Activation of the kallikrein-kinin system by cardiopulmonary bypass in humans

DUNCAN J. CAMPBELL,1 BARRY DIXON,2 ATHENA KLADIS,1 MICHAEL KEMME,3 AND JOHN D. SANTAMARIA2
1St. Vincent’s Institute of Medical Research and 2Department of Intensive Care, St. Vincent’s Hospital, Fitzroy, Victoria 3065, Australia; and 3Institut für Biochemie, Technische Universität Darmstadt, 64287 Darmstadt, Germany

Received 7 March 2001; accepted in final form 25 May 2001

Campbell, Duncan J., Barry Dixon, Athena Kladis, Michael Kemme, and John D. Santamaria. Activation of the kallikrein-kinin system by cardiopulmonary bypass in humans. Am J Physiol Regulatory Integrative Comp Physiol 281: R1059–R1070, 2001.—We used cardiopulmonary bypass (CPB) as a model of activation of the contact system and investigated the involvement of the plasma and tissue kallikrein-kinin systems (KKS) in this process. Circulating levels of bradykinin and kallidin and their metabolites, plasma and tissue kallikrein, low and high molecular weight kininogen, and kallistatin were measured before, during, and 1, 4, and 10 h after CPB in subjects undergoing cardiac surgery. Bradykinin peptide levels increased 10- to 20-fold during the first 10 min, returned toward basal levels by 70 min of CPB, and remained 1.2- to 2.5-fold elevated after CPB. Kallidin peptide levels showed little change during CPB, but they were elevated 1.7- to 5.2-fold after CPB. There were reductions of 80 and 60% in plasma and tissue kallikrein levels, respectively, during the first minute of CPB. Kininogen and kallistatin levels were unchanged. Angiotensin-converting enzyme inhibition did not amplify the increase in bradykinin levels during CPB. Aprotinin administration prevented activation of the KKS. The changes in circulating kinin and kallikrein levels indicate activation of both the plasma and tissue KKS during activation of the contact system by CPB.

Contact system; bradykinin; kallidin; angiotensin-converting enzyme inhibition; kininogen; kallistatin; aprotinin

Kinin peptides have a broad spectrum of activities (3). Kinins are potent vasodilators and also promote diuresis and natriuresis. Kinins protect against ischemia-reperfusion injury by decreasing endothelial adherence of leukocytes, leading to attenuation of postischemic leukocyte adherence, attenuation of disruption of the microvascular barrier, and reduced tissue injury. However, kinins also participate in the cardinal features of inflammation, producing vasodilatation, vascular permeability, neutrophil chemotaxis, and pain (3).

Study of the kallikrein-kinin system (KKS) in humans offers the advantage that the plasma and tissue KKS can be studied separately because they generate different kinin peptides, in contrast to some other species. Plasma kallikrein forms bradykinin (BK)-(1–9) from high molecular weight kininogen (HMWK), whereas tissue kallikrein forms kallidin [Lys0-BK-(1–9), KBK-(1–9)] from HMWK and low molecular weight kininogen (LMWK) (3). Bradykinin peptides may also be generated by aminopeptidase-mediated cleavage of kallidin peptides. A proportion of kinins is hydroxylated on proline3 (Hyp3) of the BK-(1–9) sequence (20, 21), and hydroxylated kinins have similar biological activity to nonhydroxylated kinins (33). Kinins act via two types of kinin receptor, the type 1 (B1) and the type 2 (B2) receptors. The B2 receptor normally predominates, whereas B1 receptors are induced by tissue injury. BK-(1–9) and KBK-(1–9) peptides are more potent at the B2 receptor, whereas their carboxypeptidase (kininase I) metabolites BK-(1–8) and Lys0-bradykinin-(1–8) [KBK-(1–8)], respectively, are also bioactive and more potent on B1 receptors (33). The angiotensin-converting enzyme (ACE; kininase II) and neutral endopeptidase 24.11 metabolites BK-(1–7) and KBK-(1–7) are inactive.

Kinin production in vivo is controlled, in part, by endogenous inhibitors of the kallikrein enzymes. The main inhibitors of plasma kallikrein are C1 inhibitor, α2-macroglobulin, and antithrombin III (3). An important inhibitor of tissue kallikrein is kallistatin (7).

Recent studies in humans indicate a role for endogenous kinin peptides in the regulation of coronary vascular tone (17) and in mediating the hypotensive effects of ACE inhibition (16). However, the mechanisms by which kinins are produced in vivo are unknown. Whereas kallidin peptides are more abundant than bradykinin peptides in urine, bradykinin peptide levels are higher than kallidin peptide levels in blood and tissue (5, 11). One potential mechanism of kinin peptide production is the contact system (9). The proteins HMWK, plasma prekallikrein, and factor XII are grouped together as the “contact system,” because they require contact with artificial, negatively charged surfaces for zymogen activation in vitro (38). In vivo, the contact system is assembled on endothelial (39) and
neutrophil cell membranes (18) where it may participate in basal kinin peptide production. In addition to kinin peptide formation, the contact system activates the intrinsic coagulation, complement, and fibrinolytic systems (9). There is little appreciation of how this system is activated in vivo, although C1-inhibitor deficiency can result in unpredictable activation of the contact system, leading to angioedema and increased circulating BK-(1–9) levels (29). The initial consensus that the sole function of the contact system is to activate plasma prekallikrein leading to BK-(1–9) generation has been supplanted by the current view of the contact system as a biological modulator of the vascular system with anticoagulant, profibrinolytic, antiadhesive, and proinflammatory activity (9).

Cardiopulmonary bypass (CPB) elicits a systemic inflammatory response that causes tissue injury and contributes to significant perioperative and long-term clinical morbidity (4, 47). During CPB, exposure of blood to bioincompatible surfaces of the extracorporeal circuit, as well as tissue ischemia and reperfusion associated with the procedure, induces the activation of several major humoral pathways of inflammation. These various pathways include the complement, coagulation, and cytokine cascades. Activation of the contact system is an important component of this inflammatory response to CPB (9). In the present study, we used CPB as an experimental model to study changes in the circulating components of the plasma and tissue KKS during activation of the contact system in humans.

Our laboratory recently developed HPLC-based RIA for the specific measurement of hydroxylated and non-hydroxylated bradykinin and kallidin peptides and their metabolites (11). We are able to measure both the B1-receptor agonist peptides BK-(1–9), Hyp3-BK-(1–9), KBK-(1–9), and Hyp3-KBK-(1–9), and the B1-receptor agonist peptides BK-(1–8), Hyp3-BK-(1–8), KBK-(1–8), and Hyp3-KBK-(1–8). Previous studies showed that CPB is associated with activation of the KKS (10, 24, 31, 36, 42, 48). None of these studies examined the possible role of the tissue KKS. Furthermore, no previous studies examined the generation of the B1-receptor agonist peptides. B1-receptor agonist peptides may contribute to the inflammatory response to CPB by stimulating B1 receptors induced by tissue injury (1). Moreover, activation of plasmin during CPB may, through activation of procarboxypeptidase B (27), accelerate the formation of B1-receptor agonist peptides.

Many subjects undergoing cardiac surgery are receiving ACE inhibitor therapy, and there has been some concern that this may lead to hemodynamic instability during CPB, possibly due to increased kinin peptide levels (26). We therefore compared kinin peptide levels during CPB in subjects who had received or not received ACE inhibitor therapy before surgery. A proportion of subjects undergoing cardiac surgery is administered aprotinin, and we studied the effects of aprotinin on bradykinin, kallidin, and kallikrein levels during CPB. Aprotinin is a broad-spectrum inhibitor of serine proteases, including plasma and tissue kallikrein and plasmin (15, 19). Although not inhibiting activated factor XII, aprotinin inhibits its activation by blocking its ability to bind to surfaces and to autoactivate (25). Thus aprotinin effectively blocks activation of the contact system and the KKS.

**METHODS**

This study was performed in subjects scheduled for routine cardiac surgery using CPB at St. Vincent’s Hospital, Melbourne, Australia. The surgery was coronary artery graft surgery and/or aortic or mitral valve surgery. Subjects with diabetes and/or heart failure were excluded from this study. Subjects with heart failure were excluded because these subjects have suppressed circulating levels of kallidin peptides (11). Subjects with diabetes were excluded because we have preliminary evidence that diabetes may influence kallidin peptide levels (unpublished data). This was a convenience sample of subjects collected over a 24-mo period, with median age 66 yr (range 44–83), of whom 76% were male and all were Caucasian. Subjects were taking a variety of medications for their ischemic heart disease and/or coexistent clinical conditions including hypertension and hyperlipidemia. These medications included ACE inhibitors (31%), calcium antagonists (34%), β-blockers (52%), hydroxymethylglutaryl coenzyme A reductase inhibitors (45%), gemfibrozil (7%), nitrates (38%), digoxin (7%), and diuretics (24%). The Human Research Ethics Committee of St. Vincent’s Hospital approved this protocol, and all subjects gave informed consent.

All subjects had a radial artery cannula and a six-lumen Swan Ganz VIP catheter (Baxter Healthcare, Irvine, CA) inserted before surgery. Anesthesia was induced with fentanyl (10–25 µg/kg), propofol (0.5–1 mg/kg), and a muscle relaxant, and it was maintained with isoflurane or propofol infusion. The standard procedure was for subjects to receive aminocaproic acid (AMICAR, Lederle Laboratories, Wyeth Australia, Baulkham Hills, Australia), with 5 g given as a loading dose following induction of anesthesia and 5 g given into the pump prime. For subjects who had had previous cardiac surgery, aminocaproic acid was not administered, and these subjects received aprotinin (Trasylol, Bayer Australia, Pymble, Australia) administered as a loading dose of 2 × 106 kallikrein inactivator units (KIU), a pump prime of 2 × 105 KIU, and a constant infusion dose of 0.5 × 106 KIU/h. Both aminocaproic acid and aprotinin are plasmin inhibitors that reduce postoperative bleeding by inhibiting fibrinolysis. It was hospital policy to administer aprotinin rather than aminocaproic acid to patients who had had previous cardiac surgery because of the increased risk of postoperative bleeding in these patients. CPB was performed using a Cobe membrane oxygenator (Cobe Cardiovascular, Arvada, CO) with a 40-micron arterial line filter and a heparin-coated polyvinyl chloride plastic circuit (Lovell Surgical Supplies, Carrum Downs, Victoria, Australia). A roller pump was used for 90% of the subjects, and a centrifugal pump was used for the remainder. Pump prime was with 1,200 ml Plasmalyte, 500 ml Haemaccel, 30 mmol sodium bicarbonate, 5 mmol calcium chloride, and 10,000 U heparin. Subjects were heparinized before CPB to maintain an activated clotting time above 400 s and reversed with protamine at the conclusion of CPB. Systemic heparinization was necessary to prevent clotting of blood in the pump. One of two methods of cardioplegia was used. Arrest of the heart was managed either using a combination of antegrade and retrograde blood cardioplegia without active cooling, and central temperatures plateaued at 32–33°C, or by cold crystalloid antegrade cardioplegia.
with active cooling to 28°C. All subjects were actively rewarmed to 37°C before discontinuation of CPB.

Kinin peptide levels were measured in 29 subjects who were divided into four groups based on whether they were receiving ACE inhibitor therapy before surgery and whether they had had previous cardiac surgery and therefore received aprotinin instead of aminocaproic acid during CPB. The four groups consisted of the following: 1) control subjects not receiving ACE inhibitor therapy and given aminocaproic acid during CPB; 2) subjects not receiving ACE inhibitor therapy and given aprotinin during CPB; 3) subjects receiving ACE inhibitor therapy with a presurgery ANG II/ANG I ratio <0.15 mol/mol and given aminocaproic acid during CPB; and 4) subjects receiving ACE inhibitor therapy with a presurgery ANG II/ANG I ratio >0.15 mol/mol and given aminocaproic acid during CPB.

Sample collection. Blood was collected into plastic syringes. For measurement of angiotensin and bradykinin peptides, 2 ml of blood were immediately transferred to a tube containing 10 ml 4 M guanidine thiocyanate and 1% (vol/vol) trifluoroacetic acid (GTC/TFA) and mixed thoroughly. For measurement of kallidin peptides, 10 ml of blood were immediately transferred to a tube containing 20 ml 1 M HCl. Blood for measurement of kallikrein, kininogen, and kallistatin levels was collected into heparinized tubes, immediately centrifuged, and the plasma was snap-frozen in dry ice.

Arterial blood was sampled via a threeway tap close to the arterial cannula to avoid activation of plasma prekallikrein during blood sampling. Mixed venous blood was sampled from the distal port of the pulmonary artery catheter. Blood was also collected from the mixed venous line arriving at the bypass pump and from the arterial line leaving the pump. Blood collection was timed to avoid occasions when shed blood was reinfused. Blood for measurement of bradykinin and kallidin peptides was collected from the arterial cannula before surgery and at 1, 4, and 10 h after CPB. Blood for measurement of bradykinin peptides was also collected from mixed venous blood arriving at and from arterial blood leaving the bypass pump at 10 and 70 min of CPB. In addition, mixed venous blood for measurement of kallidin peptides was collected from the pulmonary artery catheter before surgery and at 1, 4, and 10 h after CPB, and from mixed venous blood arriving at the pump at 10 and 70 min of CPB. Not all peptides were measured in all subjects because of the need to limit the amount of blood collected from each subject. Blood for measurement of kallikrein, kininogen, and kallistatin levels was collected from the arterial cannula before surgery and at 1, 4, and 10 h after surgery. When analysis of these samples showed a marked fall in circulating levels of plasma and tissue kallikrein levels after CPB, kallikrein levels were measured in a further three groups of subjects to determine the time course of fall in kallikrein levels during CPB. In the first group (n = 7), arterial blood was sampled from the radial artery cannula before commencement of surgery, immediately before bypass after 80 min of surgery (after heparinization), and after 1 min of bypass. In the second and third groups, in addition to arterial blood taken from the radial artery cannula before surgery, samples were collected from mixed venous blood arriving at the pump and from arterial blood leaving the pump at 1 and 2 min of bypass in the second group (n = 9) and at 15 and 60 min of bypass in the third group (n = 8).

Extraction and HPLC of kinin and kallidin peptides. The GTC/TFA and HCl blood samples for measurement of angiotensin, bradykinin, and kallidin peptides were processed and assayed as described previously using HPLC-based RIA (11). These assays enabled the specific measurement of the bradykinin (BK)-(1–9), Hyp3-BK-(1–9), BK-(1–8), Hyp3-BK-(1–8), BK-(1–7), and Hyp3-BK-(1–7) sequences. Nonhydroxylated and hydroxylated peptides were summed to simplify presentation of kinin peptide levels. Angiotensin peptides were measured using HPLC-based RIA as described elsewhere (6).

Measurement of kallistatin, kininogen, and kallikrein. Plasma kallistatin levels were measured by Western blot analysis of reduced plasma samples run on 5–15% gradient gels.
RESULTS

Baseline kinin peptide levels in arterial blood. As we previously described (11), many subjects receiving ACE inhibitor therapy do not have suppression of the ANG II/ANG I ratio in arterial blood. On the basis of the measurement of angiotensin peptides in arterial blood collected immediately before surgery, subjects receiving ACE inhibitor therapy were divided into two groups, those with suppression of the ANG II/ANG I ratio below 0.15 mol/mol and those without this degree of suppression. Compared with control subjects, arterial bradykinin and kallidin peptide levels measured before surgery were increased in subjects receiving ACE inhibitor therapy who had ANG II/ANG I ratio <0.15 mol/mol, but not in those with less suppression of ANG II/ANG I ratio (Fig. 1).

Effect of CPB on bradykinin peptide levels in arterial blood. Whereas CPB did not affect bradykinin peptide levels in subjects who received aprotinin during surgery, there was a marked increase in bradykinin peptide levels in the other subject groups at the commencement of CPB (Fig. 2). Although baseline bradykinin peptide levels were higher in subjects receiving ACE inhibition with ANG II/ANG I ratio <0.15 mol/mol, the bradykinin peptide levels in the three groups of subjects who did not receive aprotinin were similar at all time points during and after CPB. When the three groups that did not receive aprotinin were pooled, the increase in bradykinin peptide levels was ~22-fold for Hyp3-BK(1–7) levels and 12-fold for Hyp3-BK+(1–8) and Hyp3-BK+(1–9) levels (P < 0.0001 compared with baseline for all peptides). The increase in bradykinin peptide levels was not sustained. By 70 min of CPB, the increase in bradykinin peptide levels was ~3.7-fold for Hyp3-BK+(1–7) levels (P < 0.0001) and twofold for Hyp3-BK+(1–8)
After CPB, Hyp3-BK1BK-(1–7) and Hyp3-BK1BK-(1–8) levels remained ~2.5-fold elevated above baseline \( (P < 0.05 \text{ for } 1, 4, \text{ and } 10 \text{ h post-CPB}) \), and Hyp3-BK+BK-(1–9) levels were 1.2- to 1.5-fold elevated \( (P < 0.05 \text{ at } 4 \text{ and } 10 \text{ h post-CPB}) \).

Hyp3-BK+BK-(1–8) levels increased parallel with Hyp3-BK+BK-(1–9) levels without change in the Hyp3-BK+BK-(1–8)/Hyp3-BK+BK-(1–9) ratio during and after CPB.

Bradykinin peptide levels across the pump. Given the similarity of changes in blood bradykinin levels during CPB in the three groups of subjects who did not receive aprotinin, we pooled data for these three groups to determine whether bradykinin levels changed during transit of blood through the pump at 10 and 70 min of bypass. During CPB, mean Hyp3-BK+BK-(1–9) levels for the three groups who did not receive aprotinin were 17% higher in arterial blood leaving the pump than in mixed venous blood delivered to the pump at 10 min bypass (mean absolute difference 6.3 fmol/ml, \( P = 0.001 \)) and 74% higher at 70 min bypass (mean absolute difference 3.2 fmol/ml, \( P = 0.001 \)) (Fig. 3).

Effect of CPB on kallidin peptide levels in arterial blood. Arterial kallidin peptide levels were measured before and after, but not during, CPB. Although baseline kallidin peptide levels were higher in subjects receiving ACE inhibition with ANG II/ANG I ratio \( <0.15 \text{ mol/mol} \), analysis of arterial blood kallidin peptide levels across all time points showed no differences among the four groups of subjects (Fig. 4). However, there were statistically significant differences between time points for the pooled analysis of all four subject groups, with increases in kallidin peptide levels after CPB. Compared with presurgery levels, aprotinin-treated subjects showed a 1.7-fold increase in Hyp3-KBK+KBK-(1–7) levels at 1 h post-CPB \( (P < 0.05) \). The control group showed a 1.9-fold increase in Hyp3-KBK+KBK-(1–9) levels at both 1 and 10 h post-CPB \( (P < 0.05) \). Moreover, the ACE inhibitor without suppressed ANG II/ANG I ratio group showed a 1.7-fold increase in Hyp3-KBK+KBK-(1–7) levels at 4 h and a 2.5-fold increase in Hyp3-KBK+KBK-(1–8) and 5.2-

Fig. 3. Hyp3-BK+BK-(1–9) levels in mixed venous blood arriving at the bypass pump and arterial blood leaving the pump at 10 (A) and 70 (B) min of CPB. Pooled data for subjects who did and did not receive ACE inhibition, excluding subjects who received aprotinin. Data are means ± SE, \( n = 16 \text{ at } 10 \text{ min and } n = 14 \text{ at } 70 \text{ min}. *P < 0.01, ***P < 0.001, \) in comparison of levels in mixed venous blood arriving at bypass pump and arterial blood leaving the pump.

Fig. 4. Kallidin peptide levels in arterial blood sampled from the radial artery cannula Pre and after CPB in control subjects (○, \( n = 7 \)), subjects receiving aprotinin (○, \( n = 7 \)), and subjects receiving ACE inhibition with ANG II/ANG I ratio \( >0.15 \text{ mol/mol} \) (●, \( n = 5 \)) and with ANG II/ANG I ratio \( <0.15 \text{ mol/mol} \) (♦, \( n = 5 \)). Nonhydroxylated and hydroxylated peptides were summed to simplify presentation of kallidin peptide levels. Data are means ± SE. *\( P < 0.05 \), compared with presurgery levels for control subjects; †\( P < 0.05 \), compared with presurgery levels for aprotinin-treated subjects; #\( P < 0.05 \), compared with presurgery levels for subjects receiving ACE inhibition with ANG II/ANG I ratio \( >0.15 \text{ mol/mol} \).
fold increase in Hyp-KBK(+1–9) levels at 10 h post-CPB (P < 0.05).

Effect of CPB on kallidin peptide levels in mixed venous blood. Kallidin peptide levels were measured presurgery, during, and after CPB in mixed venous blood of control subjects and in subjects receiving aprotinin. Analysis of mixed venous blood kallidin peptide levels across all time points showed no differences between the two groups of subjects (Fig. 5). Compared with presurgery levels, analysis of the pooled data for the two groups showed no change in mixed venous kallidin peptide levels during or after CPB, except for a 1.5-fold increase in Hyp3-KBK(+1–9) levels at 70 min of CPB in aprotinin-treated subjects (P < 0.01) (Fig. 5).

Plasma protein levels. Plasma protein levels were monitored as a measure of the degree of dilution that occurred with CPB. Plasma protein levels fell to ~50% of control at the commencement of CPB and remained at this level for the duration of CPB. Plasma protein levels partially recovered following CPB and were 69, 76, and 76% of control levels at 1, 4, and 10 h post-CPB.

Plasma kininogen levels. Kininogen levels were measured in control subjects (Fig. 6). CPB had no effect on HMWK levels. There was a small decrease in LMWK levels at 1 and 4 h post-CPB. This decrease in LMWK levels was most likely a consequence of plasma dilution during bypass. When expressed per milligram protein, LMWK levels did not show a decrease, but instead showed an increase above presurgery levels at 10 h post-CPB (Fig. 6). Plasma kininogen levels are not reported for the subjects who received aprotinin, because aprotinin interferes with the enzymatic assay for kininogen.

Plasma kallistatin levels. Plasma kallistatin levels were measured in control subjects and in subjects who received aprotinin (Fig. 7). There was a small decrease in kallistatin levels at 1 and 10 h post-CPB. This decrease in kallistatin levels was most likely a consequence of plasma dilution during bypass, because kallistatin levels were unchanged when expressed per milligram protein. There were no differences between control subjects and subjects who received aprotinin.

Circulating levels of plasma and tissue kallikrein. Circulating levels of total plasma kallikrein were measured in control subjects and in subjects who received aprotinin (Fig. 8). Plasma kallikrein levels were reduced to 15% of presurgery levels at 1 h post-CPB, and levels slowly increased thereafter. Plasma kallikrein levels were also reduced in aprotinin-treated subjects, although not to the extent seen in control subjects (P = 0.01 for kallikrein U/ml; P < 0.01 for kallikrein U/mg for comparison of control and aprotinin-treated subjects at 1 and 4 h post-CPB) (Fig. 8).

Circulating levels of total tissue kallikrein and tissue prokallikrein were measured in control subjects and in subjects who received aprotinin (Fig. 9). In control subjects, total tissue kallikrein levels were reduced to 28%, and tissue prokallikrein levels were reduced to 42% of presurgery levels at 1 h post-CPB, and levels slowly increased thereafter. There was a trend for changes in total tissue kallikrein and tissue prokallikrein levels to be less marked in the subjects who received aprotinin, although the levels were not statistically significantly different from those seen in control subjects.

The reduction in circulating levels of plasma and tissue kallikrein at 1 h post-CPB led us to perform further experiments to investigate the time course of these changes in a further three groups of subjects (Fig. 10). In the first group, we studied kallikrein levels in arterial blood sampled from the radial artery catheter Pre and after CPB. Blood samples during CPB were taken from mixed venous blood arriving at the bypass pump. Nonhydroxylated and hydroxylated peptides were summed to simplify presentation of kallikrein peptide levels. Data are means ± SE. ††P < 0.01, compared with presurgery level for aprotinin-treated subjects.
Plasma protein levels fell to an average of 88% of control immediately before CPB after 80 min of surgery and to 54% of control within 1 min of CPB. Plasma total kallikrein levels (corrected for dilution) fell to 65% of the presurgery level during the 80 min of surgery before CPB, with a further fall to ~20% of control during the first minute of CPB. Subsequently, there was a slow rise in plasma kallikrein levels during CPB with no difference in levels in mixed venous blood arriving at and in arterial blood leaving the pump at 1 and 2 min. However, at 15 min, plasma kallikrein levels were lower in mixed venous blood arriving at the pump than in arterial blood leaving the pump, whereas the opposite was the case at 60 min (Fig. 10).

Plasma levels of tissue kallikrein (corrected for dilution) showed a nonsignificant 10% fall during the 80 min of surgery before CPB and then fell to ~40% of control during the first minute of CPB. Tissue kallikrein levels remained at this level throughout CPB, with no significant differences between levels in mixed venous blood arriving at and in arterial blood leaving the pump (Fig. 10).

**DISCUSSION**

The main finding from this study was evidence for activation of both the plasma and tissue KKS during activation of the contact system by CPB. Evidence for activation of the plasma KKS was the marked increase in circulating bradykinin peptide levels and the reduction in circulating plasma kallikrein levels at the commencement of CPB. Bradykinin peptide levels were higher in arterial blood leaving the pump than in mixed venous blood arriving at the pump, indicating bradykinin peptide formation within the pump. Evidence for activation of the tissue KKS was the reduction in circulating tissue kallikrein levels, although kallidin peptide levels showed little change during CPB. Reductions in plasma kallikrein and tissue kallikrein levels during CPB (corrected for plasma protein concentration) were specific for these proteins and were not observed for kininogens or kallistatin. Raab and Kemme (32) recently reported that tissue prokallikrein binds to HMWK. Thus the colocalization of tissue prokallikrein with other components of the contact system provides support for the participation of tissue prokallikrein in contact system activation. We demonstrated that the levels of the B₁-receptor agonists Hyp³-BK₁-BK-(1–8) increased parallel with the levels of the B₂-receptor agonists Hyp³-BK₁-BK-(1–9), without evidence for enhanced conversion of Hyp³-BK₁-BK-(1–9) to Hyp³-BK₁-BK-(1–8). These data emphasize the potential for B₁-receptor agonist peptides to promote inflammation by acting on B₁ receptors induced by tissue injury due to surgery and the systemic inflammatory response to CPB. In addition, we showed that ACE inhibition did not amplify the kinin peptide response and that aprotinin blocked activation of the KKS by CPB.

The zymogen plasma prekallikrein is the main circulating form of plasma kallikrein (43, 46), and our data suggest that circulating tissue kallikrein is also predominantly in the zymogen form. Once activated, plasma kallikrein is rapidly inactivated by forming complexes with C1 inhibitor, α₂-macroglobulin, and...
antithrombin III (43, 46). The importance of C1 inhibitor is shown by the increased BK-(1–9) levels and angioedema that develop in subjects with C1-inhibitor deficiency (29). These complexes are rapidly cleared from plasma in vivo (46). In addition, tissue kallikrein is inactivated by complex formation with kallistatin (8). However, heparin inhibits complex formation between tissue kallikrein and kallistatin (8), and all of our subjects were fully heparinized; 10 min before CPB, thus preventing complex formation. We collected blood into heparinized tubes to prevent the formation of tissue kallikrein-kallistatin complexes during sample processing, although already formed tissue kallikrein-kallistatin complexes are stable in the presence of heparin (12, 13).

We used Western blots to quantify circulating kallikrein. The antisera 13G11 and anti-TproK-AS recognized both the zymogen and the activated forms of plasma and tissue kallikrein, respectively (22, 44), whereas antisera APP-11-AS recognized only tissue prokallikrein (22). Plasma prekallikrein and active kallikrein migrate similarly on nonreduced SDS-PAGE (44), as do tissue prokallikrein and active kallikrein on reduced SDS-PAGE (22). Thus migration on SDS-PAGE did not allow us to differentiate zymogen from active kallikrein. Given that the zymogens are the major forms of circulating kallikrein, the reduction in immunoreactive kallikrein levels observed in this study represents both the activation of plasma prekallikrein and tissue prokallikrein and the rapid clearance of active kallikreins from plasma. The essential role of activation of plasma prekallikrein and tissue prokallikrein in this process is demonstrated by the attenuation of the reduction in immunoreactive kallikrein levels by aprotinin. Previous studies have shown that aprotinin inhibits activation of factor XII (25), and by inhibiting the activation of plasma prekallikrein, it reduces the formation of C1 inhibitor-kallikrein complexes (2, 23, 45) and thus prevents the fall in circulating kallikrein levels. It is of note that plasma kallikrein levels fell to 65% of the presurgery level before CPB. This was likely to have been due to activation and clearance of plasma prekallikrein subsequent to activation of the contact system as part of the inflammatory response to tissue trauma during surgery (9).

Although markedly elevated at 10 min, the increase in circulating bradykinin peptide levels was not sustained, with the levels having fallen toward basal by 70 min of CPB. Given that kininogen levels were not modified by CPB, our data suggest that the reduction in bradykinin peptide levels was due to the marked fall in circulating plasma kallikrein levels at the commencement of CPB. Reduction in plasma kallikrein levels may be a major mechanism for the termination of bradykinin peptide formation during activation of the contact system. Given the rapidity of the fall in...
kallikrein levels, our data also suggest that the bradykinin peptide levels at the commencement of CPB may have been much higher than the levels measured after 10 min bypass. Kallikrein activation was terminated before significant consumption of kininogen occurred, and much higher and more prolonged elevation of kinin levels may have occurred if the activation of the KKS had not been terminated as rapidly as it was. For example, C1-inhibitor deficiency is associated with prolonged elevation of circulating BK-(1–9) levels due to impaired termination of kallikrein activation (29).

In contrast to the bradykinin peptide levels, kallidin peptide levels showed little change during CPB. The much lower levels of kallidin than bradykinin peptides during CPB were likely to have been due to the much lower circulating levels of tissue kallikrein than plasma kallikrein. When quantified by RIA, plasma levels of immunoreactive tissue kallikrein are reported to be 4 ng/ml (40), in contrast to immunoreactive plasma kallikrein levels of 50 μg/ml (37). Thus, when activated, the potential for circulating plasma kallikrein to contribute to kinin peptide formation is more than 1,000-fold greater than that for circulating tissue kallikrein. However, tissue kallikrein is mainly located at tissue sites (49), where it may participate in the local formation of kallidin peptides.

The circulating levels of both bradykinin and kallidin peptides were elevated during 10 h after CPB, indicating continued activation of the KKS after cessation of CPB. It is well recognized that CPB induces a systemic inflammatory response. CPB has been shown to induce complement activation, endotoxin release, leukocyte activation, the expression of adhesion molecules, and the release of many inflammatory mediators (47). The importance of CPB in this process is illustrated by the much lower levels of inflammatory mediators when coronary artery surgery is performed without CPB (41). The continuing activation of the KKS after CPB may be the consequence of contact system activation due to the systemic inflammation, and KKS activation may also contribute to the inflammatory process. Aprotinin is of value in cardiac surgery because it reduces postoperative bleeding by inhibiting plasmin (15). In addition, aprotinin may reduce the inflammatory response to CPB (47), and the present data indicate that part of this anti-inflammatory action of aprotinin may be due to inhibition of kinin peptide formation. Chao et al. (7) reported that plasma kallistatin levels are reduced in sepsis syndrome. However, we found that the more mild inflammation associated with CPB was not associated with change in plasma kallistatin levels.

ACE inhibition, although increasing basal kinin peptide levels, did not amplify the kinin peptide response to CPB, indicating that kinin peptides are unlikely to contribute to any hemodynamic instability during CPB associated with ACE inhibition. The effects of ACE inhibition on kinin peptide levels are dependent on the relative contribution of ACE to kinin peptide metabolism. We used a presurgery ANG II/ANG I ratio <0.15 mol/mol as evidence of effective ACE inhibition. Subjects with suppressed ANG II/ANG I ratio (<0.15 mol/mol) had increased bradykinin and kallidin peptide levels before surgery, indicative of a role for ACE in
kinin peptide metabolism in the basal state. We previously reported that ACE inhibition does not affect kallidin peptide levels in arterial blood (11). However, our previous study included both diabetic and nondiabetic subjects. We have preliminary data suggesting that diabetes may influence kallidin peptide levels, and we therefore excluded diabetic subjects from the present study to avoid any confounding influence of diabetes. We also excluded subjects with heart failure, because heart failure is associated with suppressed kallidin peptide levels (11). The failure of ACE inhibition to amplify the kinin peptide response to CPB may have been due to a greater role for kininases other than ACE in kinin peptide metabolism during CPB. It is evident from Figs. 2 and 4 that subjects receiving ACE inhibitor therapy tended to have higher kinin levels after CPB than subjects not receiving this therapy.

In previous studies of the activation of the KKS during CPB, plasma kallikrein, prekallikrein, and kallikrein-inhibitor levels were measured by enzymatic assays (24, 36, 48). However, the specificity of these assays is dependent on the substrate used, and there are difficulties in relating these in vitro assays to the activity of the KKS in vivo. In the present study, we used circulating kinin peptide levels as an index of the activity of the KKS. Moreover, by using highly specific Western blot procedures, we were able to make precise quantification of the circulating levels of kallikrein and kallistatin proteins. The enzymatic kininogen assay enabled measurement of intact HMWK and LMWK. In agreement with our findings, Kongsgaard et al. (24) reported an ~40% reduction in plasma prekallikrein levels (corrected for hemodilution) during CPB and recovery of these levels during the postoperative phase.

Most previous studies of circulating kinin peptide levels during CPB measured immunoreactive material without adequate precaution against artefactual generation of kinin peptides during blood collection and processing, as indicated by the very high basal levels of immunoreactive kinin reported in these studies (31, 42). More recently, using specific methodology, Cugno et al. (10) reported an increase in plasma BK-(1–9) levels from 1.9 to 5.7 fmol/ml at 15 min and a peak of 9.8 fmol/ml at the end of CPB. The BK-(1–9) level remained elevated at 7.1 fmol/ml at the end of the operation but returned to basal levels by 24 h after surgery. These authors measured only BK-(1–9) and did not measure Hyp3-BK-(1–9) or the B1-receptor agonists BK-(1–8) and Hyp3-BK-(1–8). The study of Cugno et al. (10) showed a smaller increase in circulating BK-(1–9) levels than that observed in the present study, suggesting a milder degree of activation of the contact system such that rapid depletion of plasma kallikrein may not have occurred, and therefore bradykinin peptide formation was not terminated early in CPB. The smaller increase in BK-(1–9) levels reported by these authors may have been due to the use of a different CPB circuit with less potential for activation of the contact system during CPB. Moreover, we provided evidence that the processes observed during CPB may apply to other causes of systemic inflammation such as bacterial sepsis (9). We showed that the activation of the KKS by CPB is self-limited and accompanied by a marked fall in circulating kallikrein levels. An important consequence of the marked reduction in plasma kallikrein levels is the attenuation of contact system activation, given the essential role of plasma kallikrein in this process (30). Moreover, we provide evidence that the tissue KKS participates in contact system activation. Although activation of tissue kallikrein did not cause any detectable increase in circulating kallidin peptide levels during CPB, we observed a small rise in arterial
kallidin levels post-CPB. Our data raise the hypothesis that activated tissue kallikrein may increase kallidin peptide formation at local tissue sites and thereby contribute to the inflammatory process. Although studies in animal models indicate a role for kinin peptides in sepsis-induced tissue injury (34), studies in humans show little effect of a B2-receptor antagonist in severe systemic inflammatory response syndrome and sepsis (14). If the kinin response to generalized sepsis were terminated as rapidly as seen in the present experiments with CPB, then this may explain why therapy with kinin receptor antagonists has little effect in this condition. Moreover, our demonstration that the activated KKS generates both B1- and B2-receptor agonist peptides in similar levels indicates that antagonists of both the B1 and B2 receptors may be required to block the inflammatory effects of these peptides.

**Perspectives**

Many aspects of the function of the KKS in vivo remain to be defined. There is uncertainty concerning the relative role of the circulating and tissue-bound forms of kallikrein in kinin peptide formation. Both plasma prekallikrein and tissue prokallikrein bind to HMWK, which binds to the surfaces of endothelial cells and neutrophils, suggesting that the contact system may play a major role in basal kinin peptide production in vivo. However, it is also possible that other mechanisms of kinin peptide production operate in vivo. An area for future research is the question of the relative benefit and harm that may result from activation of the KKS. Whereas the proinflammatory actions of kinin peptides are well recognized, activation of the KKS during cardiac surgery may produce benefits by protecting the heart from ischemia-reperfusion injury. Kinin receptor antagonists may be contraindicated in the setting of CPB and coronary artery surgery, given that B2-receptor inhibition impairs recovery from acute myocardial ischemia (28). Therefore, there may be situations where the use of kinin receptor antagonists to block the inflammatory effect of KKS activation may be detrimental to patient survival. However, specific antagonists of the B1 receptor may reduce the inflammatory response to KKS activation while preserving the beneficial effects of B2-receptor activation by this system.

We are grateful to Dr. J. Chao for the gift of monoclonal antibody G4C11. We are grateful to N. Tenis for technical assistance and to the cardiothoracic, anesthetic, perfusion, and intensive care staff for assistance with this project.

Dr. Chao is funded by National Institutes of Health Grant HL-44083. This project was funded by a grant from the National Health and Medical Research Council of Australia.

**REFERENCES**


35. Rothschild AM, Boden G, and Colman RW. Kininogen changes in human plasma following a test meal or insulin ad-


AJP-Regulatory Integrative Comp Physiol • VOL 281 • OCTOBER 2001 • www.ajpregu.org