

# Adenosine $A_{2A}$ Receptor Hyperexpression in Patients With Severe SIRS After Cardiopulmonary Bypass

François Kerbaul, MD, PhD,\* Frédéric Bénard, MD,\* Roch Giorgi, MD, PhD,†  
By Youlet, MD, PhD,‡ Louis Carrega, MD, PhD,‡ Ibrahim Zouher, MD,‡ Laurence Mercier, MD,‡  
Victoria Gérolami, MD,‡ Vincent Bénas, MD,\* Dorothée Blayac, MD,\* Vlad Gariboldi, MD, PhD,§  
Frédéric Collart, MD, PhD,§ and Régis Guieu, MD, PhD‡||

## ABSTRACT

**Objective:** Adenosine (ADO) is an endogenous nucleoside, which has been involved in blood pressure failure during severe systemic inflammatory response syndrome (severe SIRS) after cardiac surgery with cardiopulmonary bypass (CPB). Adenosine acts via its receptor subtypes, namely  $A_1$ ,  $A_{2A}$ ,  $A_{2B}$ , or  $A_3$ . Because  $A_{2A}$  receptors are implicated in vascular tone, their expression might contribute to severe SIRS. We compared adenosine plasma levels (APLs) and  $A_{2A}$  ADO receptor expression (ie,  $B$ ,  $K$ , and mRNA amount) in patients with or without postoperative SIRS.

**Patients:** This was a prospective comparative observational study. Forty-four patients who underwent cardiac surgery involving CPB. Ten healthy subjects served as controls.

**Measurements and Results:** Among the patients, 11 presented operative vasoplegia and postoperative SIRS (named complicated patients) and 33 were without vasoplegia or SIRS (named uncomplicated patients). Adenosine plasma levels,  $K$ ,  $B$ , and mRNA amount (mean  $\pm$  SD) were measured on peripheral blood mononuclear cells. Adenosine plasma levels,  $B$ , and  $K$  were significantly higher in complicated patients than in uncomplicated patients (APLs:  $2.7 \pm 1.0$  vs  $1.0 \pm 0.5$   $\mu\text{mol l}^{-1}$ ,  $P < 0.05$ ;  $B$ :  $210 \pm 43$  vs  $65 \pm 26$  fmol/mg,  $P < 0.05$ ;  $K$ :  $35 \pm 10$  vs  $2 \pm 1$  nM,  $P < 0.05$ ). In uncomplicated patients, APLs remain higher than in controls ( $1 \pm 0.5$  vs  $0.6 \pm 0.25$   $\mu\text{mol/L}$ ;  $P < 0.05$ ).

Mean arterial pressure was inversely correlated to APLs ( $R = -0.58$ ;  $P < 0.001$ ) and  $B$  ( $R = -0.64$ ;  $P < 0.001$ ) leading to an increased requirement of vasoactive

drugs during the postoperative period in vasoplegic patients.

**Conclusions:** High expression of  $A_{2A}$  ADO receptor and high APLs may be a predictive factor of postoperative severe SIRS after CPB.

**Key Words:** adenosine, SIRS,  $A_{2A}$  receptors, cardiopulmonary bypass, hemodynamics

## INTRODUCTION

Systemic inflammatory response syndrome (SIRS) can complicate cardiopulmonary bypass<sup>1</sup> after cardiac surgery. This inflammatory syndrome is often induced by a proinflammatory cytokine release and can result in organ dysfunction (severe SIRS), such as myocardial reperfusion damage, lung injury, and generalized profound vasodilation, thus increasing postoperative morbidity.<sup>2–4</sup>

During cardiopulmonary bypass (CPB), hemodynamic instability associated with low vascular resistance due to a systemic release of cytokines leads to a delay in extubation and a prolonged stay in the intensive care unit (ICU).<sup>1,5</sup> However, a lot of molecules play a role in the physiopathology of the systemic inflammatory response to cardiac surgery. Among these, adenosine (ADO) may participate in hemodynamic disturbance in critical illness, especially in severe SIRS. Adenosine is an endogenous nucleoside that is released by endothelial cells and myocytes during metabolic stress associated with ischemia or systemic inflammation.<sup>6–8</sup> Thus, it is not surprising that systemic ADO levels were recently been involved in blood pressure failure induced by severe SIRS after CPB<sup>9</sup> or septic shock.<sup>10</sup>

Adenosine acts on blood vessel tone and sinoatrial nodes via 4 G-protein-coupled receptor subtypes ( $A_1$ ,  $A_{2A}$ ,  $A_{2B}$ , and  $A_3$ ) depending on their pharmacological properties.<sup>11,12</sup> Stimulation of cardiac  $A_1$  and  $A_3$  receptors is associated with cardioprotection and ischemic conditioning,<sup>13,14</sup> whereas activation of  $A_{2A}$  receptors results in vasodilation.<sup>7,15</sup> The role of  $A_{2A}$  receptors in the control of blood pressure may be crucial. Indeed,

From the \*Pôle d'Anesthésie-Réanimation, Groupe Hospitalier de La Timone; †LERTIM, Faculté de Médecine; ‡Laboratoire de Biochimie and §Service de Chirurgie Cardiaque, Groupe Hospitalier de La Timone; and ||Université de la Méditerranée, Marseille, France.

Reprints: Régis Guieu, MD, PhD, Laboratoire de Biochimie, Groupe Hospitalier de La Timone, 264, rue Saint Pierre, 13385 Marseille Cedex 05, France. E-mail: guieu.regis@numericable.fr.

the lack of receptors in animals results in hypertension and tachycardia.<sup>16</sup> The increase in A<sub>2A</sub> ADO receptor expression contributes to hypotension during hemodialysis<sup>17</sup> or during neurocardiogenic syncope.<sup>18</sup> Furthermore, A<sub>2A</sub> ADO receptors are strongly expressed in brain area implicated in the control of arterial blood pressure.<sup>19</sup> However, the action of ADO on vascular tone depends on the number of receptors expressed at the cell surface.

Thus, the aim of this study was to evaluate adenosine plasma levels (APLs) and A<sub>2A</sub> ADO receptor expression (*B*), receptor affinity (*K*), and receptor synthesis (mRNA) in patients with or without SIRS after cardiac surgery and in controls.

## METHODS

This study protocol was approved by our institutional Ethics Committee and informed consent was obtained from every patient included in the study.

### Patients

From September 2005 to October 2005, patients with no active infection, inflammatory disease, or pulmonary hypertension, undergoing valve replacement, coronary artery surgery, combined, or other (see Table 1), were prospectively and consecutively included. Among the 70 patients hospitalized during this period, 44 filled the inclusion criteria (see Inclusion criteria section). We included at the same time 10 healthy volunteers as controls, who were recruited among hospital workers with no previous cardiac surgery (6 females and 4 males, 54 ± 11 years; range, 39–68).

Patients and volunteers were instructed to avoid coffee and tea for the 72 hours preceding the study. Patients who had been treated with papaverine, dipyridamole, immunosuppressive, or antibiotic agents during the preceding 6 weeks were not included.

### Inclusion Criteria

Patients were studied by Doppler echocardiography before surgery. A preoperative left ventricular ejection fraction (LVEF) was obtained from either a left ventricular angiogram or a 2-dimensional echocardiography (Teicholz method). Patients found to have a stenotic native aortic valve with a peak aortic-jet velocity of at least 4 m·s<sup>-1</sup> and an aortic valve area less than 0.5 cm<sup>2</sup>·m<sup>-2</sup> were included in the study.<sup>20</sup> Patients found to have a stenotic native mitral valve with a peak aortic-jet velocity more than 4 m·s<sup>-1</sup> and a mitral valve area less than 1.4 cm<sup>2</sup>·m<sup>-2</sup> were included. Symptomatic patients with one or more stenotic coronary arteries (>70%) underwent coronary revascularization. Recent myocardial infarctions were defined as an acute coronary syndrome with or without ST modification and were associated with troponin I plasma

**TABLE 1.** Demographic and Baseline Patient Characteristics (Means ± SD)

	Uncomplicated Course, n = 33	Vasoplegia, n = 11	P*
No. patients, n (%)	33 (75)	11 (25)	NS
Age, yr	58 ± 17	57 ± 10	NS
Male, n (%)	18 (57)	6 (56)	NS
Physical findings			
Body weight, kg	73 ± 22	76 ± 19	NS
NYHA, median <sup>†</sup>	2 (2–3)	2 (2–3)	NS
Preoperative medication, n (%)			
Inhibition-converting enzyme	9 (33)	4 (36)	NS
Medical history, n (%)			
Hypertension	13 (39)	5 (45)	NS
Peripheral vascular disease	2 (6)	1 (9)	NS
Congestive heart failure	7 (22)	2 (19)	NS
Recent myocardial infarction	2 (6)	1 (9)	NS
Unstable angina	3 (9)	1 (10)	NS
COPD	2 (7)	1 (6)	NS
Diabetes mellitus	10 (31)	3 (23)	NS
Hypercholesterolemia	11 (32)	4 (32)	NS
Renal dysfunction	11 (32)	3 (29)	NS
Results of diagnostic tests, n (%)			
LVEF, %	56 ± 13	48 ± 18	NS
Mean PAP, mm Hg	22 ± 2	21 ± 4	NS
Creatinine CL, mL·min <sup>-1</sup>	75 ± 28	72 ± 25	NS
Results of scoring tests			
EuroSCORE	5 ± 2	7 ± 2	NS
Parsonnet Index	12 ± 6	13 ± 5	NS
Type of surgery, n (%)			
Valvular surgery	20 (61)	6 (55)	NS
CABG	7 (21)	2 (18)	NS
Combined	3 (9)	1 (9)	
Other	3 (9)	2 (18)	

\**P* < 0.05 versus uncomplicated population. <sup>†</sup>Data are median values and 25–75th percentiles in parentheses. NYHA indicates New York Heart Association (classification for cardiac insufficiency); LVDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; mean PAP, mean pulmonary arterial pressure; creatinine CL, creatinine clearance; unstable angina, acute coronary syndrome without troponin I modification; COPD, chronic obstructive pulmonary dysfunction; CABG, coronary artery bypass grafting; combined surgery, valvular surgery associated with CABG; other, Bentall Tyron-David surgery, interventricular communication closure; NS, not significant.

level modifications.<sup>21</sup> These were observed over a 6-week period after the operation. Preoperative renal function was assessed by baseline creatinine clearance, calculated according the Cockcroft-Gault formula.<sup>22</sup>

### Exclusion Criteria

Patients with active infection, inflammatory disease, or pulmonary hypertension were excluded. Patients under immunosuppressive agents were also excluded.

## Anesthesia and Surgery

Patients were premedicated with 25 mg of orally administered oxazepam 1 hour before surgery. After preoxygenation, anesthesia was induced with intravenous midazolam (Hypnovel®; Roche Laboratories, Neuilly sur Seine, France), etomidate (Etomidate-Lipuro®; P Braun, Boulogne, France), sufentanil (Sufenta®; Solvay Pharma, Suresnes, France), and atracurium (Tracrium®; Glaxo-Wellcome, Marly-le-Roi, France) and maintained with sufentanil, atracurium, sevoflurane (Sevorane®; Abbott, Rungis, France) (0.5–1.5 MAC), and midazolam during CPB. Lungs were mechanically ventilated via an endotracheal tube. Prophylactic antibiotics were administered intravenously, with 1.5 g of cefamandole (Céfamandole®; Panpharma, Fougères, France) given after induction and then 750 mg given every 2 hours during surgery. Mild hypothermic (33°C) nonpulsatile CPB was performed after administration of intravenous heparin (300 IU·kg<sup>-1</sup>), always using the same model of membrane oxygenator (BARD Quantum; Bard Ltd, Crawley, UK) and a roller pump (COBE Optima Cardiovascular, Inc, Arvada, CO). A blood flow of 2.4 L·min<sup>-1</sup>·m<sup>-2</sup> was maintained with the aim of keeping arterial blood pressure between 50 and 75 mm Hg during the entire CPB. Myocardial preservation was performed with intermittent infusion of colloid-crystalloid solution (Buckberg; FRESSENIUS Laboratories Kabi, Sevres, France). Immediately after induction of anesthesia, patients were infused with 1,000,000 U of aprotinin. A further 1,000,000 U were added to the cardiopulmonary bypass prime solution, and patients were continuously infused with 250,000 U/hour during surgery.<sup>23</sup> During CPB, vasoplegia was considered to have occurred if the mean arterial pressure was less than 50 mm Hg with a duration of more than 5 minutes despite a normal blood flow rate of 2.4 L·min<sup>-1</sup>·m<sup>-2</sup>.<sup>24</sup>

All patients had pacing wires placed but none of them required permanent pacing at the postoperative period.

## Hemodynamics and Blood Gas Measurements

Patients were monitored by ECG, pulse oximetry, end-tidal carbon dioxide capnography, systemic arterial line (mean blood pressure; MBP), and transesophageal echocardiography. Hemodynamics, arterial blood gases, and hemoglobin were measured during the whole study.

In patients with low preoperative LVEF (<50%), epinephrine or dobutamine was administered when postoperative mean arterial pressure was less than 50 mm Hg. In patients with normal preoperative LVEF (>50%), norepinephrine was added if postoperative MBP was less than 50 mm Hg. The dosage was adjusted to give an MBP of approximately 70 mm Hg.

## Definition of SIRS and Severe SIRS

SIRS was defined, according to the classification of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference, when 2 or more of the following signs are found: body temperature abnormalities (>38 or < 36°C); persistent tachycardia (heart rate >90 beats/min); tachypnea or hyperventilation (breathing frequency >20/min or PaCO<sub>2</sub> < 32 mm Hg), and leukocytosis or leukopenia (leukocyte count >12 Gpt/L or <4 Gpt/L).<sup>25</sup> Tachycardia was only considered valid if it lasted for at least 2 hours with no significant modification of central venous pressure or mean systemic arterial pressure.

Severe SIRS was defined as SIRS with one or more postoperative organ dysfunction as defined in the ODIN model.<sup>26</sup> SIRS and severe SIRS were classified in all patients at 5:00 AM on the first postoperative day taking into account the entire postoperative condition of the patient. For patients who remained longer in the ICU, evaluation and classification were repeated daily.

Cardiac operative risk was evaluated by the same physician, blinded to the APL values, using the EuroSCORE scoring system on the day of surgery.<sup>27</sup>

Patients were discharged from the ICU if they met the following criteria: tracheal extubation performed since 12 hours, adequate muscle strength, hemodynamic stability without inotropic or vasopressive support, full consciousness, stable body temperature (within the range, 37–38.5°C), and adequate ventilation (breathing rate between 10 and 30/min, PaO<sub>2</sub>/FiO<sub>2</sub> >60/0.21, PaCO<sub>2</sub> between 30 and 50 mm Hg). Mediastinal drainage was systematically removed before ICU exit.

## Collection of Blood Samples

**Timing of Sample Collection.** In a previous clinical study, we performed kinetics of APLs, before surgery, during aorta cannulation, during CPB (before and after aortic cross-clamp release), and finally 30 minutes after the end of surgery. No influence of time was evidenced concerning APLs.<sup>9</sup> Here we have chosen collecting samples 30 minutes after surgery because it is the most easy and reproducible time for sample collection in our protocol. In volunteers, samples were collected at 8:00 AM from a peripheral vein.

## Adenosine Plasma Levels

The lumen of the arterial catheter was washed out and filled with a solution of 1 mL of papaverine and 1 mL of dipyridamole, injected through the lateral entry of a 3-way stopcock just prior blood sampling.<sup>28</sup> Blood (3 mL each) was taken through the axial entry of the stopcock using an ice-cold syringe containing 7 mL of the cold stop solution to prevent nucleotidases action, ADO uptake by red blood cells, and deamination into inosine.<sup>29,30</sup>

### Isolation of Peripheral Blood Mononuclear Cells and Membrane Preparation

Peripheral mononuclear cells were used to assess ADO A<sub>2A</sub> receptors because it has been established that peripheral blood circulating cells express A<sub>2A</sub> receptors changes that closely mirror those occurring in the heart itself.<sup>31</sup> Mononuclear cells were isolated from peripheral blood using Ficoll-based CPT system (Becton Dickinson, NJ). After 3 freeze-thaw cycles, the pellets were resuspended in a Tris buffer prior binding assay. Protein concentration was determined using a Beckman Synchron LX<sup>®</sup> apparatus (Beckman Coulter, Villepinte, France).

### B and K Determinations

The methodology has been previously described.<sup>18,31,32</sup> We used a selective A<sub>2A</sub> receptor ligand: [<sup>3</sup>H]-ZM 241385.<sup>33</sup> Saturation binding experiments were performed in triplicate by incubating homogenates of mononuclear cell membranes (200 μL in a total volume of 250 μL; 90 minutes, 4°C) with increasing concentrations of ligand. Bound and free radiolabeled ligands were separated by vacuum filtration of the sample through Whatman GF/C glass-fiber filters. A cold binding buffer (1 mL) was added to the sample before filtering. The filter was washed 3 times, and bound radioactivity was measured with a Beckman LS-1800 liquid scintillation spectrometer. A weighted nonlinear least-square curve fitting program (Graph Pad Prism<sup>®</sup>, Graph Pad Software Inc, San Diego, CA) was used for analysis. Nonspecific binding value of [<sup>3</sup>H]-ZM 241385 was defined as the binding observed in the presence of 10 μM of unlabeled ligand.

The *K* is the concentration of ligand at which binding sites are 50% occupied (equilibrium constant, a measure of affinity). *B* represents the total number of binding sites expressed as fmol/mg of protein.

### Quantification of ADO A<sub>2A</sub> Receptor mRNA

Total ribonucleic acids (RNA) were extracted from purified mononuclear cells using Bio-Robot, M48 (Qiagen, Courtaboeuf, France). Complementary DNA was synthe-

sized from 250 ng of total RNA. Real-time quantitative PCR was performed with a Light-Cycler (Roche<sup>®</sup>) according to the manufacturer's recommendation. The mRNA relative amount expression was quantified using the ratio between A<sub>2A</sub> mRNAs and mRNAs from house-keeping gene18S.

### Statistical Analysis

Association between vasoplegia and *K* or *B* were analyzed using the *U* test of Mann-Whitney for continuous variables.  $\chi$  test or Fisher exact test when appropriate and Mann-Whitney *U* test were performed to analyze association between vasoplegia and other prognostic factors. Spearman correlation coefficient was used for correlation data. For all tests, *P* values less than 0.05 (2-tailed tests) were considered as statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences for Windows (Version 13.1; SPSS, Inc, Chicago, IL).

## RESULTS

### Clinical and Biological Parameters During Surgery

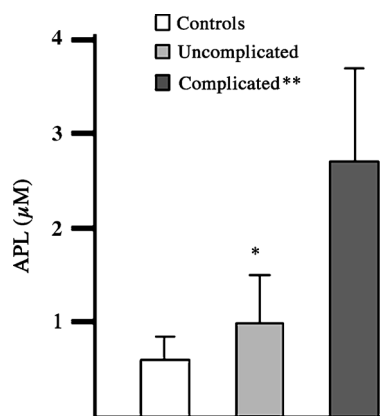
Forty-four patients were prospectively included in the study and underwent cardiac surgery with CPB. We observed a preoperative vasoplegia in 11 of them. All of these 11 patients (complicated patients) developed postoperative SIRS, with 10 developing severe SIRS. Mean age, body weight, NYHA classification status, preoperative echocardiographic, and biological parameters were not significantly different in these patients compared with those without vasoplegia (Table 1). None of the patients had unstable angina.<sup>21</sup>

Duration of endotracheal intubation (since induction of anesthesia) was significantly longer in patients with vasoplegia who developed postoperative SIRS compared with patients without vasoplegia (224 ± 87 vs 14 ± 9 hours, respectively; *P* < 0.05). The duration of stay in the ICU was significantly longer for patients

**TABLE 2.** Perioperative Patient Characteristics (Means ± SD)

	Uncomplicated Course, n = 33	Vasoplegia, n = 11	P
Mean duration of CPB, min	91 ± 37	98 ± 38	NS
Mean duration of aorta clamping, min	64 ± 28	72 ± 37	NS
Minimal core temperature, CPB	33.3 ± 1.4	33.1 ± 1.6	NS
Postoperative inotropic support			
Dobutamine (μg·kg <sup>-1</sup> ·min <sup>-1</sup> ), n (%)	3.0 ± 2.1 (10)	9.0 ± 4.0 (60)	<0.05
Epinephrine (μg·kg <sup>-1</sup> ·min <sup>-1</sup> ), n (%)	0.04 ± 0.02 (3)	0.32 ± 0.12 (49)	<0.05
Norepinephrine (μg·kg <sup>-1</sup> ·min <sup>-1</sup> ), n (%)	0.0 ± 0.0 (0)	0.15 ± 0.02 (42)	<0.05
Blood transfusion >1 U, n (%)	9 (28)	3 (29)	NS
Duration of ventilation, h	14 ± 9	224 ± 87	<0.05
Duration of stay in ICU, d	3 ± 1	16 ± 13	<0.05
Death, n (%)	0 (0)	2 (18)	<0.05

CPB indicates cardiopulmonary bypass; ICU, intensive care unit; NS, not significant.



**FIGURE 1.** Means  $\pm$  SD of adenosine plasma concentrations in complicated patients (with per operative vasoplegia or postoperative SIRS;  $n = 11$ ), in patients with no complications ( $n = 33$ ), and in 10 healthy subjects. \* $P < 0.05$  versus uncomplicated group. \*\*Preoperative vasoplegia or postoperative severe systemic inflammatory response syndrome. APL indicates adenosine plasma level.

with vasoplegia compared with patients without vasoplegia ( $16 \pm 13$  vs  $3 \pm 1$  days;  $P < 0.05$ ; Table 2).

The duration of CPB and aorta cross-clamping, the body temperature, and the  $\text{PaO}_2/\text{FiO}_2$  ratio between the 2 groups were not different. Conversely, the administration of postoperative inotropic drugs was significantly higher in the patients with severe SIRS (Table 2). Dobutamine and epinephrine were simultaneously administered to 5 patients, and dobutamine and norepinephrine were simultaneously administered to 4 patients to main-

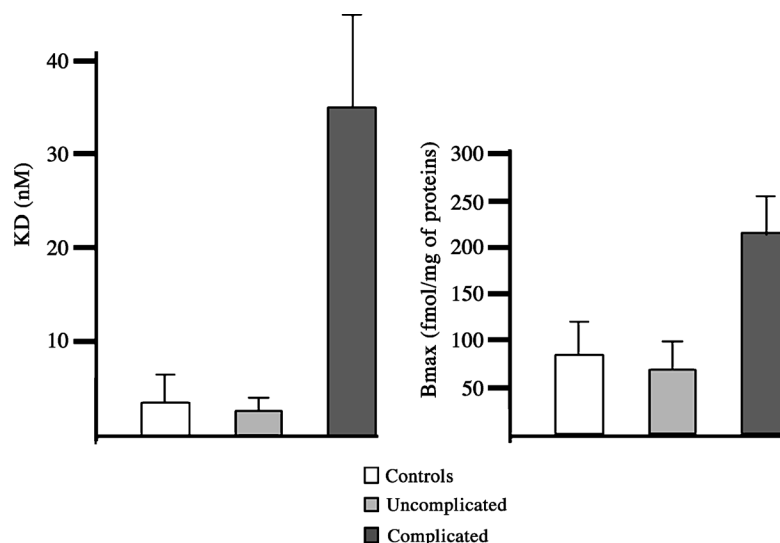
tain systolic arterial pressure. All these patients experienced postoperative severe SIRS.

### Adenosine Plasma Levels

Adenosine plasma levels in uncomplicated patients were higher than those of healthy subjects ( $1 \pm 0.5$  vs  $0.6 \pm 0.25$   $\mu\text{mol/L}$ ;  $P < 0.05$ ). In complicated patients, APLs were significantly higher than those observed in uncomplicated patients ( $2.7 \pm 1$  vs  $1 \pm 0.5$   $\mu\text{mol/L}$ ;  $P < 0.05$ ; Fig. 1). Baseline MBPs were not different between the group of patients ( $80 \pm 6$  mm Hg in patients with vasoplegia vs  $81 \pm 6$  mm Hg in uncomplicated). However, during surgery, MBPs strongly decreased in the vasoplegic patients ( $55 \pm 8$  mm Hg in complicated patients with vasoplegia vs  $68 \pm 11$  mm Hg in uncomplicated patients at the end of surgery;  $P < 0.002$ ). Among the 44 patients, MBPs were inversely correlated with arterial APL (Spearman  $r = -0.58$ ;  $P < 0.001$ ) and  $B$  ( $r = -0.64$ ;  $P < 0.001$ ).

### $A_{2A}$ Receptors Expression

$B_{\text{max}}$  and  $K_D$  were significantly higher in complicated patients compared with uncomplicated patients ( $B_{\text{max}}$ :  $210 \pm 43$  vs  $65 \pm 26$  fmol/mg,  $P < 0.05$ ;  $K_D$ :  $35 \pm 10$  vs  $2 \pm 1$  nM,  $P < 0.05$ ; Fig. 2). But  $K_D$  and  $B_{\text{max}}$  were not different between uncomplicated patients and healthy volunteers. Finally, mRNA amount was not different between the 2 groups of patients (mRNA  $A_{2A}$ /mRNA beta-actin; uncomplicated vs complicated patients:  $19.84 \pm 0.05$  vs  $20.05 \pm 0.04$ ;  $P > 0.05$ ) and remains in the range of those measured in controls  $19.98 \pm 0.06$ ;  $P > 0.05$ ).



**FIGURE 2.**  $K_D$  and  $B_{\text{max}}$  values.  $K_D$  (nM) of [ $^3\text{H}$ ]-ZM 241385, a specific ligand of  $A_{2A}$  adenosine receptor evaluated using peripheral blood mononuclear cell membranes. Samples were collected 30 minutes after surgery.  $K_D$  was defined as the concentration of ligand at which binding sites are 50% occupied.  $B_{\text{max}}$  represents the total number of binding sites expressed as femtomole per milligram of proteins.  $K_D$  and  $B_{\text{max}}$  were evaluated in patients with uncomplicated operative course in patients with per operative vasoplegia or postoperative severe SIRS and in 10 healthy subjects. Data are expressed as mean and SD. \* $P < 0.05$  versus uncomplicated group.

## ■ DISCUSSION

Our study showed that hyperexpression of A<sub>2A</sub> receptors in a context of high APLs measured after surgery could be a predictive factor for postoperative severe SIRS. This resulted in a delayed extubation, a higher dose of catecholamines, and a prolonged stay in the ICU.

Vasoplegia during and immediately after cardiac surgery involving CPB occurs in between 5% and 20% of patients.<sup>1,5,9</sup> Twenty-five percent of our patients developed postoperative SIRS. However, the number of patients included was lower than in previous reports.<sup>1,5</sup>

The vasoplegia is one of the main factors influencing the extent of the SIRS.<sup>1</sup> During CPB, the exposure of blood to the extracorporeal circuit activates the complement system that initiates the proinflammatory process.<sup>1,3,4</sup> Neutrophil activation or free radical, endotoxin, or cytokine release is thought to participate in the inflammatory process of SIRS.<sup>3,34,35</sup> However, the role of ADO in this process has been poorly investigated. In a prospective clinical study, we have shown that high APL was found to be associated with postoperative complications after cardiac surgery using CPB.<sup>9</sup> But the level of A<sub>2A</sub> ADO receptor expression has never been evaluated in this population.

### Possible Mechanisms of High APL in Vasoplegic Patients

Adenosine is a nucleoside that is released by endothelial cells and myocytes during ischemia or oxidative stress.<sup>6-8</sup> Adenosine has been implicated in the drop of blood pressure that occurs during tilt-test-induced syncope<sup>18,36</sup> in vasoplegia of septic shock,<sup>10</sup> but ADO may be also an endogenous modulator that plays a role in triggering vasoplegia during heart surgery in predisposed patients. Adenosine is released by sympathetic fibers and poorly myelinated C fibers.<sup>37,38</sup> Moreover, ADO has been reported to modulate baroreflex activation.<sup>39</sup> Therefore, high APLs may lead to dysfunction of the baroreflex, as observed in some patients during surgery who were preoperatively treated with angiotensin-converting enzyme inhibitors.<sup>9</sup> However, the number of such patients between the vasoplegic and the nonvasoplegic groups was not different. High APLs do not seem to be related to myocardial necrosis, as we found no difference in troponin concentrations between the vasoplegic and the nonvasoplegic groups at postoperative time.

### Effects of A<sub>2A</sub> Receptor Modulation on Infectious and Noninfectious SIRS

In an animal model of infectious SIRS, deoxycoformycin, an inhibitor of ADO deaminase that increases ADO levels in the extra cellular spaces, has been shown to improve survival time and to suppress the inflammatory response indices.<sup>40</sup> Moreover, it was demonstrated that activation

of A<sub>2A</sub> receptors diminishes phagocytosis and augments secretion of anti-inflammatory cytokines in invasive bacterial infection.<sup>41,42</sup> Recently, Nemeth et al.<sup>43</sup> demonstrate that ADO A<sub>2A</sub> receptor inactivation increases animal survival after polymicrobial sepsis. This protection after A<sub>2A</sub> receptor blockade was paralleled by a decrease in apoptosis and proinflammatory cytokines production associated with an increase of phagocytosis. Many of the effects of ADO may also involve modulating oxyradical-mediated response. This occurs via increased oxyradical production via ADO to xanthine degradation or limiting inflammatory oxyradical generation.<sup>44</sup>

Adenosine has also been shown to clinically increase the systemic vasodilation that characterizes the hemodynamic profile of patients with noninfectious SIRS during CPB.<sup>9</sup> High APLs can result in a very low blood pressure and bradycardia, which is often resistant to vaso-pressive amines.<sup>10</sup> These are secondary effects of the activation of A<sub>2A</sub> receptors, which are primarily implicated in blood pressure control.<sup>16</sup> A<sub>2A</sub> receptor activation could therefore also result in systemic vasodilation occurring with severe SIRS.

The cardiovascular consequences of high APLs effectively seem to depend on the expression of ADO receptors. This expression may be different among patients and from 1 receptor subtype to another.<sup>11</sup> We observed a decrease in mean arterial pressure in vasoplegic patients with high APLs and A<sub>2A</sub> receptor hyperexpression. Thus, the effects of ADO on the cardiovascular system are probably due to a balance between A<sub>2A</sub> receptor hyperexpression and APL. A<sub>2A</sub> receptor number was 4-fold greater in complicated than in uncomplicated patients after CPB. Furthermore, we found that in the complicated group, the ADO plasma level is increased 2.5- to 3-fold. This increase may be sufficient to activate A<sub>2</sub> spare receptors<sup>45</sup> and to precipitate vasoplegia.

Thus, the great expression of these receptors in a context of high APLs may precipitate vasoplegia in our patients.

## ■ CONCLUSION

These results strongly suggest that high expression of A<sub>2A</sub> ADO receptor, in a context of high APLs, may be a predictive factor of postoperative severe SIRS after CPB. Further clinical studies are needed to determine if preventive blockade of A<sub>2A</sub> receptors may offer a new strategy for reducing cardiovascular vasoplegia and non-infectious postoperative SIRS occurring after CPB.

## ■ REFERENCES

1. Laffey JG, Boylan JF, Cheng DCH. The systemic inflammatory response to cardiac surgery. *Anesthesiology*. 2002;97:215-252.

2. Kerbaul F, Guidon C, Lejeune PJ, et al. Hyperprocalcitonemia is related to postoperative severe SIRS associated with coronary artery bypass grafting. *J Cardiothorac Vasc Anesth.* 2002;16:47–53.
3. Wan S, Leclerc JL, Vincent JL. Inflammatory response to cardiopulmonary bypass. Mechanisms involved and possible therapeutic strategies. *Chest.* 1997;112:676–692.
4. Menasché P. The inflammatory response to cardiopulmonary bypass and its impact on postoperative myocardial function. *Curr Opin Cardiol.* 1995;10:597–604.
5. Carrel T, Englberger L, Mohacsi P, et al. Low systemic vascular resistance after cardiopulmonary bypass: incidence, etiology, and clinical importance. *J Card Surg.* 2000;15:347–353.
6. Newholmes EA, Leech AR. Pathways for the degradation of purines and pyrimidine nucleotides. In: *Biochemistry for the Medical Sciences*, 5th ed, edited by Chichester: John Wiley and Sons, 1992, pp 507–508.
7. Sollevi A. Cardiovascular effects of adenosine in man: possible clinical implications. *Prog Neurobiol.* 1986;27:319–349.
8. Hack B, Witting PK, Rayner BS, et al. Oxidant stress and damage in post-ischemic mouse hearts; effects of adenosine. *Moll Cell Biochem.* 2006;287:165–175.
9. Kerbaul F, Collart F, Giorgi R, et al. Role of endogenous adenosine as a predictive marker of vasoplegia during cardiopulmonary bypass and postoperative severe SIRS. *Crit Care Med.* 2006;34:640–645.
10. Martin C, Leone M, Viviani X, et al. High adenosine plasma concentration as a prognostic index for outcome in patients with septic shock. *Crit Care Med.* 2000;28:3198–3202.
11. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev.* 1998;50:413–492.
12. Fredholm BB, Arslan G, Halldner L, et al. Structure and function of adenosine receptors and their genes. *Naunyn Schmiedebergs Arch Pharmacol.* 2000;362:364–374.
13. Picano E, Abbracchio MP. Adenosine, the imperfect endogenous anti-ischemic cardio-neuroprotector. *Brain Res Bull.* 2000;52:75–82.
14. Baxter GF. Role of adenosine in delayed preconditioning of myocardium. *Cardiovasc Res.* 2002;55:483–494.
15. Shryock JC, Belardinelli L. Adenosine and adenosine receptors in the cardiovascular system: biochemistry, physiology and pharmacology. *Am J Cardiol.* 1997;79:2–10.
16. Ledent C, Vaugeois JM, Schiffmann SN, et al. Aggressiveness, hypoalgesia and high blood pressure in mice lacking the A2A receptor. *Nature.* 1997;388:674–678.
17. Giaime P, Carrega L, Fenouillet E, et al. Relationship between A2A adenosine receptor expression and intradialytic hypotension during hemodialysis. *J Investig Med.* 2006;54:473–477.
18. Carrega L, Saadjian A, Mercier L, et al. Increased expression of A2A adenosine receptors in patients with spontaneous and head up tilt test syncope. *Heart Rhythm.* 2007;4:870–876.
19. Thomas T, St Lambert JH, Dashwood MR, et al. Localization and action of A2A receptors in regions of the brainstem important in cardiovascular control. *Neuroscience.* 2000;95:513–518.
20. Rosenhek R, Binder T, Porenta G, et al. Predictors of outcome in severe, asymptomatic aortic stenosis. *New Engl J Med.* 2000;343:611–617.
21. The Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined. *Eur Heart J.* 2001;21:1502–1513.
22. Provenchère S, Plantefève G, Hufnagel G, et al. Renal dysfunction after cardiac surgery with normothermic cardiopulmonary bypass: incidence, risk factors, and effect on clinical outcome. *Anesth Analg.* 2003;96:1258–1264.
23. Feindt P, Volkmer I, Seyfert U, et al. Activated clotting time, anticoagulation, use of heparin, and thrombin activation during extracorporeal circulation: changes under aprotinin therapy. *Thorac Cardiovasc Surg.* 1993;41:9–15.
24. Arkin DB, Saidman LJ, Benumof JL. Hypotension following cardiopulmonary bypass. *Anesth Analg.* 1977;56:720–724.
25. Muckart DJJ, Bhagwanjee S. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions of the systemic inflammatory response syndrome and allied disorders in relation to critically injured patients. *Crit Care Med.* 1997;25:1789–1795.
26. Fagon JY, Chastre A, Novara P, et al. Characterization of intensive care unit patients using a model based on the presence or absence of organ dysfunctions and/or infection: the ODIN model. *Intensive Care Med.* 1993;19:137–144.
27. Nashef SAM, Roques F, Michel P, et al. The EuroSCORE study group European system for cardiac operative risk evaluation (EuroSCORE). *Eur J Cardiothorac Surg.* 1999;16:9–13.
28. Saadjian AY, Paganelli F, Gaubert ML, et al. Adenosine plasma concentration in pulmonary hypertension. *Cardiovasc Res.* 1999;43:228–236.
29. Guieu R, Paganelli F, Sampieri F, et al. The use of HPLC to evaluate the variations of blood coronary adenosine levels during percutaneous transluminal angioplasty. *Clin Chim Acta.* 1994;230:404–409.
30. Guieu R, Peragut JC, Roussel P, et al. Adenosine and neuropathic pain. *Pain.* 1996;68:1–5.
31. Varani K, Laghi-Pasini F, Camurri A, et al. Changes of peripheral A2A adenosine receptors in chronic failure and cardiac transplantation. *FASEB J.* 2003;17:280–282.
32. Carrega L, Fenouillet E, Giaime P, et al. Influence of hemodialysis and left ventricular failure on peripheral A2A

- adenosine receptors. *Nephrol Dial Transplant*. 2007;22: 851–856.
33. Poucher SM, Keddie JR, Singh P, et al. The in vitro pharmacology of ZM 241385, a potent, non xanthine A2A selective adenosine receptor antagonist. *Br J Pharmacol*. 1995;115:1096–1102.
34. Partrick DA, Moore EE, Fullerton DA, et al. Cardiopulmonary bypass renders patients at risk for multiple organ failure via early neutrophil priming and late neutrophil disability. *J Surg Res*. 1999;86:42–49.
35. Seghaye MC, Duchateau J, Grabitz RG, et al. Complement activation during cardiopulmonary bypass in infants and children: relation to postoperative multiple system organ failure. *J Thorac Cardiovasc Surg*. 1993;106: 978–987.
36. Saadjian A, Levy S, Francheschi F, et al. Role of endogenous adenosine as a modulator of syncope induced during tilt testing. *Circulation*. 2002;106:569–574.
37. Von Kugelgen I, Allgaier C, Schobert A, et al. Co-release of noradrenaline and ATP from cultured sympathetic neurons. *Neuroscience*. 1994;61:199–202.
38. Pelleg A, Burnstock G. Physiological importance of ATP release from nerve terminals and its degradation to adenosine in humans. *Circulation*. 1990;82:2269–2272.
39. Mosqueda-Garcia R, Furlan R, Tank J, et al. The elusive pathophysiology of neurally mediated syncope. *Circulation*. 2000;102:2898–2906.
40. Law R, Valli VE, Conlon BA. Therapeutic potential for transient inhibition of adenosine deaminase in systemic inflammatory response syndrome. *Crit Care Med*. 2003;31: 1475–1481.
41. Cronstein BN, Daguma L, Nichols D, et al. The adenosine/neutrophil paradox resolved: human neutrophils possess both A1 and A2 receptors that promote chemotaxis and inhibit O<sub>2</sub> generation respectively. *J Clin Invest*. 1990;85: 1150–1157.
42. Eppell BA, Newell AM, Brown EJ. Adenosine receptors are expressed during differentiation of monocytes to macrophages in vitro: implications for regulation of phagocytosis. *J Immunol*. 1989;143:4141–4145.
43. Nemeth ZH, Csoka B, Wilmanski J, et al. Adenosine A2A receptor inactivation increases survival in polymicrobial sepsis. *J Immunol*. 2006;176:5616–5626.
44. Conlon BA, Ross JD, Law WR. Advances in understanding adenosine as a plurisystem modulator in sepsis and the systemic inflammatory response syndrome. *Front Biosci*. 2005;10:2548–2565.
45. Shryock JC, Snowdy S, Baraldi PG, et al. A2A adenosine receptor reserve for coronary vasodilation. *Circulation*. 1998;98:711–718.