**Increase of Bradykinin in Plasma of Patients Undergoing Cardiopulmonary Bypass**

*The Importance of Lung Exclusion*

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**Study objectives:** Hemodynamic complications including hypotensive episodes are frequently associated with cardiopulmonary bypass (CPB) and can be attributed to a generalized inflammatory response in which bradykinin may be a mediator. The purpose of this study was to determine the plasma levels of bradykinin-(1–9)nonapeptide in patients during CPB and the physiologic elimination of bradykinin by the lungs.

**Design:** Prospective, observational study.

**Setting:** University hospital, cardiac surgery unit.

**Patients and methods:** Intra-arterial BP was monitored and serial blood samples were obtained from 27 patients undergoing CPB for cardiac surgery. We measured plasma bradykinin and parameters of coagulation, fibrinolysis, complement, contact system, and the cytokine tumor necrosis factor (TNF).

**Results:** Mean arterial pressure fell progressively until the end of CPB (−18 mm Hg, p = 0.001) but returned to baseline by the end of surgery. The venous bradykinin level, normal in basal conditions (median, 1.90 fmol/mL), was increased (p = 0.001) from 15 min after the beginning of CPB (5.71 fmol/mL) to the end of the operation (7.07 fmol/mL), with a peak at the end of CPB (9.81 fmol/mL; p = 0.0001); it was normal at recovery 24 h later (2.81 fmol/mL). Bradykinin plasma levels fell 60% across the lung when the pulmonary circulation was fully restored while the patients were still receiving CPB. Activated-factor XII, thrombin-antithrombin complexes, prothrombin fragment F1 + 2, plasmin-antiplasmin complexes, C5a, and TNF increased significantly after the beginning of the surgical procedure, rising further during CPB, and remained elevated until the end of surgery, but they all returned to normal within 24 h. Changes in plasma bradykinin levels were not correlated with any of the other variables.

**Conclusions:** During CPB, there is a progressive increase of plasma bradykinin that is at least partially due to reduced catabolism as a consequence of shunting the lungs. The increase in bradykinin may contribute to the fall in BP.

**Key words:** angiotensin-converting enzyme; bradykinin metabolism; coagulation; complement; extracorporeal circulation; fibrinolysis; hypotension

**Abbreviations:** ACE = angiotensin-converting enzyme; CPB = cardiopulmonary bypass; ECC = extracorporeal circulation; ELISA = enzyme-linked immunosorbent assay; FXIIa = activated-factor XII; HK = high-molecular-weight kininogen; MAP = mean arterial pressure; PAP = plasmin-antiplasmin; TAT = thrombin-antithrombin; TNF = tumor necrosis factor

Cardiovascular surgery needing cardiopulmonary bypass (CPB) is often complicated by hypoten-
sive episodes and accumulation of extravascular fluid. These side effects may reflect a generalized inflammatory response involving the successive activation of various biochemical pathways (complement, contact system, fibrinolysis, cytokines) and cells (white cells, platelets, endothelial cells) with the release of vasoactive substances. Many components of coagulation and inflammation are activated when blood comes into contact with a large synthetic surface, but the precise relationships between the...
activations of the different protease systems are not well defined. The vasoactive peptide bradykinin, generated through cleavage of high-molecular-weight kininogen (HK) by kallikrein during contact system activation, could contribute to the inflammatory response of CPB. This mediator is a potent vasoconstrictor that increases vascular permeability. In the present study, we used a recently developed sensitive and specific method to measure plasma levels of bradykinin in patients with coronary artery disease undergoing CPB. In view of the close links among the kinin system, hemostasis, and inflammation, changes in bradykinin levels were compared with activation of the contact system, coagulation, fibrinolysis, complement system, and cytokines, and analyzed in relation to changes in BP. Moreover, because bradykinin is assumed to be mainly inactivated in the pulmonary vascular tree, we evaluated its clearance during the restoration of the circulation across the natural lungs when extracorporeal circulation (ECC) was still ongoing. This study completes the preliminary data presented at the 15th International Conference on Kinins.

**Materials and Methods**

In 27 patients (21 men and 6 women; 63 ± 9 years old, mean ± SD) scheduled for coronary surgery, CPB was conducted with a standard circuit and membrane oxygenator at mild hypothermia. Non–heparin-coated tygon tubing, membrane oxygenator, and centrifugal pump were used (Medtronic, Minneapolis, MN). A single double-stage right atrial cannulation was performed, and suction tubing was assembled on the heart-lung machine via two roller pumps (Stockert; Munich, Germany). Anesthesia was induced with thiopental, curare, and isoflurane, and was maintained with curare and fentanyl at standard doses in all patients. For myocardial protection, St. Thomas II solution was used (1,000 mL for induction and 200 mL every 20 min) and allowed to drain into the circulation. The duration of ECC was 136 ± 23 min, and aortic cross-clamping lasted 92 ± 23 min. During CPB, the blood flow was 3,719 ± 455 mL/min, rectal temperature was 32.5 ± 1.2°C, and esophageal temperature was 31.0 ± 1.8°C. Intra-arterial BP was measured in the radial artery by a pressure transducer. Systemic vascular resistance was calculated in absolute units using the following equation: (mean arterial pressure [MAP] – pressure in right atrium)/blood flow ± 80. None of the patients were receiving treatment with angiotensin-converting enzyme (ACE) inhibitors. The study protocol was approved by the ethics committee of the University of Milan, and informed consent was obtained from all patients.

In 21 patients, blood samples were obtained from a central venous catheter, and during CPB from the venous line of the bypass system. The sampling times were as follows: before surgery (baseline), after heparin infusion before ECC, 15 min after the beginning of ECC, at the end of ECC, at the end of surgery, and on the day after surgery (recovery). We compared changes in bradykinin with several parameters of the contact system (activated-factor XII [FXII], cleavage of HK), the coagulation cascade (prothrombin fragment F1 + 2 and thrombin-antithrombin [TAT] complexes), the fibrinolytic system (plasmin-antiplasmin [PAP] complexes), the complement system (C3a), and the cytokine tumor necrosis factor (TNF). These patients were disconnected from ECC after a weaning time of 30 ± 20 min. The weaning time, which follows the rewarming period, is defined as the time between aortic declamping and the end of ECC. This period is needed for the accomplishment of proximal anastomoses with partial clamping of the ascending aorta and then for the gradual restoration of satisfactory cardiac output.

The remaining six patients were studied in order to evaluate the generation and clearance of bradykinin by the artificial circuit and by the natural lung. In these six patients, we took paired samples from entrance and exit of the artificial circuit during CPB, and from the right atrium and aorta still during CPB after rapidly restoring the filling pressures and pulmonary blood flow.

Plasma bradykinin levels were measured specifically by radioimmunoassay after liquid-phase extraction and subsequent high-performance liquid chromatography, as described in detail. The detection limit was 0.2 fmol/mL. Intra-assay and interassay coefficients of variation were 18% at the low endogenous concentrations.

FXIIa was measured intrusion citrated plasma with a sandwich enzyme-linked immunosorbent assay (ELISA) [Shield Diagnostic Ltd; Dundee, UK], which uses a specific monoclonal antibody for human FXIIa (2/215) as capture antibody and a sheep polyclonal anti-FXIIa antibody conjugated to alkaline phosphatase as second antibody. Intra-assay and interassay coefficients of variation were 5.1% and 8.2%, respectively.

Cleavage of HK was assessed in plasma obtained from blood drawn into polypropylene tubes containing acid-citrate-dextrose (100 nM trisodium citrate, 67 mM citric acid, and 2% dextrose, pH 4.5), benzamidine (100 mM), 400 µg/mL hexadimethrine bromide, 2 mg/mL soybean trypsin inhibitor, 263 µM leupeptin, and 20 mM aminooxybenzenesulfonfylfluoride (nine parts of blood into one part of anticoagulant). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting analysis was carried out by a method based on that described by Berrettini et al. After electrophoretic transfer of proteins from gel onto a polyvinylidene difluoride membrane (Immobilon; Millipore Corporation; Milford, MA), HK was identified with goat polyclonal anti-light chain HK (Nordic; Tullburg, Netherlands) and visualized by a biotinylated rabbit anti-rabbit antibody (Sigma Chemical; St. Louis, MO). The apparent molecular masses of proteins were estimated by comparison with the high-molecular-weight protein markers from Bio-Rad Laboratories (Richmond, CA). With this method, native HK appears as a band with molecular weight of 130,000, and cleaved HK is represented by two bands with molecular weights of 107,000 and 98,000. The density of the bands was evaluated by computerized image analysis (ImageMaster; Pharamacia, Uppsala, Sweden). The amount of cleaved HK (bands with molecular weights of 107,000 and 98,000) was expressed as a percentage of the total HK (sum of the three bands).

Prothrombin fragment F1 + 2 was assessed in citrated plasma with a sandwich ELISA (Enzygnost F1 + 2; Behring Diagnostics GmbH; Marburg, Germany). The method uses a rabbit antibody to human prothrombin fragment F1 + 2 as capture antibody and a rabbit peroxidase conjugated antiprothrombin as second antibody. Intra-assay and interassay coefficients of variation were 8%.

TAT complexes were measured in citrated plasma with a sandwich ELISA (Enzygnost TAT micro; Behring Diagnostics GmbH). The method uses a rabbit antibody against human prothrombin fragment F1 + 2 as capture antibody and a rabbit peroxidase-conjugated antiprosthetic as second antibody. Intra-assay and interassay coefficients of variation were 6% and 9%, respectively.

PAP complexes were measured in citrated plasma with a sandwich ELISA (Enzygnost PAP micro; Behring Diagnostics GmbH). The method uses a rabbit antibody against human PAP as capture antibody and a rabbit peroxidase-conjugated antihuman.
Plasma bradykinin increased during ECC and returned to baseline in the recovery period (Fig 1, Table 1). During ECC, two patients needed vasoconstrictors (norepinephrine [Noradrenaline], 0.1 to 1.0 μg/kg/min) and five patients needed vasodilators (sodium nitroprusside, 3 to 4 μg/kg/min). If we exclude the two patients treated with vasoconstrictors, who tended to have high levels of bradykinin (5.7 to 40.1 fmol/mL) and the five patients treated with vasodilators, who tended to have low bradykinin levels (3.8 ± 1.1 fmol/mL), there was a significant inverse correlation between bradykinin plasma levels and MAP at the end of ECC ($r = -0.574; p = 0.032$). At that time, systemic vascular resistances (1,288 ± 265 U) were inversely correlated with bradykinin plasma levels ($r = -0.451; p = 0.04$). The increase in plasma bradykinin was not paralleled by enhanced cleavage of HK. A typical example of analysis of plasma HK and HK fragments in one patient during ECC is shown in Figure 2.

Table 1 summarizes the time course of plasma coagulation, fibrinolysis, and inflammation parameters. The surgical procedure alone increased FXIIa, TAT complexes, prothrombin fragment F1 + 2, C3a, and TNF. All these rose further during ECC, remaining elevated until the end of the operation, but had returned to normal 24 h later. PAP complexes increased only after the beginning of the ECC, remaining elevated until the end of the operation, and fell to below basal values 24 h later (Table 1).

During ECC, there was no correlation between changes in bradykinin and in the other parameters. At the end of ECC, there was an inverse correlation between bradykinin levels and the weaning time (the time during which the pulmonary circulation is progressively restored while ECC continues) [$r = -0.636; p = 0.003$; Fig 3]. Interestingly, at the same time (end of ECC), bradykinin levels were also inversely correlated with the polymorphonuclear blood cell count (neutrophils) [Fig 4]. Neutrophil counts were directly correlated with the weaning time ($r = 0.565; p = 0.008$). None of the other parameters were correlated with bradykinin levels at the end of ECC. The only correlation between the various parameters at any time was the strong one between prothrombin fragment F1 + 2 and TAT complexes ($r = 0.801; p < 0.001$).

Plasma bradykinin levels from the six patients studied for generation and clearance of bradykinin by the artificial circuit and natural lung are summarized in Table 2. Bradykinin levels more than doubled (+115%) after passage through the artificial circuit ($p = 0.028$) and fell to less than half (−60%) across the natural lung ($p = 0.028$).

**Discussion**

Our study demonstrates that CPB raises plasma bradykinin levels and confirms the inflammatory
response and activation of the coagulation and fibrinolysis systems. Earlier studies of the involvement of the kinin system in ECC gave contradictory results. However, immunoreactive kinin levels measured in these studies, during control conditions at baseline and during the bypass procedure, were several orders of magnitude higher than the plasma concentrations of immunoreactive bradykinin obtained by more recent assays. Specific measurement of the plasma bradykinin-(1–9) nonapeptide concentration is difficult since small amounts of this short-lived peptide have to be quantitated in the presence of large amounts of precursor and metab-

olite peptides, and rapid generation and degradation of bradykinin may lead to estimation of sampling artifacts.

In the present study, we measured plasma bradykinin specifically after liquid-phase extraction and high-performance liquid chromatography separation of kinins using a method that had previously detected increased plasma bradykinin concentrations during attacks of angioedema. This method is based on careful sample handling and uses efficient inhibitors of bradykinin-generating and bradykinin-degrading enzymes. Baseline bradykinin levels in our patients with coronary artery disease are in the low

Table 1—Contact System, Fibrinolysis, and Inflammation Parameters in Plasma Samples From 21 Patients With Coronary Artery Disease Undergoing CPB

<table>
<thead>
<tr>
<th>Variables</th>
<th>Basal</th>
<th>Before ECC</th>
<th>ECC 15 min</th>
<th>End of ECC</th>
<th>End of Operation</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin, fmol/mL</td>
<td>1.90 (1.70–4.65)</td>
<td>2.68 (1.79–6.87)</td>
<td>5.71 (2.62–8.31)</td>
<td>9.81 (4.51–28.25)</td>
<td>7.07 (2.94–12.22)</td>
<td>2.81 (1.75–4.78)</td>
</tr>
<tr>
<td>Cleaved HK, % of total HK</td>
<td>16 (13–18)</td>
<td>14 (10–17)</td>
<td>12 (10–14)</td>
<td>12 (10–16)</td>
<td>14 (8–17)</td>
<td>17 (13–18)</td>
</tr>
<tr>
<td>FXIIa, ng/mL</td>
<td>1.53 (1.16–1.96)</td>
<td>2.05 (1.69–3.15)</td>
<td>2.82 (2.24–3.94)</td>
<td>3.85 (3.30–5.41)</td>
<td>3.36 (2.72–3.78)</td>
<td>1.83 (1.51–2.22)</td>
</tr>
<tr>
<td>PAP, ng/mL</td>
<td>460 (355–534)</td>
<td>433 (305–598)</td>
<td>322 (353–1,152)</td>
<td>1,313 (824–1,619)</td>
<td>1,133 (717–1,729)</td>
<td>202 (150–352)</td>
</tr>
<tr>
<td>TAT, ng/mL</td>
<td>6.70 (3.70–10.40)</td>
<td>10.68 (7.85–24.66)</td>
<td>20.64 (13.47–49.19)</td>
<td>79.29 (45.80–98.34)</td>
<td>77.72 (44.67–96.45)</td>
<td>11.50 (5.47–19.14)</td>
</tr>
<tr>
<td>F1+2, ng/mL</td>
<td>1.20 (1.10–1.50)</td>
<td>1.65 (1.36–2.39)</td>
<td>3.09 (1.76–3.90)</td>
<td>6.29 (4.35–8.48)</td>
<td>6.60 (5.11–9.23)</td>
<td>1.62 (1.04–2.16)</td>
</tr>
<tr>
<td>C3a, ng/mL</td>
<td>337 (179–903)</td>
<td>629 (475–907)</td>
<td>3,869 (2,218–5,589)</td>
<td>2,960 (1,812–5,375)</td>
<td>3,938 (3,375–6,946)</td>
<td>345 (232–647)</td>
</tr>
<tr>
<td>TNF, pg/mL</td>
<td>4.79 (2.00–13.00)</td>
<td>12.70 (2.24–23.14)</td>
<td>18.34 (3.03–35.79)</td>
<td>59.70 (19.66–74.98)</td>
<td>55.04 (33.57–103.77)</td>
<td>12.62 (4.17–33.56)</td>
</tr>
</tbody>
</table>

*Data are presented as median (25 to 75% interquartile range). Plasma samples were collected from the right atrium in basal conditions, before ECC after the surgical procedure of connection, after 15 min of ECC, at the end of ECC, at the end of operation, and 24 h later.

*p = 0.0001 vs basal conditions.

*p = 0.001 vs basal conditions.

*p = 0.003 vs basal conditions.

*p = 0.01 vs basal conditions.

Figure 2. Immunoblotting analysis of HK in plasma from a patient undergoing CPB. Plasma samples were collected in basal conditions, before ECC after the surgical procedure of connection, after 15 min of ECC, at the end of ECC, at the end of the operation, and 24 h later. In all samples, the pattern of HK remained similar to that of normal plasma (N) with a major molecular weight band of 130,000 and a minor one with molecular weight of 107,000. Maximal cleavage of HK (disappearance of the 130,000 molecular weight band, increase of 107,000 molecular weight band, and appearance of a molecular weight band of 98,000) was obtained by activation of human normal plasma with kaolin *in vitro* (K). See Figure 1 legend for definitions of abbreviations.
picomolar range, thus comparable to those of healthy control subjects. From the beginning of CPB, the plasma bradykinin level rises. It is increased fourfold at the end of CPB and returns to normal by 24 h later. Thus, the hypothesis of increased plasma bradykinin concentrations during CPB was verified. The increase could be due to enhanced activation of the contact system as previously described in clinical CPB. In our patients undergoing CPB, FXIIa was indeed increased in parallel with bradykinin (Table 1), but no direct correlation was found between individual changes in bradykinin and FXIIa levels. HK cleavage was not enhanced enough for immunoblotting to show an increase in HK breakdown products in plasma (Fig 2), in contrast with overactive states of the kallikrein system, like hereditary angioedema. Nevertheless, a smaller degree of activation of the kallikrein-kinin system, beyond the sensitivity of Western blot analysis, cannot be excluded because HK has a micromolar plasma concentration but bradykinin has only a picomolar plasma concentration. Cleavage of a small fraction of HK (below the detection limit of Western blotting) might generate the few femtomols of bradykinin clearly detected by the bradykinin assay. Another reason for the increase in plasma bradykinin during CPB could be reduced degradation. Bradykinin is efficiently degraded in the pulmonary vascular bed by kininase II, i.e., ACE. In animal experiments, bradykinin disappeared mostly in a single passage through the pulmonary circulation, and a clearance rate of 95% was extrapolated from bradykinin infusions in humans. CPB completely bypasses the pulmonary circulation, so bradykinin may well accumulate. In agreement with this reduced metabolism of bradykinin is our observation that plasma bradykinin levels at the end of CPB were inversely correlated with the weaning time, i.e., the time it took for the pulmonary circulation to be gradually restored while CPB was still in progress (Fig 3). If CPB is terminated after a long weaning time, the lungs are perfused for long enough to metabolize a large amount of bradykinin. If the weaning time is short, therefore, the decrease in plasma bradykinin may be small. To verify this, in six patients undergoing CPB, we took paired samples from the right atrium and the aorta after declamping the aorta and restoring the pulmonary circulation while ECC was ongoing. Bradykinin plasma levels were indeed 60% lower after passage through the natural lungs. Thus, in humans too, endogenous bradykinin is largely eliminated in the pulmonary vascular tree. Another reason for nondegradation of circulating bradykinin during CPB could be the absence of endothelium and thus of ACE in the tubings of the extracorporeal circuit. This was in fact found to cause artifically increased angiotensin I levels in instrumented dogs. For bradykinin, this artifact has never been demonstrated, but our results are in full agreement with the idea of nondegradation, since CPB patients’ plasma bradykinin levels more than doubled from the entrance to the exit of the artificial circuit (Table 2). Whatever the reason, however, for the increased plasma levels of bradykinin in tubings (lack of endothelium or activation of the contact system), the impressive 60% extraction of bradykinin across the

![Figure 3](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21970/)

Figure 3. Correlation between plasma bradykinin levels at the end of CPB and the time of weaning from ECC in 21 patients. $r = $ Spearman’s correlation coefficient. See Figure 1 legend for definition of abbreviation.

![Figure 4](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21970/)

Figure 4. Correlation between plasma bradykinin levels and neutrophil counts at the end of CPB in 21 patients. $r = $ Spearman’s correlation coefficient. See Figure 1 legend for definition of abbreviation.
natural lungs leads us to assume that the exclusion of the lungs during CPB is the most important reason for the increase in plasma bradykinin.

At the end of CPB, blood neutrophil counts were inversely related to plasma bradykinin. This would be compatible with bradykinin-mediated extravasation of neutrophils. Trafficking of neutrophils between the intravascular and extravascular compartment may be induced by the bradykinin-activation platform on the neutrophil surface, enhancing vasopereation. This mechanism could contribute to the sequestration of neutrophils in the lungs at the end of bypass, also produced by activation of the fifth component of the complement system.7

Bradykinin is well-known as a vasodilator and hypotensive peptide. However, many other factors can induce hypotension during CPB, and in this condition the BP is maintained by the continuous intervention of the anesthetist. The drop in MAP observed before ECC was not associated with an increase of bradykinin and appeared after the induction of anesthesia, which is known to have a hypotensive effect. During CPB, if we exclude the seven patients who received vasoactive agents, the inverse correlation between bradykinin plasma levels and MAP suggests an important role of bradykinin. The inverse correlation between plasma bradykinin and the systemic vascular resistance further supports the hemodynamic effect of bradykinin during CPB.

Bradykinin levels were not correlated with the parameters of activation of coagulation, fibrinolysis, complement, and cytokines. The activation of coagulation during CPB is well-known and requires systemic heparin therapy to prevent gross clotting. However, the exact mechanism of activation is not completely understood, and both contact and tissue factor pathways may be involved.31 Our data show an increase of markers of thrombin generation (prothrombin fragment F1 + 2 and TAT complexes) not correlated with markers of contact system activation (cleaved HK and FXIIa), supporting the view of Boisclair et al31 that contact activation by exposure to foreign surfaces is not the only procoagulant stimulus during CPB. The increase of PAP complexes confirms the activation of fibrinolysis, already described in many studies32–34 and associated with blood loss during CPB.35 The significant reduction of PAP complexes we observed 24 h after CPB could be due to the fibrinolytic shutdown described in the postoperative period.36 The increases in C3a and TNF are in agreement with the activation of complement37 and cytokines38 during CPB. We did not find any correlations between the various parameters, except for prothrombin fragment F1 + 2 and TAT complexes, which were strongly correlated (r = 0.801; p < 0.001) as expressions of thrombin generation.38

In conclusion, our data demonstrate a progressive increase of plasma bradykinin levels during CPB, at least partially due to reduced elimination by the lungs. This increase, which may partially contribute to hypotension, is not correlated with activation of the contact system, coagulation, fibrinolysis, complement, or TNF. The effectiveness of the recently developed bradykinin-receptor antagonists in preventing complications of CPB remains to be established.

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CHEST / 120 / 6 / DECEMBER, 2001 1781

Table 2—Plasma Bradykinin Concentrations at the Entrance and Exit of Artificial and Natural Lungs in Six Patients Undergoing CPB*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Artificial Lung</th>
<th>Natural Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entrance of the Circuit</td>
<td>Exit of the Circuit</td>
</tr>
<tr>
<td>Bradykinin, fmol/mL</td>
<td>1.60 (0.91–2.55)</td>
<td>2.05 (0.95–4.38)</td>
</tr>
</tbody>
</table>

*p = 0.028

*Data are presented as median (25 to 75% interquartile range). Samples from the entrance and exit of the circuit were obtained after 15 min of CPB. Samples from right atrium and from aorta were obtained at the end of CPB after restoration of the pulmonary circulation.


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