LABORATORY INVESTIGATIONS

Esmolol Improves Left Ventricular Function via *Enhanced β-Adrenergic Receptor Signaling in a Canine Model of Coronary Revascularization*

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Background: Recent American Heart Association guidelines highlight the paucity of data on effectiveness and/or mechanisms underlying use of β-adrenergic receptor (βAR) antagonists after acute coronary syndromes in patients subsequently undergoing revascularization. It is important to assess whether βAR antagonists might protect the heart and improve ventricular function in this scenario. The authors therefore used esmolol (an ultra–short-acting βAR antagonist) to determine whether βAR antagonist treatment improves left ventricular function in a canine model of acute reversible coronary ischemia followed by coronary reperfusion during cardiopulmonary bypass (CPB). The authors also tested whether the mechanism includes preserved βAR signaling.

Methods: Dogs were randomized to either esmolol or saline infusions administered during CPB (n = 29). Pre-CPB and end-CPB transmyocardial left ventricular biopsies were obtained; plasma catecholamine concentrations, myocardial βAR density, and adenylyl cyclase activity were measured. In addition, left ventricular systolic shortening and postsystolic shortening were determined immediately prior to each biopsy.

Results: While βAR density remained unchanged in each group, isoproterenol-stimulated adenylyl cyclase activity decreased 26 ± 6% in the control group but *increased* 38 ± 10% in the esmolol group (pre-CPB to end-CPB, mean ± SD, P = 0.0001). Left ventricular systolic shortening improved in both groups after release of coronary (LAD) ligature; however, the esmolol group increased to 72 ± 23% of pre-CPB values compared to 48 ± 12% for the control group (P = 0.0008).

Conclusions: These data provide prospective evidence that esmolol administration results in improved myocardial function. Furthermore, the mechanism appears to involve enhanced myocardial βAR signaling.

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β-ADRENERGIC receptor (βAR) antagonists have been shown to improve mortality and reduce reinfarction in many randomized clinical trials after acute myocardial infarction (AMI).[1-3] However, the vast majority of these trials excluded patients who subsequently underwent surgical coronary revascularization. As a result, a paucity of studies exist on effectiveness, rationale, and/or mechanisms underlying the use of βAR antagonists in this setting. This paucity of data has recently been raised as a concern by both the American College of Cardiology (ACC) and the American Heart Association (AHA).[4] In fact, current ACC/AHA guidelines for coronary artery bypass graft (CABG) surgery state that "there is no universally applicable myocardial protection technique" in reducing the risk of perioperative myocardial dysfunction.[5]

Preoperative left ventricular (LV) dysfunction is one of the strongest predictors of mortality after CABG surgery.[5,6] In addition, an already poorly functioning left ventricle is often further impaired in the perioperative period.[7,8] One possible mechanism underlying acute LV dysfunction in this setting is decreased βAR signaling. Previous studies have demonstrated that βAR function decreases after 15 min of acute ischemia.[9] This is due, in part, to rapid induction and translocation of agonist-induced β-adrenergic receptor kinase (βARK), a kinase which phosphorylates agonist occupied myocardial βARs during ischemia, resulting in receptor phosphorylation and binding of the inhibitor protein β arrestin.[10,11] Functionally, this process has been shown to result in acute global myocardial dysfunction in mice[12,13] and humans[14] and very recently has been shown to be reversible by administration of βARK inhibitor constructs.[15]

Dampened myocardial βAR signaling has also been shown to occur during isolated cardiopulmonary bypass (CPB) in the absence of ischemia.[7,16] Cardiac surgery is accompanied by a 2- to 20-fold elevation of serum catecholamines (epinephrine > norepinephrine).[7,16-18] Elevations in catecholamines have been shown in both cellular and animal models to induce βAR desensitization. Decreased isoproterenol-stimulated adenylyl cyclase suggests acute myocardial βAR desensitization is a mechanism underlying CPB-induced myocardial dysfunction. This process appears to be heterologous since dampened βAR signaling occurs in the absence of altered βAR density and is primarily localized to the adenylyl cyclase moiety itself.[7,16]

Fig. 1. Schematic of study protocol. Timing of ischemia, cardiopulmonary bypass (CPB), aortic cross-clamping (x), and esmolol infusion are shown. Timing of measurements is also shown, including start of anesthesia, systolic shortening (SS), and each transmyocardial biopsy (B). See text for details.
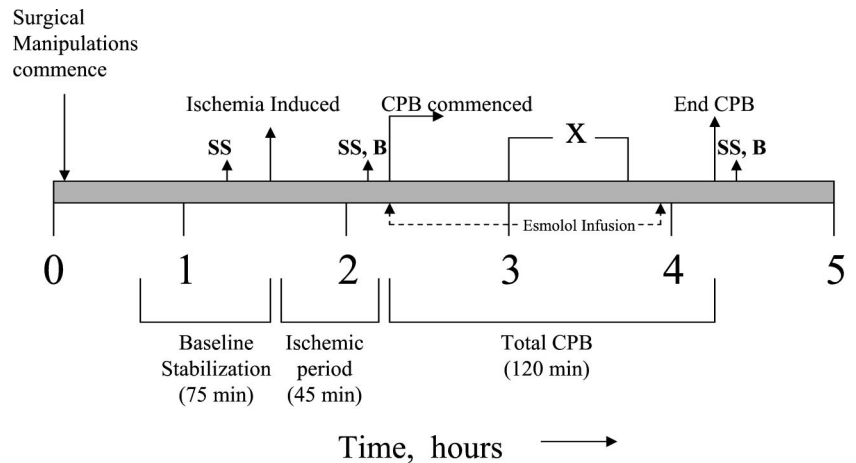
Given our understanding of catecholamine-induced impaired βAR signaling in the presence of ischemia and/or CPB, we tested the hypothesis that βAR antagonist administration improves ventricular function by preventing agonist-induced βAR desensitization. Ironically, βAR antagonists tend to be avoided during cardiac surgery because of concern that negative inotropic effects may make it difficult to terminate CPB. Esmolol (an ultra–short-acting βAR antagonist) is rapidly metabolized by an aryl esterase in the blood,[19] resulting in an elimination half-life of 9 min;[20] therefore, the negative inotropic effects of βAR antagonism are minimized during CPB. As a result, we chose esmolol to determine whether βAR antagonist treatment improves LV function in a canine model of acute coronary ischemia–reperfusion. We also examined potential mechanisms by determining isoproterenol-stimulated myocardial adenylyl cyclase response in each animal.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee (Duke University, Durham, North Carolina), and all animals used in these experiments received humane care conforming to the guiding principles formulated by the National Society for Medical Research in "Principles of Laboratory Animal Care" and published by the National Institutes of Health (NIH Publication No. 80-23, revised 1985).

Canine Preparation

To produce a canine model that mimics the human scenario of acute coronary syndrome with subsequent CABG, a protocol was designed to produce acute myocardial ischemia in the LAD area of the left ventricle followed by coronary reperfusion (fig. 1). Anesthesia was induced in 29 mongrel dogs weighing 20–26 kg with thiopental (5–10 mg/kg intravenously). Tracheal intubation was performed, and the animals were ventilated with oxygen (Ohio V5 Airco; Ohmeda, Madison,

WI) at a rate designed to achieve normocapnia. Anesthesia was maintained with halothane (1.0–2.0% end-tidal) until the aortic root was cannulated. End-tidal concentrations of both halothane and carbon dioxide were continuously measured by infrared spectroscopy (Datex model 254; Puritan Bennett Corp., Wilmington, MA). A continuous infusion of 0.9% saline ($6-8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was commenced through an intravenous cannula introduced in the hind limb, and the animals were placed in a supine position. Aortic root pressure and blood samples for arterial gas analysis and catecholamine analysis were obtained by inserting a 7-French pressure transducer-tipped catheter (Millar Instruments, Inc., Houston, TX) into the carotid artery and advancing until the tip was in the aortic root. Immediately prior to aortic root cannulation, loading doses of midazolam (100 μg/kg intravenously) and fentanyl (10 μg/kg intravenously) were administered followed by continuous infusions (midazolam at 2 μg $\cdot$ kg^{-1} $\cdot$ min^{-1} and fentanyl at 0.2 μg $\cdot$ kg^{-1} $\cdot$ min^{-1}). After aortic root cannulation, halothane was discontinued, and a minimum time of 135 min passed before any βAR assays were performed, to ensure stable low baseline circulating catecholamine concentrations prior to initiating the protocol. LV and pulmonary arterial pressures were measured by inserting 5-French pressure transducer-tipped catheters (Millar Instruments, Inc.) *via* stab incisions into the left ventricle and pulmonary artery. Two pairs of ultrasonic crystals (diameter, 1.5–2 mm) were placed in the anterior apical LV and posterior apical LV subendocardium, respectively, and oriented in the short axis of the heart to obtain circumferential data. Continuous assessment of the regional myocardial contraction pattern was performed by measuring the segment length between the two sonomicrometer crystals, based on the measurement of ultrasonic transit time (Sonomicrometer model 120; Triton Technologies, Inc., Townsend, MA).[21,22] Heparin (300 U/kg) was administered to the animals, and both the right atrium and left subclavian artery were cannulated for CPB.

Cardiopulmonary Bypass Protocol and Induction of Myocardial Ischemia

To standardize hemoglobin concentrations before, during, and after CPB, normovolemic hemodilution to 8 g/dl (IL482 Co-Oximeter, specifically calibrated for dog hemoglobin; Instrumentation Laboratory, Lexington, MA) was performed prior to induction of ischemia using 6% Dextran in 0.9% sodium chloride (Abbott Laboratories, North Chicago, IL). Blood withdrawn during hemodilution was used along with 0.9% sodium chloride to prime the CPB circuit. The pre-CPB inspired oxygen fraction was controlled to achieve arterial oxygen partial pressures (Pao$_2$) of 100–200 mmHg. Transmyocardial biopsies, including both endocardium and epicardium, were obtained from the apex of the left ventricle with a 7-French Tru-cut biopsy needle. Transmyocardial biopsies are essential due to a gradient of βAR density that exists across the myocardium from epicardium to endocardium[23]; previous studies in our laboratory demonstrate that biopsies of this size in the LV apex do not induce measurable regional or global changes in myocardial function. Ninety minutes from the commencement of anesthesia, myocardial ischemia was produced by a gradual ligature on the left anterior descending coronary artery (LAD), which was tightened under sonomicrometry control until regional myocardial function in the anterior and posterior apical regions of the left ventricle decreased by 75%. LAD blood flow was confirmed by the placement of a Doppler flow transducer (Triton, San Diego, CA) distal to the ligature, thus ensuring that the LAD was not completely occluded throughout the ischemic interval. After 45 min of myocardial ischemia, the first transmyocardial biopsy was obtained (defined as pre-CPB), and animals were placed on CPB as previously described.[24] On initiation of CPB, 12 dogs received esmolol therapy (loading dose 500 μg/kg, continuous infusion 100 μg · kg · min^{-1}) defined as treatment group, while 17 dogs received an infusion of 0.9% sodium chloride at the same rate (ml/min) defined as placebo group. Both infusions were discontinued 20 min prior to termination of CPB. The mean arterial pressure during CPB was maintained at 50–60 mmHg by means of phenylephrine or nitroprusside as necessary while CPB flow rate was set at 1.7 l · min^{-1} · m^{-2}. Partial pressure of arterial carbon dioxide (Paco$_2$) was maintained at 30–40 mmHg; Pao$_2$ was maintained at 100–200 mmHg; pH was maintained at 7.35–7.45; and base excess was kept less than −5 mEq/l using alpha stat blood gas management.

Core body temperature was lowered to 28–30°C during CPB. After 45 min of CPB, the aorta was cross-clamped, and the heart was arrested by an initial dose (400 ml) of cold (4°C) cardioplegia solution (120 mEq/l sodium, 16 mEq/l potassium, 2.4 mEq/l calcium, 32 mEq/l magnesium, 160 mEq/l chloride, 20 mEq/l bicarbonate, 1,000 U/l heparin, 50 mg/l procainamide; osmolality 300 mOsm, pH 7.8 at 4°C) infused into the aortic

root using a 14-gauge DLP aortic root cannula with vent (DLP, Inc., Grand Rapids, MI). A septal myocardial temperature of less than 10°C (Shelly, Inc., Irvine, CA) was produced when the heart was packed in crushed ice. A second dose of cardioplegia solution (300 ml) was infused 20 min later. The ligature was released around the LAD after this second dose of cardioplegia to mimic revascularization during human aortocoronary surgery. The aortic cross-clamp was then released after a further 20 min (total cross-clamp time of 40 min), and rewarming commenced. When the dogs' core temperatures were stable at 36–37°C (a period of approximately 20 min), the animals were weaned from CPB (mean total CPB time, 105 ± 9 min) without the use of inotropic drugs. A subsequent transmyocardial biopsy was performed at this time (defined as end-CPB). Each myocardial biopsy was placed immediately in liquid nitrogen and stored at −70°C until analysis. Blood for analysis of serum catecholamine concentrations was collected at each transmyocardial biopsy time point.

Data Processing and Calculations

The pressure signals were amplified by a low-noise DC preamplifier (Grass Instruments, Quincy, MA). All analog data signals were digitally converted (analog–digital converter, model 16AF; MetraByte Corp., Taunton, MA) and were recorded with a personal computer (model 386; Compaq Computer Corp., Houston, TX) at a sampling rate of 500 Hz over a period of 6 s. The end-diastolic segment length and end-systolic length were determined. Systolic shortening and postsystolic shortening, respectively, were calculated as follows[25]:

$$SS = [(EDL - LminS)/EDL] \cdot 100 \qquad (1)$$

$$PSS = [(LminS - LminD)/(EDL - LminD)] \cdot 100 \qquad (2)$$

where SS is systolic shortening, PSS is postsystolic shortening, EDL is end-diastolic segment length, LminS is minimum length during systole, and LminD is minimum length obtained during early diastole. For subsequent statistical analyses, negative values for postsystolic shortening were listed as zero.

Myocardial Tissue Samples and Analysis

Snap-frozen transmyocardial biopsy samples were weighed (300–600 mg) and then homogenized with a Polytron PT 3000 using a foam-reducing generator (Brinkman Instruments, Westbury, NY) at 20,000 rpm for 30 s in 1 ml lysis buffer (5 mM Tris, 2 mM EDTA) with protease inhibitors (10 mg/ml soybean trypsin inhibitor, 10 mg/ml benzamidine, 10 mg/ml leupeptin). After homogenization, 10 ml lysis buffer was added, and the homogenate was centrifuged at 19,000 rpm (36,000g) for 10 min at 4°C. The pellet was resuspended in assay buffer (75 mM Tris, 5 mM MgCl$_2$, 2 mM EDTA; pH 7.4 with

Table 1. Hemodynamic Data

	Baseline		Pre-CPB		End-CPB	
	Control	Esmolol	Control	Esmolol	Control	Esmolol
SBP	90 ± 9	97 ± 27	92 ± 17	92 ± 21	81 ± 41	101 ± 39
MBP	70 ± 14	75 ± 29	74 ± 21	69 ± 21	51 ± 27	78 ± 36
DBP	54 ± 14	62 ± 24	59 ± 19	56 ± 19	34 ± 20	62 ± 27
LVEDP	2.1 ± 2.6	2.3 ± 2.0	4 ± 2.1	5 ± 3.3	4.7 ± 1.7	5.7 ± 2.5
LVdP/dt	1,400 ± 280	1,400 ± 330	1,500 ± 560	1,600 ± 360	1,500 ± 270	1,400 ± 480
CO	3.0 ± 0.9	2.5 ± 1.0	3.1 ± 1.7	2.3 ± 0.6	1.8 ± 0.7	2.5 ± 0.7
HR	106 ± 18	115 ± 32	116 ± 16	101 ± 12	136 ± 20	117 ± 22

Data are mean ± SD.

SBP = systolic blood pressure (mmHg); MBP = mean blood pressure (mmHg); DBP = diastolic blood pressure (mmHg); LVEDP = left ventricular end-diastolic pressure (mmHg); LVdP/dt = left ventricular dP/dt maximum; CO = cardiac output (l/min); HR = heart rate.

protease inhibitors), 10 ml/mg tissue wet weight, and was then filtered through a 210-μm mesh filter (Spectramesh; VWR, Boston, MA) to remove any remaining tissue chunks. Protein concentrations were determined by the bicinchoninic acid method (Pierce, Rockford, IL), using bovine serum albumin as a standard.

Ligand Binding Assays

Ligand binding assays were performed in triplicate using 10 μg of total membrane protein. Assays were conducted using a final volume of 500 μl assay buffer with a saturating concentration (300 pM) of [^{125}I]-CYP (Dupont, Boston, MA). Propranolol, 1 μM (Sigma Chemical Co., St. Louis, MO), was used to determine nonspecific binding. The reaction was incubated for 2 h at room temperature (25°C). Bound tracer was separated from free by rapid vacuum filtration onto glass fiber (GF/C) filters (Whatmann International, Maidstone, United Kingdom). Filters were rinsed three times with 3 to 4 ml ice-cold 50 mM Tris, pH 7.4, using a Brandell cell harvester. Filters were counted in a gamma counter at 70% efficiency. We chose a one-point binding assay due to limitations in biopsy size available.

Adenylyl Cyclase Assays

Ventricular membrane adenylyl cyclase activities were assessed by the method of Salomon[26] as modified and described previously.[27,28] Twenty-milligram ventricular membranes were incubated in triplicate with various agents in a reaction mixture consisting of 75 mM Tris, 5 mM MgCl$_2$, 2 mM EDTA, 0.12 mM ATP, 0.06 mM GTP, 2.8 mM phosphoenolpyruvate, 50 mg/ml myokinase, 0.1 mM cAMP, 10 mg/ml pyruvate kinase, and 1 mCi α[^{32}P]ATP in a final volume of 50 μl normalized to 15 min at 37°C. Reactions were performed in the presence of water (basal), 100 mM isoproterenol (ISO-MAX), and 500 nM isoproterenol (ISO-EC$_{50}$). The ISO-MAX dose was chosen as a dose known to supramaximally stimulate adenylyl cyclase activity. The ISO-EC$_{50}$ dose was chosen as an isoproterenol dose known to provide submaximal stimulation of adenylyl cyclase. Although this dose is not the ED$_{50}$ dose for every dog, it is a reasonable

estimate of ED$_{50}$ from our other experiments and manuscripts. [^{32}P]cAMP was isolated by sequential chromatography over 1 ml Dowex and alumina columns. Individual column recovery was normalized on the basis of recovery of a known amount of [^{3}H]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 scintillation cocktail and were counted with a dual-channel liquid scintillation counter. This resulted in a linear accumulation of [^{32}P]cAMP with respect to time, protein, and temperature.

Statistical Analysis

Desensitization was defined *a priori* as a reduction in ISO-MAX– or ISO-EC$_{50}$–stimulated adenylyl cyclase activity at the end of CPB using mean percent change. Power calculations were performed using a two-tailed paired t test to test the null hypothesis using Solo Power Analysis (NCSS Statistical Software, Kaysville, UT), and Muller's software (UNC, Chapel Hill, NC). Data were tested for skewness, and a logarithmic transformation was utilized to normalize data distribution when required prior to inferential analysis. Data were then fitted to a generalized linear multivariate model, and the effect of esmolol on ISO-MAX and ISO-EC$_{50}$ was tested. Systolic shortening and postsystolic shortening were analyzed using a Wilcoxon two-sample rank sum test. Data are expressed as mean ± SD to two significant figures, with $P < 0.05$ defined as significant.

Results

Of the 29 dogs initially commencing the protocol, 2 did not complete the study. One dog died upon commencement of CPB (randomized to esmolol group, but prior to drug administration), and the other could not be weaned from CPB (control group). This resulted in 16 dogs in the control group and 11 in the esmolol group who completed the entire study. There was no difference between groups with respect to heart rate, LV end-diastolic pressure, LV dP/dt max, cardiac output, or

Table 2. Adenylyl Cyclase Assays, βAR Density, and LV Functional Data

Treatment Group	Measure	Preischemia	Pre-CPB	End-CPB	Mean % Change from Pre-CPB
Control	Iso-MAX	—	960 ± 37	710 ± 63*	−26 ± 6%
	Iso-EC$_{50}$	—	580 ± 120	400 ± 89*	−21 ± 4%
	Systolic shortening	26 ± 6.9	8.2 ± 8	12 ± 5.5*	+48 ± 12%
	Postsystolic shortening	0.23 ± 0.08	49 ± 37	17 ± 25	−65 ± 19%
	βAR density	—	52 ± 17	54 ± 15	+1.5 ± 12%
Esmolol	Iso-MAX	—	840 ± 71	1,200 ± 110*	+38 ± 10%
	Iso-EC$_{50}$	—	550 ± 25	680 ± 33*	+39 ± 8%
	Systolic shortening	31 ± 7.0	12 ± 7.04	22 ± 7.0*	+72 ± 23%
	Postsystolic shortening	1.3 ± 1.7	61 ± 9.9	5.5 ± 3.6	−91 ± 15%
	βAR density	—	49 ± 21	51 ± 16	+2.3 ± 31%

Data are mean ± SD.

blood pressure at each time point. Hemodynamic data are presented in table 1.

Plasma Catecholamines and Adenylyl Cyclase Assays

Plasma catecholamine concentrations increased significantly during CPB (esmolol group: 180 ± 151 to 1,300 ± 840 pg/ml epinephrine, $P = 0.021$; 150 ± 130 to 300 ± 75 pg/ml norepinephrine, $P = 0.034$, pre-CPB to end-CPB; control group: 230 ± 180 to 1,100 ± 1,000 pg/ml epinephrine, $P = 0.01$; 220 ± 140 to 390 ± 80 pg/ml norepinephrine, $P = 0.02$). There was no difference between groups with respect to catecholamine changes.

Maximal isoproterenol-stimulated adenylyl cyclase activity in control animals decreased approximately 26% during CPB (960 ± 37 to 710 ± 63 fmol cAMP · mg^{-1} protein · 15 min^{-1}, pre-CPB to end-CPB, $P = 0.003$, table 2 and fig. 2A), similar to previous studies in humans and dogs.[7,16,29] In contrast, animals receiving esmolol demonstrated a 38% *increase* in adenylyl cyclase activity upon isoproterenol stimulation (840 ± 71 to 1,200 ± 110 fmol cAMP · mg^{-1} protein · 15 min^{-1}, $P = 0.001$). In addition, stimulation of adenylyl cyclase with a submaximal isoproterenol dose demonstrates a 39% improvement in activity at the end of CPB with esmolol
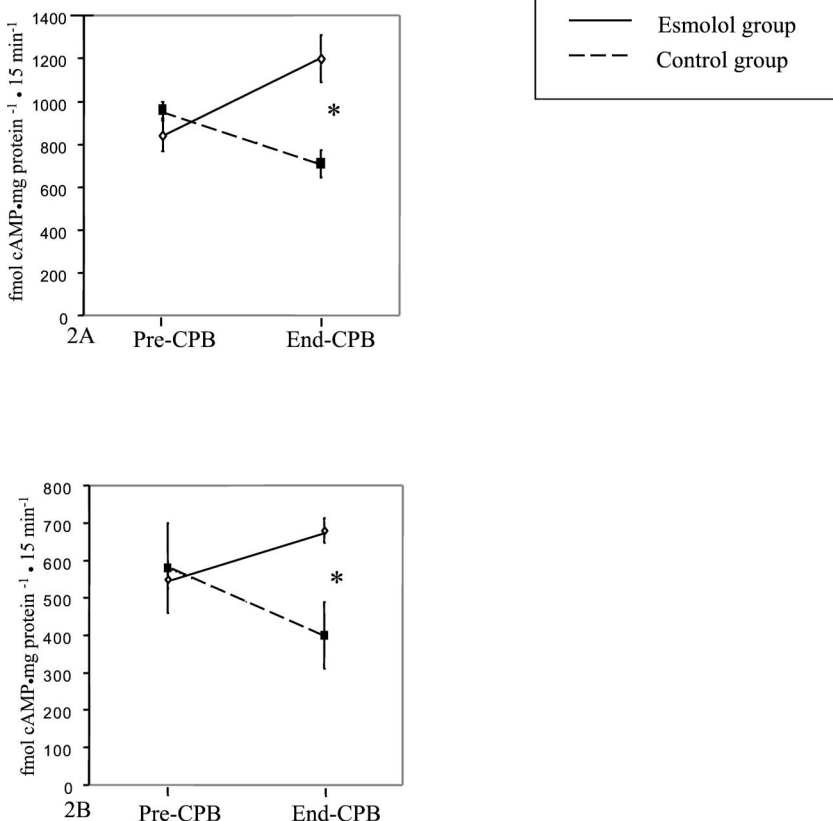
Fig. 2. Results for adenylyl cyclase assays for ISO-MAX (*A*) and ISO-EC50 (*B*) demonstrate improved left ventricular βAR transmembrane signaling, end-CPB compared to pre-CPB, in the esmolol group. Data are expressed as mean ± SD. *$P < 0.05$.
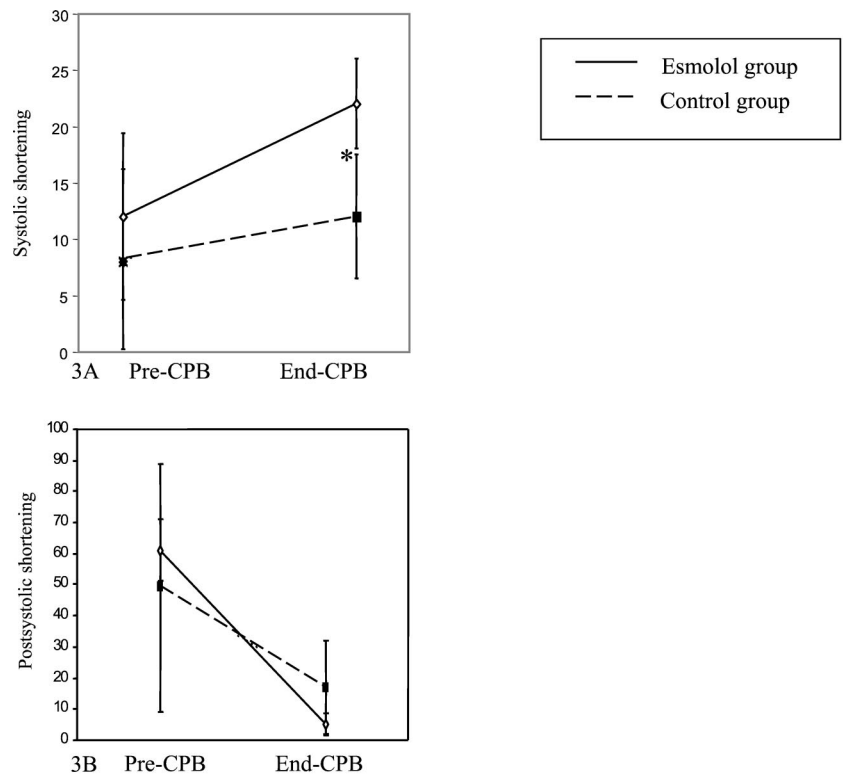
Fig. 3. Graph showing that (*A*) left ventricular regional systolic function (systolic shortening) improves and (*B*) left ventricular regional diastolic function (postsystolic shortening) returns to normal, in the esmolol compared with the placebo group, end-CPB *versus* pre-CPB. Data are expressed as mean ± SD. *P < 0.05.

(P = 0.0001, table 2 and fig. 2B), suggesting esmolol therapy resulted in a shift to the left of the isoproterenol dose–response curve. There was no difference in βAR density between groups during CPB (table 2).

Systolic Shortening

As expected, systolic shortening over the anterior left ventricle decreased in both groups after ischemia induced by the LAD ligature (table 1, P = 0.007). The decrease in LV function induced by LAD ischemia was similar in both groups (esmolol group: 39% of preischemic function, control group: 34% of preischemic function). LV systolic shortening improved in both groups at the end of CPB as expected from improved coronary blood flow to the myocardium following release of the LAD ligature. However, systolic shortening is substantially greater in the esmolol group compared to control group at the end of CPB (esmolol group: 72% of preischemic function, control group: 48% of preischemic function, P = 0.0008, fig. 3A and table 2). Multivariate analysis of the effect of esmolol on systolic shortening and isoproterenol stimulated adenylyl cyclase activity demonstrated a significant improvement in the esmolol group compared to the control group at the end of CPB (P = 0.0002).

Postsystolic Shortening

Postsystolic shortening was close to zero in both groups prior to ischemia as commonly seen in normal myocardium, and increased in both groups after regional

LV ischemia was induced (control group: 49 ± 37%, esmolol group: 61 ± 10%). At end-CPB, the esmolol group had returned to low values (5.5 ± 3.6%), whereas the control group still demonstrated significant postsystolic shortening (17 ± 25%, table 2 and fig. 3B). However, direct comparison of the change from pre-CPB to end-CPB between groups resulted in borderline significance (P = 0.055) in this measure.

Discussion

The present study documents that following acute myocardial ischemia and subsequent revascularization, intravenous administration of the short-acting βAR antagonist esmolol results in improved systolic shortening, a measure of LV function. Simultaneously, intravenous esmolol administration during CPB improves isoproterenol-stimulated AC activity without change in receptor density, therefore resulting in improved receptor-activated transmembrane signaling at the end of surgery. Furthermore, since postsystolic function returned close to zero (5.5%) in the esmolol group as compared to 17% for controls, we surmise esmolol may have an effect in improving LV diastolic function after CPB as well.

Currently, only two studies (both retrospective in design) have examined the effect of βAR antagonists administered following acute coronary syndromes in patients who subsequently undergo CABG surgery. Hekmat *et al.*[30] showed that cardioplegia using esmolol-enriched blood during CABG surgery resulted in fewer

inotropic requirements postoperatively. In addition, perioperative myocardial infarctions occurred less frequently in the esmolol group. Chen *et al.*[31] documented that βAR antagonist therapy was associated with a reduction in 1-yr mortality for elderly patients undergoing CABG or percutaneous transluminal coronary angioplasty after AMI. While suggestive of benefit since both studies utilized a retrospective chart review design, neither addressed the issue of potential mechanisms underlying βAR antagonist therapy.

We have chosen to use a model of CPB that includes myocardial ischemia with subsequent reperfusion. This model was specifically designed to more closely mimic the physiology that occurs in humans during CABG surgery. With this model, the effects of CPB alone or ischemia–reperfusion alone on the left ventricle cannot be separated. It is a complex model that mimics the various aspects of human CABG operations and thus has a number of uncontrolled variables. In fact, it is also impossible to separate the effects of esmolol on myocardial metabolism, on ischemia, or at the βAR–G protein–adenylyl cyclase moiety. Unfortunately, most animal models to date have focused on CPB alone. The criticism of those models is that no human undergoes CPB alone, and therefore, the model does not reflect CABG surgery. Thus, we have attempted to overcome these criticisms of CPB-alone studies with the use of LAD ligatures in our model. This makes the model more complex but inherently more aligned with the process of CABG surgery. In addition, we were limited by the small size of the biopsy sample; therefore, we performed one-point βAR binding, which is inherently less accurate than full-saturation binding. Also, the use of propranolol to assess nonspecific binding precludes the detection of "internalized" receptors. Therefore, there may have been some "internalization" of βARs that we were unable to detect using our methodology. We do not believe that these aspects of the research compromise the validity of the work, but they should be taken into account when interpreting the data.

In spite of the paucity of data available on the effectiveness, rationale, or mechanisms behind βAR antagonist administration after AMI–acute coronary syndromes in patients undergoing revascularization, several studies have examined mechanisms underlying the effect of ischemia on βAR signal transduction. The capacity of βAR agonists to stimulate adenylyl cyclase activity is enhanced during the first 15 min of myocardial ischemia due to acutely increased βAR density.[9,32] With sustained ischemia, however, isoproterenol-stimulated adenylyl cyclase activity decreases to below control values, although βAR density remains elevated.[9] Likewise, aortic cross-clamping with subsequent anoxia during CPB surgery induces a reversible functional impairment of βAR signaling in myocardium *in vivo*, and we have previously demonstrated acute reduction in LV myocardial

βAR responsiveness at CPB termination despite stable βAR density in both dogs and humans.[7,16,29] In these studies, isoproterenol-stimulated adenylyl cyclase activity (receptor level), sodium fluoride (Gs level), and manganese (adenylyl cyclase level) is dampened; therefore, impaired myocardial βAR responsiveness to agonists during CPB is due to heterologous desensitization since the dampened responsiveness (or desensitization) includes impairment of the nonreceptor components of the signal transduction cascade.[33]

A recent study by Ungerer *et al.*[10] provides a possible underlying mechanism for βAR dysfunction in the setting of acute myocardial ischemia. This study, utilizing isolated perfused rat hearts, demonstrates time-dependent (10–15 min) increased βARK activation corresponding to functional inactivation of the βAR system occurring within the 15th–30th min of isolated ischemia.[9] In the same time frame, other serine/threonine kinases, such as protein kinase C, have also been shown to be activated during myocardial ischemia.[34] Ungerer *et al.*[10] also found that norepinephrine, perfused through the heart, increases membrane βARK activity during normoxia, implying that receptor activation itself triggers translocation of the enzyme. Furthermore, in perfusion of ischemic hearts treated with desipramine prior to ischemia (desipramine suppresses ischemic norepinephrine release by almost 75%),[35] βARK activity was suppressed compared to ischemic untreated hearts. Thus, agonist occupation of cardiac βARs during ischemia leads to induction and intracellular translocation of βARK to the cell membrane. In adult humans, βAR dysfunction after cardiac surgery occurs in the clinical setting of significant acute myocardial ischemia, resulting in increases (2- to 20-fold) in catecholamine concentrations.[7,16–18,29] Taken together, these data indicate that increased myocardial catecholamine concentrations may, at least in part, be the stimulus for myocardial hyporesponsiveness seen during cardiac surgery. In support of this, blockade of βARs with low-dose βAR antagonist therapy has been shown to improve myocardial function in congestive heart failure,[36–38] a clinical setting in which attenuation of chronic βAR desensitization has been postulated as a possible mechanism.

We demonstrate that intravenous esmolol administration during coronary artery revascularization after acute myocardial ischemia results in improved LV myocardial βAR function immediately after CPB. We chose esmolol for our study due to its short half-life (9 min) in blood, to minimize prolonged post-CPB residual negative inotropic effects.[39–41] Esmolol infusion during CPB in adult humans undergoing elective aortocoronary surgery without prior AMI or acute coronary syndromes results in improved stroke volume index and LV stroke work index during the first 30 min after CPB as measured by transesophageal echocardiography.[39,40] We now provide evidence that improved myocardial function coin-

cides with restoration of agonist-induced βAR transmembrane signaling, providing a possible mechanism for this observation. These data therefore provide the first prospective evidence for the rational use of βAR antagonists in the setting of AMI–acute coronary syndromes and subsequent revascularization.

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