoproterenol (ISO-EC50),[9,10] 100 μM zinterol (a selective β_2AR agonist), 10 mM sodium fluoride (mixed in a glass tube), or 5 mM manganese for 15 min at 37°C in a 50-μl reaction mixture (fig. 1). Manganese (as opposed to forskolin) was chosen as the direct stimulant for adenylyl cyclase in these experiments because it stimulates adenylyl cyclase without being influenced by the presence or activity of G proteins.[11,12] The reaction mixture contained the following final drug concentrations: 30 mM Tris, 5 mM $MgCl_2$, 0.8 mM EDTA, 0.12 mM adenosine triphosphate, 0.06 mM guanosine triphosphate, 2.8 mM phosphoenolpyruvate, 50 μg/ml myokinase, 0.1 mM cyclic adenosine monophosphate (cAMP), 10 μg/ml pyruvate kinase, and 1 μCi α[^{32}P]adenosine triphosphate. The reaction was stopped with 1 ml stop buffer (360 μM adenosine triphosphate, 285 μM cAMP, and 25,000 cpm/ml [^{3}H]-cAMP. [^{32}P]cAMP was isolated by sequential chromatography over Dowex columns using 1 ml alumina, and individual column recovery was normalized based on the recovery of a known amount of [^{3}H]cAMP added to the stop buffer; routine recovery is approximately 75–80%. Samples were eluted off alumina columns with 0.1 M imidazole into 15 ml scintillation cocktail and counted with a dual-channel liquid scintillation counter (Wallac Inc., Gaithersburg, MD). This resulted in a linear accumulation of [^{32}P]cAMP with respect to time, protein, and temperature. Final results were reported as pmol cAMP · mg total protein^{-1} · 15 min^{-1}.

Ligand Binding Assays

The βAR density was determined using standard ligand-binding techniques. Briefly, ligand binding was performed in triplicate (20–30 μg of membrane protein per tube) in a final volume of 500 μl assay buffer (75 mM Tris, 12.5 mM $MgCl_2$, 2 mM EDTA, *p*H 7.4 with protease inhibitors [10 μg/ml soybean trypsin inhibitor, 10 μg/ml benzamidine, and 5 μg/ml leupeptin] at 4°C) using a saturating concentration (275 pM) of [^{125}I]-cyanopindolol (Dupont, Boston, MA); Propranolol (1 μM; Sigma Chemical Co., St. Louis, MO) was used to determine nonspecific binding. The reaction was incubated and agitated for 2 h at room temperature (25°C). Bound [^{125}I]-cyanopindolol was separated from free by rapid vacuum filtration onto glass fiber (GF/C) filters (Whatmann International, Maidstone, UK). Filters were rinsed rapidly three times with 3 or 4 ml ice-cold 50 mM Tris, *p*H 7.4, using a Brandel cell harvester (Brandel, Gaithersburg, MD) and counted in a gamma counter (Packard, Downers Grove, IL).

Plasma Catecholamines

Blood samples obtained during surgery were collected on ice and immediately centrifuged (834*g* at 4°C); plasma was placed, using a pipette, into storage vials, and immediately placed in liquid nitrogen; plasma was stored at −70°C until analysis. Catecholamines were assayed by high-pressure liquid chromatography using

on-line trace enrichment onto a cation exchange resin, followed by elution onto a C18 column and thin-layer electrochemical detection as previously described by Kilts.[13] Lower limits of assay detection are 10 pg for both norepinephrine and epinephrine; intraassay variability is ±3–5%.

Statistical Analysis

Changes in serum catecholamine concentrations before CPB and at the end of CPB were tested using paired *t* tests; $P < 0.05$ was considered significant. βAR dysfunction was defined *a priori* as decreased (≥15% based on our original canine study[4]) ISO-MAX or ISO-EC50-stimulated adenylyl cyclase activity.

Mechanisms underlying impaired βAR dysfunction were examined using βAR density as well as isoproterenol-, zinterol-, sodium fluoride-, and manganese-stimulated adenylyl cyclase activity. The mean percentage change was used for the analysis. Power calculations for each hypothesis were performed using a two-tailed paired *t* test to test the null hypothesis of no mean percentage change using Solo Power Analysis[14] and Muller's software.[15] Because data in some of the adenylyl cyclase subgroups was not normal, logarithmic transformation was performed to ensure normality, followed by analysis using paired *t* tests and a general linear multivariate model. To determine whether receptor-mediated or G protein-mediated changes in adenylyl cyclase activity differed in magnitude from direct manganese stimulation of the adenylyl cyclase moiety, equality of mean percentage change of variates was tested using a generalized estimating equation model[16,17] with identity link and exchangeable correlation. To evaluate the effect of long-term use of preoperative βAR antagonists on myocardial βAR dysfunction after CPB, the data were divided into two subsets and characteristics of the two groups were inspected to assess similarity. Unless otherwise stated, data are presented to two significant figures as means ± SD; because some subgroups of adenylyl cyclase data are not normally distributed, these data are presented as medians [interquartile range].

Results

Patient Characteristics

Fifty-two patients were enrolled in the study. Patient characteristics (demographics, history of chronic disease, and preoperative medications) are shown in table 1 for all 52 patients and for subgroups of patients receiving (n = 31) or not receiving (n = 21) long-term preoperative βAR therapy. The CPB time was 90 ± 26 min (91 ± 28 βAR antagonist group, 88 ± 21 non-βAR antagonist group). The aortic cross-clamp time was 45 ± 11 min (43 ± 10 βAR antagonist group, 46 ± 14 non-βAR antagonist group), reflecting use of the first 20–30 min of CPB to dissect distal coronary artery anastomosis sites and size coronary grafts before the aortic cross-clamp was applied, thus minimizing myocardial ischemic time. No difference in patient characteristics existed between groups, except for the use of preoperative βAR antagonists.

Table 1. Preoperative Patient Characteristics

Characteristics*	Total (n = 52)	Preop βAR Antagonists (n = 31)	No Preop βAR Antagonists (n = 21)
Age (yr)	62 ± 10	62 ± 9.0	63 ± 5.0
Sex (% male)	63	64	61
Preoperative LVEF	55 ± 13	55 ± 14	55 ± 6.0
Race (% black)	23	23	24
Co-existing diseases (%)			
Diabetes	27	29	24
Congestive heart failure	2.0	3.2	0
Hypertension	59	61	57
Medication (%)			
βAR antagonist†	60	100	0
Calcium channel antagonist	33	32	33
ACE inhibitor	10	6.4	9.5
Nitroglycerin	21	23	19
Diuretic	58	68	43
Digoxin	12	13	9.5

Values are mean ± SD. Preop = preoperative; βAR = β-adrenergic receptor; LVEF = left ventricular ejection fraction determined at preoperative cardiac catheterization; ACE = angiotensin-converting enzyme.

* Specific definitions were used to define the presence of co-existing diseases. Documentation of congestive heart failure required at least three of the following: standard chest x-ray evidence of increased cardiothoracic ratio (>0.5:1) or pulmonary edema; evidence on physical examination of audible rales, dependent edema, congestive hepatomegaly, elevated jugular venous pulse, or presence of third heart sound; increased left ventricular end-diastolic pressure > 18 mmHg at the time of cardiac catheterization or pulmonary artery occlusion pressure > 18 mmHg immediately prior to surgery. Hypertension was defined as diastolic blood pressure > 90 mmHg documented on at least three occasions, or a history of increased blood pressure requiring antihypertensive medication. Diabetes mellitus was considered present if a patient required oral hypoglycemic or insulin medication to control serum glucose concentrations.

† Chronic preoperative βAR antagonists administered included metoprolol (~70% of patients) and atenolol. βAR antagonist dose was determined by the patient's primary physician as efficacious for the treatment of hypertension or symptoms of coronary artery disease. Doses were generally as follows: metoprolol 100–200 mg/day (divided twice daily) and atenolol 50–100 mg/day (given once daily).

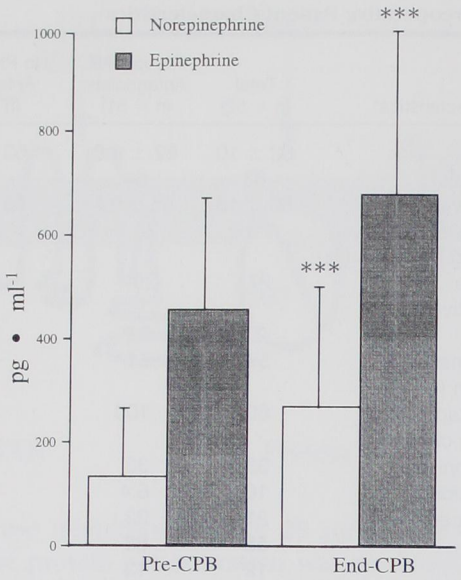

Fig. 2. Plasma catecholamine concentrations during CPB. Both plasma epinephrine and norepinephrine levels increased significantly during CPB. ***$P < 0.005$ compared with before cardiopulmonary bypass. Mean ± SD.

Plasma Catecholamines and Acute Myocardial βAR Desensitization during Cardiopulmonary Bypass

Plasma catecholamine levels increased significantly ($P < 0.005$) during CPB (fig. 2). Isoproterenol-stimulated adenylyl cyclase activity decreased 20% during CPB (19% decrease ISO-EC50, $P = 0.0032$; 21% decrease ISO-MAX, $P = 0.0062$; table 2, fig. 3). Zinterol-stimulated adenylyl cyclase activity (β_2AR-selective agonist) was similar to that mediated by an ISO-EC50 concentration, decreasing 24% during CPB ($P = 0.0020$, table 2). The CPB also decreased basal (unstimulated) adenylyl cyclase activity ($P < 0.0001$, table 2).

To determine whether myocardial βAR dysfunction during CPB occurs at the receptor or more distal in the βAR signal transduction cascade, βAR density, sodium fluoride, and manganese were used. βAR density did not change during CPB (table 2, fig. 3). Sodium fluoride-stimulated adenylyl cyclase (representing G protein activity) decreased 14% ($P = 0.0044$, table 2) and manganese-stimulated adenylyl cyclase activity (representing direct stimulation of the adenylyl cyclase moiety itself) decreased 21% ($P = 0.0001$, table 2). Taking into account individual variability in various adenylyl cyclase determinations, the power to detect the observed difference (percentage change) with the final patient number is as follows: ISO-MAX, 81.5%; ISO-EC50, 86.4%; zinterol, 90.4%; sodium fluoride, 84.9%; manganese, 99.9%. The observation that neither receptor-mediated nor G protein-mediated decreases in adenylyl cyclase activity were greater than the decrease seen with direct manganese stimulation suggests that the defect is localized to the adenylyl cyclase moiety itself.

Effect of Long-term Preoperative βAR Antagonist Therapy on Acute Myocardial βAR Dysfunction during Cardiopulmonary Bypass

Patients receiving long-term preoperative βAR antagonists had a small but significant increase in atrial βAR density at baseline (before CPB, $P = 0.012$, table 2). Consistent with this increased βAR density, ISO-stimulated adenylyl cyclase activity was higher before CPB in patients receiving long-term preoperative βAR antagonists (using assay conditions in which antagonist is no longer present; fig. 3B). However, myocardial βAR response to CPB was similar in both groups, with significantly decreased adenylyl cyclase activity without change in βAR density regardless of long-term preoperative βAR antagonist therapy (table 2).

Discussion

This study shows that atrial myocardial βAR signal transduction is reduced during CPB in adult humans, providing support for our first hypothesis. Dampened receptor responsiveness (also called desensitization) can occur as a result of changes in the receptor, in proteins involved in the signal transduction pathway, or both. Components of the βAR signal transduction pathway include the cell-surface βAR, intermediary G protein, and the effector (adenylyl cyclase moiety; fig. 1). Three mechanisms are involved in βAR desensitization at the receptor level—uncoupling (disruption of receptor/G protein complex), sequestration (movement of receptor from the cell surface to intracellular compartments), and downregulation (decrease in receptor number resulting from a complex interplay between depressed receptor synthesis and destruction of sequestered receptors).[2,18] These processes are thought to result from receptor phosphorylation by various kinases, such as second messenger stimulated kinases protein kinase A and protein kinase C, as well as G protein-coupled receptor kinases.[18] Receptor desensitization is considered homologous when only the stimulated receptor is desensitized, or heterologous when multiple receptors systems are desensitized indirectly as a result of second messenger activation. Although heterologous desensitization historically has been defined at the receptor level, more re-

Table 2. Adenylyl Cyclase Activity (pmol cAMP/mg Total Protein/15 min) and βAR Density (fmol/mg Protein) before and after CPB

	Total (n = 52)			Preop βAR Antagonist (n = 31)			No Preop βAR Antagonist (n = 21)		
	Pre-CPD	End-CP	% Change	Pre-CPB	End-CPB	% Change	Pre-CPB	End-CPB	% Change
Basal	250 [160]	160 [150]	−29 [55]*	270 [170]	140 [180]	−30 [44]*	230 [140]	170 [130]	−27 [67]*
ISO-MAX	750 [430]	540 [390]	−21 [49]†	770 [470]	580 [490]	−21 [58]‡	700 [360]	520 [260]	−23 [45]‡
ISO-EC50	480 [320]	300 [320]	−19 [54]*	500 [350]	310 [460]	−21 [59]‡	410 [370]	300 [220]	−19 [43]‡
Zinterol	480 [260]	350 [320]	−24 [51]*	500 [370]	330 [400]	−30 [51]*	470 [250]	370 [200]	−12 [44]
NaF	1,300 [600]	1,000 [820]	−14 [50]*	1,300 [670]	1,000 [800]	−28 [59]‡	1,400 [430]	1,000 [870]	−7.5 [50]
Mn	850 [410]	570 [440]	−21 [39]*	910 [380]	690 [470]	−20 [36]*	570 [520]	440 [310]	−28 [43]‡
βAR density	50 [19]	47 [26]	No change	53 [15]	47 [25]	No change	47 [20]	44 [19]	No change

% Change = (End-CPB − Pre-CPB)/Pre-CPB.

Differential activation along the βAR signal transduction pathway was performed by stimulating the production of adenylyl cyclase activity at the receptor (ISO-MAX, ISO-EC50, zinterol), Gs (NaF), and adenylyl cyclase moiety (Mn). Median [interquartile range] to two significant figures.

Significant decreases were noted after CPB for each measure of adenylyl cyclase function: * $P < 0.005$, † $P < 0.01$, ‡ $P < 0.05$, *versus* baseline.

cently the definition of heterologous desensitization has been expanded to include impairment of nonreceptor components of the signal transduction cascade[19]; in the βAR signal transduction system, this reflects changes in G protein subtypes and adenylyl cyclase isoforms.[19,20]

Work in the past two decades examining mechanisms underlying chronic βAR desensitization in heart failure suggests that sympathetic activation leads to increased transmyocardial concentrations of norepinephrine[21] and dampened βAR signal transduction.[2,22] Because these changes occur at both the receptor (decreased number and function) and signal transduction pathway (increased levels of Gi and decreased levels of adenylyl cyclase V and VI), both homologous and heterologous desensitization occurs with chronic heart failure.[22–25] Despite strong evidence for chronic βAR desensitization, a paucity of data exists indicating that acute myocardial βAR desensitization occurs clinically. Because plasma catecholamines increase dramatically during CPB (from 2 to 20 times),[4,26] and application of the aortic cross-clamp results in myocardial ischemia (with resultant release of norepinephrine from myocardial sympathetic nerve fibers) despite protective maneuvers such as hypothermic cardioplegic arrest, CPB provides a potentially good model to examine acute myocardial βAR desensitization. Previously we found acute myocardial βAR desensitization in a canine model of CPB.[4] However, because of species differences in myocardial βAR subtype expression, functional activity, and regulation,[10,27,28] documentation that similar process are present in humans is important. One human study has reported the occurrence of acute myocardial βAR dysfunction in children during heart surgery using atrial biopsies,[29] although another study using lymphocytes contradicts this finding.[30] Furthermore, the relevance of these studies for adults is unclear, because the distribution of myocardial βAR subtypes and coupling to adenylyl cyclase is altered in children with congenital heart disease.[31,32] In the current study, although the CPB period was relatively short, doubling of both plasma norepinephrine and epinephrine occurred. Forty-five minutes of aortic cross-clamping potentially exposed myocardial βARs to ischemia and elevated myocardial catecholamines, followed by release of the cross-clamp and exposure to blood containing the highest plasma catecholamine concentrations present during CPB. Myocardial ischemia has been shown to variably affect βAR activity, with most studies demonstrating initial acute increases in βAR signaling resulting from sympathetic stimulation, externalization of sequestered receptors, and acute inhibition of Gi activity[33]; however more prolonged ischemia (10–60 min) results in dampened βAR function.[33] When βAR function was examined during CPB in the current study, decreased isoproterenol-stimulated adenylyl cyclase activity occurred, providing evidence of acute impairment of the myocardial βAR signal transduction pathway during CPB in adults undergoing heart surgery.

To elucidate the underlying mechanism(s) for myocardial βAR dysfunction during CPB, we first examined βAR density. No change in receptor density occurred with CPB. This result is not surprising because crude membrane preparations isolate βARs from both the cell membrane and sequestered vesicles. Many studies have shown that 3 h of agonist exposure are required before significant βAR downregulation is apparent using [^{125}I]-cyanopindolol in crude membrane preparations. Previously we detected downregulation in a canine model of

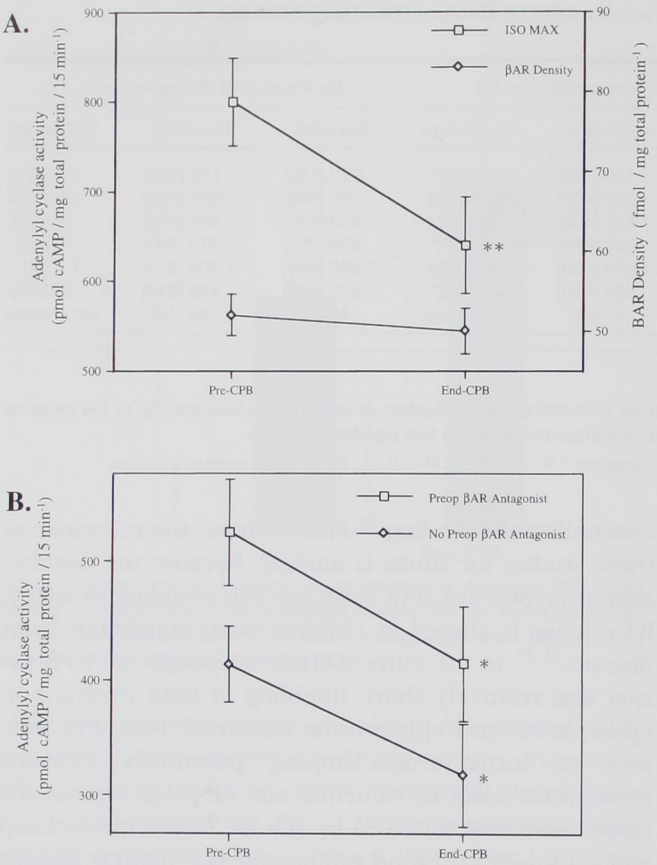

Fig. 3. Myocardial β-adrenergic receptor (βAR) function before cardiopulmonary bypass (CPB) and at the end of CPB. (*A*) Maximal isoproterenol-stimulated βAR responsiveness (100 μM, measured by adenylyl cyclase activity) is depressed at the end of CPB (end-CPB) compared with baseline (left axis). In comparison, βAR density remains constant during CPB (right axis). (*B*) Although patients receiving long-term preoperative βAR antagonist therapy have higher baseline isoproterenol-stimulated adenylyl cyclase activity median effective concentration (EC50), both groups undergo significant myocardial βAR desensitization during CPB (see the text for details); ISO-EC50 data is presented for this comparison. *$P < 0.05$, **$P < 0.01$ compared with before CPB. Mean ± SEM is used for clarity of presentation.

CPB only at the final time point 30 min after CPB; this time point represented exactly 3 h of agonist exposure.[4] However, in the current study CPB only lasted 90 min, so constant βAR density was expected (demonstrating lack of downregulation). Two methods can be used to distinguish membrane receptors from sequestered receptors. In whole-cell experiments, comparison of ligand binding with hydrophilic radioligands (which cannot cross the lipid membrane and therefore only bind receptors at the cell surface) and hydrophobic radioligands (which do cross the lipid membrane and therefore bind surface and sequestered receptors) identifies the fraction of sequestered receptors. In harvested tissues, differential centrifugation can also be used to separate membrane fractions from light vesicle fractions; unfortunately there was insufficient tissue available in the current study to perform these experiments. Furthermore, identification of sequestered receptors during CPB does not identify a potentially important subpopulation of receptors present at the cell surface that are acutely uncoupled from G proteins. Thus we chose to examine these two possibilities (uncoupling and sequestration) indirectly using functional assays; dampened βAR function above and beyond that seen for other proteins involved further downstream in the βAR signal transduction cascade (G proteins and adenylyl cyclase moiety) would provide indirect evidence of either uncoupling or sequestration.

To explore βAR function before- and at the end of CPB, we examined receptor, intermediary G protein, and manganese-stimulated adenylyl cyclase and found an ≈20% decrease ($P < 0.007$) in function at all levels after exposure to CPB. Because isoproterenol-stimulated adenylyl cyclase activity (receptor level) is dampened no further than that seen with sodium fluoride (G protein level) or manganese (adenylyl cyclase level), and >80% power existed to detect such a difference at all levels, the defect appears to be localized to the adenylyl cyclase moiety itself. Consistent with this notion, during CPB we also found a decrease in basal adenylyl cyclase activity. Thus heterologous desensitization of the βAR signal transduction pathway occurs during CPB. However, these results do not eliminate the possibility of βAR desensitization at the receptor level. Although human heart contains relatively few spare receptors compared with other animal models,[34] even a few spare receptors might prevent detection of a small population of desensitized receptors. Longer agonist exposure, as might be present during the entire postoperative period, might unmask such βAR desensitization with time. However, our data show that the primary defect associated with CPB appears to reside at the level of the adenylyl cyclase moiety.

A decrease in adenylyl cyclase activity could occur *via* several mechanisms; these include generalized decreases in catalytic function, decreased total protein concentration, or alterations in specific adenylyl cyclase isoforms. For example, decreased concentrations of adenylyl cyclase types V and VI (predominant isoforms in the heart[24]) occur in chronic pacing-induced heart failure,[25,35] whereas changes in activity of these isoforms occur with activation of PKC,[36] PKA,[19] aging,[37] and in pressure-overloaded failing right ventricles.[38] In our

study, preservation of adenylyl cyclase catalytic efficiency (evidenced by increases in manganese-stimulated activity before CPB and at the end of CPB of similar magnitudes) suggests that decreased catalytic function is not the cause of depressed adenylyl cyclase activity after CPB. Rather, changes in isoforms (density or activity) or overall decreased adenylyl cyclase concentrations may be involved. In fact, in a study published while this article was being reviewed suggests that intermittent warm blood cardioplegia preserves myocardial βAR function compared with cold crystalloid cardioplegia[39]; because cold potassium cardioplegia results in increases in intracellular calcium, a condition that inhibits adenylyl cyclase isoform VI present in heart,[24] this provides another possible mechanism for our results. Each of these mechanisms are being investigated in our laboratory.

Administration of anesthetic agents can also alter adenylyl cyclase activity. For example, fentanyl (and other opioids) couple *via* Gi to inhibit adenylyl cyclase, and so do other compounds generated with ischemia, such as adenosine. With regard to anesthetic agents, these drugs were administered as a continuous infusion during the study, so similar amounts should have been present before and at the end of CPB. However, drugs are less well metabolized during hypothermia; in contrast, fentanyl binds to the plastic tubing in the CPB circuit, which tends to result in lower than expected plasma levels during CPB. If fentanyl levels were lower at the end of CPB and significant Gi effects occur with fentanyl anesthesia, then this would have biased our results to show no desensitization. Instead we found very reproducible impaired βAR signalling in every patient. Furthermore, anesthetics and metabolic products are washed out during myocardial membrane preparation in the laboratory. Therefore in this study we do not believe that the anesthetic background influenced the results significantly.

Our second prospective hypothesis was that administration of long-term preoperative βAR antagonists would prevent the reduction in βAR signalling associated with CPB. βAR antagonist therapy has been shown to improve myocardial function in a dose-dependent manner in congestive heart failure.[40–42] Although the exact mechanism(s) for improved myocardial function have not been elucidated, regression of chronic βAR desensitization has been postulated. In support of this mechanism, metoprolol (β_1-selective antagonist) upregulates βAR receptor density and improves myocardial response to dobutamine.[41,42] However, βAR upregulation is not an absolute requirement for the beneficial effects of βAR antagonists in congestive heart failure because carvedilol improves myocardial function without changing βAR density.[43] In the current study, ~70% of patients in the βAR antagonist group received long-term metoprolol before surgery. Thus our finding of increased myocardial βAR density and concomitant higher baseline isoproterenol-stimulated adenylyl cyclase activity in patients receiving long-term βAR antagonist therapy is expected (the higher βAR density naturally results in higher adenylyl cyclase activity in an assay in which residual βAR antagonist is eliminated). However, because patients in both groups show statistically significant decreases in isoproterenol-stimulated adenylyl cyclase activity with CPB, the second hypothesis is disproved. An explanation for this finding might be that clinically effective doses of preoperative βAR antagonists may not be present in sufficient concentration during CPB to prevent binding of extremely high concentrations of myocardial catecholamines generated during aortic cross-clamp,[44,45] especially because cold inactivates monoamine oxidase and catechol-O-methyltransferase,[46] further increasing myocardial catecholamine levels. Interestingly, a recent canine study performed in our laboratory shows that acute intraoperative administration of esmolol during CPB prevents acute myocardial βAR desensitization and results in improved myocardial function after CPB.[47]

In conclusion, we provide evidence that transmembrane myocardial βAR dysfunction occurs in adults during aortocoronary surgery requiring hypothermic CPB, regardless of the administration of long-term preoperative βAR antagonists. The defect appears to be localized to the adenylyl cyclase moiety.

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