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Uncoupling of Myocardial β -Adrenergic Receptor Signaling During Coronary Artery Bypass Grafting: The Role of GRK2

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Abstract

Background—Cardiopulmonary bypass (CPB) and cardioplegic arrest during cardiac surgery leads to desensitization of myocardial β -adrenergic receptors (β -ARs). Impaired signaling through this pathway can have a detrimental effect on ventricular function and increased need for inotropic support. The mechanism of myocardial β -AR desensitization during cardiac surgery has not been defined. This study investigates the role of G protein-coupled receptor kinase-2 (GRK2), a serine-threonine kinase which phosphorylates and desensitizes agonist-occupied β -ARs, as a primary mechanism of β -AR uncoupling during coronary artery bypass grafting (CABG) with CPB and cardioplegic arrest.

Methods—Forty-eight patients undergoing elective CABG were enrolled in this study. Myocardial β -AR signaling was assessed by measuring total β -AR density and adenylyl cyclase activity in right atrial biopsies obtained prior to CPB and just before weaning from CPB. Myocardial GRK2 expression and activity were also measured pre-CPB and just prior to weaning from bypass.

Results—Myocardial β -AR signaling was significantly impaired following cardiopulmonary bypass and cardioplegic arrest during CABG. Cardiac GRK2 expression was not altered; however, there was a 2-fold increase in GRK2 activity during CABG. There was an even greater elevation in cardiac GRK2 activity in patients with severely depressed ventricular function.

Conclusions—Increased myocardial GRK2 activity appears to be the primary mechanism of impaired β -AR signaling during CABG with CPB and cardioplegic arrest. This may contribute to the greater need for inotropic support in patients with severe ventricular dysfunction. Strategies to inhibit activation of GRK2 during CABG may decrease morbidity in this patient population.

Keywords

Coronary artery bypass grafting; Biochemistry; Cardiopulmonary bypass; Cardioplegia; Molecular biology

Introduction

The use of cardiopulmonary bypass (CPB) and cardioplegic arrest during cardiac surgery leads to desensitization of myocardial β -adrenergic receptors (β -ARs) and impaired signaling through this pathway which is critical in the regulation of cardiac function [1,2,3]. Uncoupling of this signaling system may play a significant role in myocardial dysfunction which can occur following CPB and cardioplegic arrest leading to utilization of inotropic therapy despite a technically successful operation. Although β -AR desensitization has been demonstrated in animal models of cardiac surgery with CPB [2] and in human myocardium following coronary artery bypass grafting (CABG) [3], a specific mechanism has not been described. CPB leads to a significant increase in circulating catecholamine levels [4,5,6,7] and cardioplegic arrest is associated with high local myocardial catecholamine release [8,9,10]. A primary mechanism of β -AR desensitization following prolonged stimulation is phosphorylation of agonist-occupied receptors by G protein-coupled receptor kinase-2 (GRK2), a member of the family of serine-threonine kinases known as G protein-coupled receptor kinases [11]. GRK2 has been shown to be important in the modulation of cardiac function in vivo [12,13] and enhanced activity leads to uncoupling of β -ARs and impaired ventricular systolic and diastolic function. In addition, cardiac GRK2 activity is elevated 2-fold in chronic heart failure and β -ARs are desensitized and downregulated as a result of elevated catecholamine levels [14]. We hypothesize that the primary mechanism of desensitization of myocardial β -ARs during CABG with CPB and cardioplegic arrest is increased GRK2 activity. Impaired β -AR signaling and ventricular function following cardiac surgery may be of even greater importance in patients with significantly depressed left ventricular function preoperatively.

Materials and Methods

Approval for this study was granted by the Institutional Review Board of the University of Cincinnati and individual patient consent for the study was obtained.

Study design

All patients consented for this study underwent coronary artery bypass grafting utilizing cardiopulmonary bypass and hypothermic cardioplegic arrest. Clinical factors included in the study were: age, sex, left ventricular ejection fraction, cardiopulmonary bypass time, and aortic cross-clamp time. Consent was obtained from patients undergoing elective or semi-elective operations. At the time of right atrial (RA) cannulation for CPB, a RA biopsy was obtained and snap frozen in liquid nitrogen. A RA biopsy was taken again just before weaning from CPB and prior to initiating any inotropic support. This second RA sample was taken from the lateral wall. No right atrial activity was present throughout the cardioplegic arrest period.

Anesthesia protocol

General anesthesia was induced with fentanyl (1 to 3 μ g/kg), versed (0.1 mg/kg), and vecuronium (0.1 mg/kg). During the maintenance phase of the anesthetic, additional fentanyl is titrated up to a total of 20 μ g/kg and an inhalational agent (either isoflurane, sevoflurane, or desflurane) is administered in the range of 0.4 to 1 MAC. Bispectral Index (BIS) monitoring is utilized to monitor for intraoperative awareness. During CPB, the perfusionists administer sevoflurane to the pump blood at 0.5 MAC. A propofol infusion is started during placement of sternal wires and the inhalational agent is turned off by the completion of skin closure.

Conduct of operation

All coronary bypass operations were done via a median sternotomy and the left internal mammary artery was harvested as a pedicled graft. Radial artery and greater saphenous vein grafts were harvested using an endoscopic technique. Standard aortic and two-stage venous

cannulation were performed and antegrade and retrograde cardioplegia were utilized in all cases. Following initiation of CPB, systemic temperature was allowed to drift to 32°C and flow rates were maintained at 2.4 L/minute/M². Cardioplegia was standard St. Thomas solution with a 4:1 blood dilution at 4°C. In general, 1 L of cold blood cardioplegia was given antegrade followed by 500 mL retrograde after cross clamping the aorta to achieve diastolic arrest. Maintenance doses of 500 mL of both antegrade and retrograde cardioplegia were delivered between each distal anastomosis. A warm dose of cardioplegia was delivered antegrade after completion of proximal anastomoses, followed by controlled reperfusion with warm blood until sinus rhythm was regained. The cross clamp was then removed and after approximately 20 minutes, the patient was weaned from CPB. Low dose epinephrine (0.05 µg/kg/min) was initiated before weaning from CPB for patients with a left ventricular ejection fraction of less than 25% and milrinone (0.25 µg/kg/min) was added if additional inotropy was required.

Radioligand binding

Total β-AR density (B_{max}) was determined by incubating 25 µg of cardiac sarcolemmal membranes with a saturating concentration of [¹²⁵I] cyanopindolol and 20 µmol/L alprenolol to define nonspecific binding. Sarcolemmal membrane samples from all groups were done in triplicate with 80 pmol/L [¹²⁵I] cyanopindolol and 10⁻⁴ mol/L isoproterenol in 250 µL of binding buffer (50 mmol/L HEPES [pH 7.3], 5 mmol/L MgCl₂, and 0.1 mmol/L ascorbic acid). The reactions were performed at 37° C for 1 hour and then filtered over GF/C glass fiber filters (Whatman) that were washed twice and counted in a gamma counter. Data were analyzed by nonlinear least-square curve fit (GraphPad Prism).

Adenylyl cyclase activity

Myocardial sarcolemmal membranes (20 µg of protein) were incubated for 15 minutes at 37° C with [α -³²P]ATP under basal conditions, with 10⁻⁴ mol/L isoproterenol, or 10 mmol/L NaF. cAMP production was quantified by standard methods described previously [15].

Protein immunoblotting

Expression of GRK2 in myocardial sarcolemmal membranes was performed on tissue extracts. Tissue was homogenized in ice-cold lysis buffer (25 mmol/L Tris-HCl [pH 7.5], 5 mmol/L EDTA, 5mmol/L EGTA, 10 µg/mL leupeptin, 20 µg/mL aprotinin, and 1 mmol/L phenylmethylsulfonyl fluoride). Nuclei and tissue were separated by centrifugation at 800g for 20 minutes. The crude supernatant was then centrifuged at 20,000g for 20 minutes. Sedimented proteins (membrane fraction) were resuspended in 50 mmol/L HEPES (pH 7.3) and 5 mmol/L MgCl₂. The immunodetection of myocardial levels of GRK2 using a polyclonal antibody (1:5000 dilution; Santa Cruz Biotechnology, Inc, CA) was performed on an equal amount of protein from membrane extracts (70 µg) electrophoresed through 10% Tris/glycine gels and transferred to nitrocellulose. Membranes were blocked in 5% nonfat dried milk in 0.1% Tween 20 in PBS (PBS-T) for 1 hour at room temperature. The protein was visualized using a horseradish peroxidase-linked secondary antibody and ECL detection (Amersham).

GRK activity by rhodopsin phosphorylation assay

The membrane fractions of the myocardial extracts were used to determine GRK activity. Extracts (120 µg of protein) were incubated with rhodopsin-enriched rod outer-segment membranes in reaction buffer containing the following (in mmol/L): MgCl₂ 10, Tris-HCl 20, EDTA 2, EGTA 5, and ATP 0.1 (containing [γ -³²P]ATP). After incubating in white light for 15 minutes at room temperature, reactions were quenched with ice-cold lysis buffer and centrifuged for 15 minutes at 13,000g. Sedimented proteins were resuspended in 25µl of protein-gel-loading dye and treated with 12% SDS-PAGE. Phosphorylated rhodopsin was

visualized by autoradiography of dried polyacrylamide gels and quantified using a Molecular Dynamics PhosphorImager.

Statistical analysis

Repeated-measures analysis of variance (ANOVA) was used to analyze data between treatment groups. Analyses were conducted using Statview 4.01 software (Abacus Concepts Inc, Berkeley, CA). Experimental groups were compared using Student's *t* test or 1-way ANOVA, as appropriate. The Bonferroni test was applied to all significant ANOVA results using SigmaStat software. *P* values of less than 0.05 were considered statistically significant. All results are expressed as mean±SEM.

Results

Patient population

The clinical data for the study group are described in Table 1. All patients underwent primary coronary artery bypass grafting by two surgeons at the University of Cincinnati Medical Center. These were elective or semi-elective procedures and all patients had left internal mammary artery grafts to the left anterior descending coronary artery and a total of 2–5 total grafts per patient. Of the 48 total patients included in this study, 12 of them had a left ventricular ejection fraction (EF) of less than 25%. All patients were weaned from CPB without event and those with a pre-operative EF of less than 25% were started on low-dose epinephrine prior to weaning from bypass. The second RA biopsy was taken prior to the initiation of inotropic support. No patient required placement of an intra-aortic balloon pump or ventricular assist device. In all cases, cold blood cardioplegia (4:1 dilution) was delivered both antegrade and retrograde approximately every 20 minutes throughout the operation. A warm dose of cardioplegia was delivered prior to cross-clamp removal.

Myocardial β -AR signaling

We studied receptor-effector coupling by measuring myocardial sarcolemmal membrane adenylyl cyclase activity before CPB and just prior to weaning from CPB (Figure 1). Under basal conditions, there was a significant decline in cyclase activity following CPB and CP arrest [58.8 ± 14.1 versus 35.8 ± 10.0 pmol cAMP/mg/minute, $P < 0.05$]. Similarly, isoproterenol-stimulated adenylyl cyclase activity was impaired following CPB and CP arrest [124.0 ± 31.3 versus 75.6 ± 27.7 pmol cAMP/mg/minute, $P < 0.05$]. These data demonstrate impaired signaling through myocardial β -ARs which play a critical role in regulating cardiac function. Sodium fluoride-stimulated cyclase activity was not different between groups (Figure 1) indicating that the impaired basal and isoproterenol-stimulated signaling is due to altered β -AR-cyclase coupling. Sodium fluoride directly stimulates the G protein subunit, $G_{\alpha s}$, which activates adenylyl cyclase indicating that the impaired signaling is occurring at the level of the receptor.

Myocardial β -AR Density and G protein expression

We then measured total myocardial sarcolemmal membrane β -AR density ($B_{\max}$) by radioligand binding to determine a potential mechanism of impaired signaling through these receptors (Figure 2). There was no difference in cardiac β -AR density between the pre- and post-CPB groups [78.3 ± 6.5 versus 73.9 ± 5.4 fmol/mg membrane protein, $P > 0.05$]. The decrease in myocardial basal and β -agonist stimulated adenylyl cyclase activity following CPB was not due to a decline in total β -AR density. We also studied the expression of the G proteins to which β -ARs are coupled. $G_{\alpha s}$ stimulates cyclase activity following stimulation of both β_1 - and β_2 -ARs, and $G_{\alpha i}$ inhibits cyclase activity and is coupled only to β_2 -ARs. There was no change in expression of $G_{\alpha s}$ or $G_{\alpha i}$ following CPB and cardioplegic arrest (data not shown). Thus, alteration in G protein expression was not a mechanism of impaired β -AR signaling.

Myocardial GRK Expression and Activity

We then studied myocardial GRK2 expression by protein immunoblotting and found no difference between the pre-CPB and post-CPB groups (Figure 3). Interestingly, there was an approximately 2-fold increase in cardiac GRK2 activity in the post-CPB group compared to pre-CPB [167.9 ± 31.0 densitometry units (DU) versus 88.4 ± 16.4 DU, $P < 0.05$] (Figure 4). These data indicate that the impaired β -AR signaling present following CABG in these patients was due to increased activity of GRK2, resulting in β -AR desensitization. When studied separately, the group of patients with an LV ejection fraction (EF) of $< 25\%$ ($N = 12$) had elevated myocardial GRK2 activity pre-CPB compared to those with an EF of $> 25\%$ [143.8 ± 26.7 versus 70.8 ± 15.2 DU, $P < 0.05$]. In addition, the increase in cardiac GRK2 activity following CPB was significantly greater in the patients with severely reduced LV function [3.4-fold versus 2.1-fold, $P < 0.05$] (Figure 5). This may be an important mechanism that explains the greater need for inotropic and mechanical circulatory support following CABG in patients with severe LV dysfunction.

Discussion

It is well known that circulating catecholamine levels are increased during CPB [4,5] and that myocardial catecholamine levels are also upregulated during cardioplegic arrest [8,9]. Previous studies have demonstrated that cardiac β -AR signaling is impaired after cardiopulmonary bypass with cardioplegic arrest in children with acyanotic heart disease who underwent cardiac surgery [1] and in a canine model of CPB [2]. These important studies did not, however, define a specific mechanism for β -AR uncoupling in this setting. We have performed a comprehensive analysis of the myocardial β -AR signaling system in 48 adults undergoing CABG with CPB and cardioplegic arrest. We also found significant uncoupling of β -ARs from adenylyl cyclase under basal conditions and following β -agonist stimulation in this patient population following CPB and arrest. There was no alteration in the adenylyl cyclase moiety as direct stimulation of $G_{\alpha s}$ led to normal cyclase activity in the post-CPB group. Total β -AR density ($B_{\max}$) was also unchanged following CPB. There was a significant upregulation of myocardial GRK2 activity post-CPB which appears to be the primary mechanism of impaired β -AR signaling following CABG in this study group.

GRK2 is a member of the family of serine-threonine kinases known as G protein-coupled receptor kinases which are targeted to the sarcolemmal membrane via binding to the $G_{\beta\gamma}$ subunits of activated G proteins [16]. Following prolonged stimulation, GRKs phosphorylate agonist-occupied receptors leading to uncoupling of these receptors from their downstream effectors, a process known as homologous desensitization [17]. Furthermore, binding of arrestin proteins to phosphorylated receptors sterically interdicts further signaling and leads to receptor internalization, or downregulation [18]. GRK2 desensitizes agonist-occupied β -ARs leading to a decline in adenylyl cyclase activity, a decrease in intracellular cAMP, and subsequently a decrease in cardiac myocyte contractility and relaxation [19]. GRK2 is known to be a critical regulator of cardiac function in vivo as increased cardiac-specific expression of this kinase in transgenic mice led to blunted β -agonist stimulated ventricular function and inhibition of GRK2 in the heart led to enhanced basal and β -agonist stimulated function as a result of inhibiting β -AR desensitization [12,13]. In animal studies, inhibition of GRK2 has led to improved myocardial function after ischemic injury [20] and following cold preservation in a rat heterotopic transplant model [21].

Myocardial GRK2 activity is known to be elevated in patients with chronic heart failure (CHF) by approximately 2-3-fold compared to normal controls leading to impaired signaling through β -ARs and blunted inotropic reserve [14]. This is thought to be an important mechanism in the pathogenesis of CHF resulting from an increase in circulating catecholamines. Although current myocardial protection strategies during CABG provide excellent outcomes, patients

with severe LV dysfunction are at greater risk of post-operative morbidity and mortality as a result of low cardiac output despite revascularization. There is clearly a greater need for inotropic support in patients with poor LV function and this group of patients will represent a greater proportion of those requiring cardiac surgery in the future as we continue to operate on older and more complicated patients with advanced cardiovascular disease. Although gene therapy approaches have been successful in animal models to inhibit myocardial GRK2 activity and improve cardiac function [20,21,22,23], there is currently no direct pharmacological inhibitor of GRK2. This is an area of ongoing investigation and novel strategies to inhibit GRK2 activity in the heart may be beneficial in improving outcomes for patients with severe ventricular dysfunction undergoing cardiac surgery in the future.

There are some important limitations of this study. Although we have defined a specific mechanism for myocardial β -AR desensitization during these CABG operations, the true impact on ventricular function and post-operative outcomes is not addressed. This is difficult to study in a clinical setting as patients with severe LV dysfunction are started on inotropic therapy prior to weaning from CPB rather than attempting to wean and then initiating inotropic support if necessary. We do not routinely utilize inotropes for patients with an LV EF of greater than 40% unless there is a prolonged period of CPB and cardioplegic arrest. Therefore, this degree of β -AR desensitization in this group of patients with preserved ventricular function may not have much clinical sequelae. Given the significant increase in mortality with increasing inotrope use in the post-cardiotomy setting [24], impaired β -AR signaling is likely more significant in patients with heart failure and dysfunctional myocardial β -AR signaling at baseline. These patients are also more refractory to β -agonist stimulation as a result of receptor uncoupling. Animal studies will be required to better define the impact of β -AR desensitization by GRK2 on ventricular function in the setting of CPB and cardioplegic arrest, particularly with significant LV dysfunction pre-operatively. We believe that the increase in GRK2 activity and β -AR uncoupling in the right atrium during CABG is also present in the right and left ventricles although this was not able to be studied due to technical limitations associated with obtaining LV biopsies. In previous studies characterizing myocardial β -AR signaling in the failing human heart, the upregulation in GRK2 expression and activity and impaired β -AR signaling were present in all cardiac chambers [14]. Since there is an elevation in both circulating and myocardial catecholamine levels with CPB and cardioplegic arrest, we believe this has a global effect on this signaling pathway in the heart.

This study provides a mechanism for altered cardiac β -AR signaling during CABG with CPB and cardioplegic arrest, specifically, β -AR uncoupling by GRK2. Additional studies to define the clinical impact and to inhibit activation of GRK2 in this clinical setting need to be performed. It has recently been shown that there is a high degree of correlation between myocardial (right atrial) and peripheral lymphocyte (PL) GRK2 activity in patients with chronic heart failure [25]. Greater right atrial and PL GRK2 activity correlated with worse LV function and greater NYHA heart failure class. It may, therefore, be possible to prospectively identify patients at higher risk for impaired myocardial β -AR signaling and myocardial dysfunction during cardiac surgery prior to operation by measuring PL GRK2 activity. Inhibition of cardiac GRK2 activity may represent a novel strategy to improve outcomes in high risk patients undergoing cardiac surgery.

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Figure 1.

Basal and β -agonist-stimulated myocardial sarcolemmal membrane adenylyl cyclase activity pre- and post-cardiopulmonary bypass. * $P < 0.05$ between groups.

□ Pre-CPB ■ Post-CPB (n=48 in each group). ISO=Isoproterenol; NaF=Sodium Fluoride

Figure 2. Myocardial sarcolemmal membrane β -adrenergic receptor density

□ Pre-CPB ■ Post-CPB. No statistical difference between groups (n=48 in each group performed in triplicate, $P>0.05$).

Figure 3. Myocardial GRK2 expression before and after cardiopulmonary bypass

□ Pre-CPB ■ Post-CPB. No statistical difference between groups ($P>0.05$). + = positive control for GRK2.

Figure 4. Myocardial GRK2 activity before and after cardiopulmonary bypass

□ Pre-CPB ■ Post-CPB * $P < 0.05$ versus Pre-CPB. (n=48 in each group). Rho=rhodopsin.

Figure 5. Post-CPB GRK2 activity relative to pre-CPB

□ LV EF > 25% (n=36) ■ LV EF < 25% (n=12) * $P < 0.05$

Table 1
Clinical Data

Mean Age: 66.3 ± 8.2 years
Sex: 30 male 18 female
LV Ejection Fraction: 42 ± 14 (12 patients EF < 25%)
Pre-op medications (N): aspirin (48), β -blocker (48), ACE Inh (34), statin (29)
Operation: CABG X 2-5 all with LIMA to LAD graft
CPB time: 85 ± 16 minutes
Aortic clamp time: 68 ± 21 minutes
All data are mean $\pm$ SEM. N=48 total patients.