Uncoupling of Human Cardiac $\beta$-Adrenoceptors During Cardiopulmonary Bypass With Cardioplegic Cardiac Arrest

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Background. It is well known that during cardiopulmonary bypass (CPB) with cardioplegic cardiac arrest, catecholamines are vigorously increased. We therefore investigated whether this might cause desensitization of human cardiac $\beta$-adrenoceptors.

Methods and Results. We assessed in 12 children with acyanotic congenital heart disease who underwent open-heart surgery right atrial $\beta$-adrenoceptor number and subtype distribution [by (−)-[125I]iodocyanopindolol binding] and adenylate cyclase activation [by the $\beta$-adrenoceptor agonist isoprenaline (100 $\mu$M) and by the non−receptor-mediated activators 10 $\mu$M GTP, 10 mM NaF, 100 $\mu$M forskolin, and 10 mM Mn$^{2+}$] before and after CPB with cardiac arrest by means of St. Thomas' cardioplegic solution. CPB affected neither $\beta$-adrenoceptor number or subtype distribution nor GTP, NaF, forskolin, or Mn$^{2+}$-induced activation of adenylate cyclase. In contrast, activation of adenylate cyclase by 100 $\mu$M isoprenaline was significantly ($p=0.0249$) lower after CPB than before CPB.

Conclusions. CPB with cardioplegic cardiac arrest decreases $\beta$-adrenoceptor−mediated adenylate cyclase activation in a manner compatible with an uncoupling of $\beta$-adrenoceptors from the $G_{\text{i}}$−protein−adenylate cyclase complex. Such a $\beta$-adrenoceptor desensitization may be the reason why after CPB many patients need inotropic support but do not respond sufficiently to catecholamines. (Circulation 1993;87:422−426)

KEY WORDS • $\beta$-adrenoceptors, human cardiac • desensitization • cardiopulmonary bypass

Cardiopulmonary bypass (CPB) is a process routinely used during cardiac surgery. During CPB, after aortic cross-clamping, the myocardium is cooled, arrested by means of cardioplegic solution, and deprived of its native blood. It has repeatedly been reported that endogenous catecholamines are vigorously increased during CPB.1−4 In addition, it has been demonstrated that norepinephrine is released from anoxic, isolated hearts5 and that cooling maintains elevated catecholamines in ischemic myocardium.6 Accordingly, on termination of CPB, myocardial tissue may have been exposed locally to increased catecholamine levels, and rewarmed un−cross-clamped heart is perfused by blood containing markedly elevated levels of catecholamines. Since continuous exposure of $\beta$-adrenoceptors to $\beta$-adrenoceptor agonists (including elevation of endogenous catecholamines) causes desensitization and, finally, downregulation of $\beta$-adrenoceptors,7−9 it may well be that the increase in catecholamines during CPB with cardioplegic cardiac arrest causes desensitization of cardiac $\beta$-adrenoceptors. Two recent studies in patients undergoing cardiac surgery have shown that in circulating lymphocytes, $\beta_{2}$-adrenoceptors were desensitized after CPB.10,11 However, it is not known whether human cardiac $\beta$-adrenoceptors (which are mainly of the $\beta_{1}$-subtype; for a recent review, see Reference 12) are affected by CPB, and alterations of $\beta_{2}$-adrenoceptors in circulating lymphocytes are certainly not a good model to predict $\beta$-adrenoceptor changes in the human heart.13 Therefore, in the present study, we determined in 12 children undergoing open-heart surgery the effects of CPB with cardioplegic cardiac arrest on right atrial $\beta$-adrenoceptor density, $\beta$-adrenoceptor subtype distribution, and functional responsiveness (assessed by right atrial adenylate cyclase activation by the $\beta$-adrenoceptor agonist isoprenaline and various non−receptor−mediated activators).

Methods

Right atrial appendages were obtained from 12 children (six females and six males; mean age, 4.6±1.3 years; age range, 0.75−17 years) with acyanotic congenital heart disease who underwent open-heart surgery because of ventricular septal defect ($n=5$), atrioventricular septal defect ($n=2$), atrial septal defect ($n=1$), tetralogy of Fallot ($n=3$), or aortic stenosis ($n=1$). Their parents had given informed written consent. None of the children suffered from acute heart failure or had been treated with sympathomimetic (i.e., catecholamines) or parasympathomimetic drugs for at least 3
weeks before surgery. Some children were treated with spironolactone (n=3), furosemide (n=2), and digitalis glycosides (n=3), either alone or in combination.

Anesthesiological premedication usually consisted of 50-100 μg/kg flunitrazepam p.o. administered in the evening before surgery or in the morning of surgery. The operation was carried out under neuroleptic anesthesia with boluses of flunitrazepam (50 μg/kg) and fentanyl (10-25 μg/kg). Pancuronium (100 μg/kg) was used as a muscle relaxant. Controlled ventilation was performed with an inspired oxygen fraction of 50-100%. In some children, isoflurane was added up to 1.0% (vol/vol). Right atrial appendages were removed immediately after installation of the CPB before the aorta was cross-clamped (“before”). The heart was arrested by a dose (30 ml/kg) of cold (4°C) St. Thomas’ cardioplegic solution. Moderate continuous-perfusion hypothermia was maintained at a mean temperature of 28°C. The flow rate of the nonpulsatile CPB was set at 2.4 l/min/m² and maintained arterial blood pressure was held constant at 35-55 mm Hg (depending on the age of the children). PaO₂ was maintained at 30-40 mm Hg; PaCO₂, 100-200 mm Hg; pH, 7.3-7.5. In infants and children with a body weight of <15 kg, blood was added to the pediatric prime solution. A second right atrial specimen was obtained after release of the aortic cross-clamp during reperfusion of the heart and when warming was begun (“after”). The mean “before”-“after” time interval was 62.4±6.9 minutes (range, 21-95 minutes). Immediately after excision, specimens were divided into two parts (one for radioligand-binding studies and one for adenylate cyclase assay) and quickly frozen in liquid nitrogen.

**Radioligand-Binding Studies**

Tissues (mean wet weight, 25.5±3.6 mg; range, 4.3-65.3 mg) were minced with scissors and homogenized in 10 vol of ice-cold 1 mM HCO₃ with an Ultra Turrax (Janke & Kunkel, Staufen, Germany) for 10 seconds at full speed and twice for 20 seconds at half-maximal speed at 1-minute intervals. The homogenate was diluted to 20 ml with 1 mM HCO₃, passed through four layers of cheesecloth, and centrifuged at 50,000g for 30 minutes. For β-adrenoceptor determination, the final pellets were resuspended in 10 mM Tris-HCl with 154 mM NaCl buffer (pH 7.4) containing 0.55 mM ascorbic acid at a protein concentration of 0.1-0.2 mg/ml. Protein content was determined by the method of Bradford using bovine immunoglobulin G as a standard.

The density of β-adrenoceptors in atrial membranes was determined by (-)[35S]iodocyanopindolol (ICYP) binding at six concentrations of ICYP ranging from 5 to 200 pM as recently described. Nonspecific binding of ICYP was defined as binding to membranes, which was not displaced by a high concentration of the nonselective β-adrenoceptor antagonist (±)-CGP 12177 (1 μM). Specific binding of ICYP was defined as total binding minus nonspecific binding; it usually was 70-80% at 50 pM ICYP.

To determine the relative amount of β₁- and β₂-adrenoceptors, membranes were incubated with ICYP (100 pM) in the presence or absence of eight concentrations (ranging from 10⁻¹⁰ to 10⁻³ M) of the highly selective β₁-adrenoceptor antagonist CGP 20712A, and specific binding was determined as described above. CGP 20712 A competition curves were analyzed by the iterative curve-fitting program INPLOT (GraphPad software, San Diego, Calif.). Statistical analysis was performed using the F ratio test to measure the goodness of fit of the competition curves for one or two sites.

**Adenylate Cyclase Determination**

Adenylate cyclase activity was assessed as described by Salomon et al., with minor modifications as detailed elsewhere. Three different incubation conditions were used. For isoprenaline activation, membranes (30-40 μg protein) were incubated for 10 minutes at 37°C in a final volume of 100 μl containing 40 mM HEPES buffer (pH 7.4), 5 mM MgCl₂, 1 mM EDTA, 10 μM GTP, 500 μM [α-32P]ATP, 100 μM cyclic AMP (cAMP), and an ATP-regenerating system (5 mM phosphocreatine and 50 units/ml creatine phosphokinase; buffer A). For GTP, NaF, and forskolin activation, membranes were incubated in buffer A without GTP; for Mn⁺ activation, membranes were incubated in buffer A without GTP and Mg++. The reaction was stopped by adding 0.8 ml of 50 mM Tris-HCl buffer (pH 7.4) containing 40 mM ATP and 1.4 mM cAMP. [3H]cAMP (5,000-10,000 cpm) was then added to monitor the recovery of [3H]cAMP. Statistical Analysis

We included results in data evaluation only from children from whom tissue was sufficient to perform measurements simultaneously in “before” and “after” right atria. The experimental data given in the text and figures are expressed as mean±SEM of 12 (β-adrenoceptor density and subtype distribution) and 10 (adenylate cyclase) experiments. The equilibrium dissociation constants (Kᵦ) and the maximal number of binding sites (Bₘₐₓ) for ICYP were calculated from plots according to Scatchard. The significance of differences was estimated by nonpaired Student’s t test. A value of p<0.05 was considered significant.

**Study Drugs**

CGP 20712A (1-[2-(3-carbamoyl-4-hydroxy)phenoxy]-ethylamino)3-[4-((1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy)2-propanol methyl sulfate) and (±)-CGP 12177 hydrochloride (4-((3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-ony) were gifts of CIBA-GEIGY (Basel, Switzerland). (-)-[35S]Iodocyanopindolol (ICYP; specific activity, 2,200 Ci/mmol), [α-32P]ATP (specific activity, 30 Ci/mmol), and [3H]cAMP (specific activity, 44.5 Ci/mmol) were obtained from New England Nuclear ( Dreieich, Germany). Isoprenaline bitartrate and forskolin were obtained from Sigma Chemical (Deisenhofen, Germany). All other chemicals were of the highest purity grade commercially available.

**Results**

**β-Adrenoceptor Density**

The mean number of “before” right atrial β-adrenoceptors amounted to 79.4±8.8 fmol ICYP specifically bound/mg protein (n=12), and the mean β₁; β₂-adrenoceptor ratio was 67.4±3.5/32.6±3.5% (n=12). CPB with cardioplegic arrest did not significantly affect β-adrenoceptor density (after: 73.6±10 fmol/mg protein) or β₁; β₂-adrenoceptor ratio (70.3±3.1/29.9±3.1%) (Fig-
FIGURE 1. Bar graphs of the effects of cardiopulmonary bypass (CPB) with cardioplegic cardiac arrest on \( \beta \)-adrenoceptor density and \( \beta_1; \beta_2 \)-adrenoceptor ratio in right atria from children undergoing open-heart surgery. For details, see “Methods.” Left ordinate: Right atrial \( \beta \)-adrenoceptor density in fmol (\( \sim \sim \)23I]iodocyanopindolol (ICYP) specifically bound/mg protein. Right ordinate: Right atrial \( \beta_1; \beta_2 \)-adrenoceptor ratio in percent. Values are mean±SEM of 12 experiments.

ure 1). Similarly, \( K_D \) values for ICYP were not changed (before: 9.1±1.0 pM; after: 9.6±0.7 pM).

Adenylate Cyclase Activity

Basal adenylate cyclase activity determined in membranes from “before” right atria (93.6±19 pmol cAMP formed/mg protein/min, \( n=10 \)) was nearly identical with that obtained in “after” right atria (93.9±20 pmol cAMP formed/mg protein/min). Figure 2 shows the effects of CPB with cardioplegic cardiac arrest on \( \beta \)-adrenergic (isoprenaline) as well as non-receptor-mediated activation of adenylate cyclase. Activation of adenylate cyclase by non-receptor-mediated-activated activators (10 \( \mu \)M GTP, 100 \( \mu \)M forskolin, 10 mM NaF, and 10 mM Mm\( ^{2+} \)) was not significantly affected. In contrast, in all except one child, CPB reduced \( \beta \)-adrenoceptor-mediated activation of adenylate cyclase; the net increase in adenylate cyclase activity evoked by 100 \( \mu \)M isoprenaline was in “after” right atrial membranes (64.4±14 pmol cAMP formed/mg protein/min) significantly lower than in “before” right atrial membranes (128.2±22 pmol cAMP formed/mg protein/min, \( n=10 \), \( p=0.0249 \)).

FIGURE 2. Bar graph of effects of cardiopulmonary bypass (CPB) with cardioplegic cardiac arrest on adenylate cyclase activity in right atria from children undergoing open-heart surgery. For details, see “Methods.” Right ordinate: Right atrial adenylate cyclase activity in pmol cyclic AMP (cAMP) formed/mg protein/min. Values are mean±SEM of 10 experiments. B, basal adenylate cyclase activity; ISO, isoprenaline; F, forskolin. *\( p=0.0249 \) vs. before CPB.

Discussion

In the present study, CPB and cardiac arrest by cold (4°C) St. Thomas' cardioplegic solution did not affect \( \beta \)-adrenoceptor number or \( \beta_1; \beta_2 \)-adrenoceptor ratio in right atria from children undergoing corrective cardiac surgery but did significantly depress isoprenaline-induced activation of adenylate cyclase. On the other hand, activation of adenylate cyclase by 10 \( \mu \)M GTP and 10 mM NaF (which is believed, in this concentration, to activate predominantly Gs, with little effect on Gi\( ^{19} \)), 100 \( \mu \)M forskolin (acting predominantly at the catalytic unit of the enzyme but involving at least partly Gs\( ^{20} \)), and 10 mM Mn\( ^{2+} \) (acting at the catalytic unit of the enzyme) was not at all affected during CPB. \( \beta \)-Adrenoceptor number also did not change. These results, therefore, suggest that after CPB, the relation between right atrial \( \beta \)-adrenoceptors and G protein has been altered in a manner compatible with an uncoupling of the \( \beta \)-adrenoceptor from the G protein—the first step in agonist-induced \( \beta \)-adrenoceptor desensitization. In favor of this idea is the result of (unfortunately, only one was performed because of lack of tissue) one experiment performed in pooled membranes from three “before” and four “after” right atria, which revealed that in “after” right atria, the high-affinity state for isoprenaline (as assessed from competition curves with ICYP binding) that is essential for coupling \( \beta \)-adrenoceptors to adenylate cyclase\( ^{21} \) amounted only to 20.3% of the \( \beta \)-adrenoceptors compared with 44.8% in “before” right atria (Figure 3). Because we did not measure G protein amount or function directly, we cannot fully exclude the possibility that changes in G protein may—at least in part—contribute to the reduction in isoprenaline-induced adenylate cyclase activation after CPB.
In the human heart, adenylate cyclase is much more efficiently coupled to β₂-adrenoceptors than to β₁-adrenoceptors (see Reference 12). Thus, perhaps CPB-induced decrease in isoprenaline-induced activation of adenylate cyclase reflects desensitization of mainly right atrial β₁-adrenoceptors. Such a more pronounced β₂-adrenoceptor desensitization would be in agreement with recently published data showing that in rats and guinea pigs during chronic isoprenaline infusion, cardiac β₁-adrenoceptors are more rapidly and to a greater extent downregulated than are cardiac β₁-adrenoceptors.22-24 On the other hand, in ”after” atrial membranes, the proportion of isoprenaline-induced high-affinity state of the β₁-adrenoceptors was markedly reduced (Figure 3). Because isoprenaline is in functional and binding studies approximately sixfold as β₁-adrenoceptor selective,5,25 and the human right atrium contains twice as many β₁-adrenoceptors than β₂-adrenoceptors (Figure 1; see also Reference 12), this favors the idea that β₁-adrenoceptors also are desensitized.

This is, to our best knowledge, the first report describing a desensitization of cardiac β₁-adrenoceptors during CPB with cardioplastic cardiac arrest in humans. Although we could not determine plasma catecholamines in the children, it is quite likely that the well-known increase in catecholamines during CPB with cardioplastic cardiac arrest, as discussed, explains this β₁-adrenoceptor desensitization, although other factors may be also involved. Thus, during CPB, cardiac tissue undergoes hyperperfusion and hypothermia that might affect β₁-adrenoceptor function. Furthermore, a possible influence of the anesthetic agents administered cannot be completely ruled out since some anesthetics can modify the properties of membranes. However, it is quite unlikely that this contributes considerably to CPB-induced right atrial β₂-adrenoceptor desensitization since halothane, for example, has been found to decrease human lymphocyte β₁-adrenoceptor density in vitro by only approximately 10%27 and has no effect in rat brain.28

In the present study, CPB-induced changes of β₁-adrenoceptors were studied in right atria. Whether similar changes also occur with human left ventricular β₁-adrenoceptors is not known. However, the pattern of changes in β₁-adrenoceptors and β₂-adrenergic activation of adenylate cyclase found in human right atria in this study is very comparable to that recently described for canine left ventricular β₁-adrenoceptors.29 These authors found that in dogs, a 155-minute CPB did not significantly affect cardiac β₁-adrenoceptors but did significantly depress isoprenaline- and zinterol-activated adenylate cyclase. They observed, in addition, that by 30 minutes after CPB, β₂-adrenergic adenylate cyclase activation had returned to normal, whereas the β₁-adrenoceptor number decreased. Whether similar changes after CPB also occur in humans cannot be assessed. However, it is interesting to note that in the present study, one infant with tetralogy of Fallot and two with atrioventricular septal defect were treated with epinephrine in a dosage ranging from 0.1 to a maximum of 2.0 μg/kg/min for 10 minutes from CPB and treating low cardiac output. In these three patients, epinephrine had to be continuously infused during the first 16, 22, and 23, respectively, postoperative hours. Interestingly, these three children exhibited much more pronounced decreases in isoprenaline-induced adenylate cyclase activation (“before” CPB: 105±22; “after” CPB: 34±5 pmol cAMP formed/mg protein/min, i.e., 67% decrease) than the other seven children (“before” CPB: 138±31; “after” CPB: 77±18 pmol cAMP/min/mg protein, i.e., 45% decrease). These findings therefore might be taken as an indication that during CPB, β₁-adrenoceptors desensitize not only in right atria but also in left ventricular myocardium.

The finding of a desensitization of cardiac β₁-adrenoceptor function during CPB with cardioplastic cardiac arrest may be of clinical importance. The human heart contains only a few spare receptors for β₁-adrenoceptor agonist (including endogenous catecholamines)–mediated positive inotropic effects, and nearly all receptors must be occupied to reach a maximal response.15,30,31 Because of this lack of spare receptors, it is rather plausible that a reduced isoprenaline-induced activation of adenylate cyclase, which results in insufficient increases in cAMP, the second messenger for positive inotropic effects in the human heart,32 is accompanied by a reduced positive inotropic effect. In fact, it is frequently observed that during weaning from CPB and in the early phase after CPB, patients often need inotropic support that cannot be satisfactorily achieved by β₁-adrenoceptor agonists.

In conclusion, in children undergoing open-heart surgery, CPB with cardioplastic cardiac arrest causes a reduction in β₁-adrenoceptor-mediated activation of adenylate cyclase in a manner compatible with an uncoupling of β₁-adrenoceptors from the Gₛ protein–adenylate cyclase complex. As the human heart contains only a few spare receptors for β₁-adrenoceptor agonists, such a β₁-adrenoceptor desensitization may be the reason why many patients need inotropic support after CPB but respond only weakly to β₂-adrenoceptor agonists.

References


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