Carboxypeptidase N concentration during cardiopulmonary bypass in humans

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Carboxypeptidase N (CPN) is an inactivator of anaphylatoxins and kinins, peptides implicated in the pathogenesis of complications in extracorporeal circulation. To investigate whether the level of CPN is altered during cardiopulmonary bypass (CPB) we studied 15 patients undergoing cardiac surgery utilizing CPB. The concentration of CPN decreased to about 48% of the initial value upon initiation of CPB and remained low throughout the procedure. A similar decrease was observed in the level of alkaline phosphatase, an enzyme that was measured to assess the degree of haemodilution. When the data were normalized for dilution, no difference in the concentration of CPN was observed during CPB. Moreover, no changes in the concentration of CPN were observed when protamine was given to neutralize heparin and none of the 15 patients experienced any side-effects of protamine administration. We conclude that the decrease in CPN during CPB was due primarily to dilution and not to changes in CPN synthesis or catabolism. Protamine administration is not associated with significant changes in the level of CPN in patients who have an asymptomatic reversal of heparin anticoagulation.

La carboxypeptidase N (CPN) est un inactivateur des anaphylatoxines et des kinines impliqué dans la pathogénèse des complications lors d'une circulation extracorporelle. Afin d'investiguer si le niveau de CPN est altéré durant la CEC, on a étudié 15 patients devant subir une chirurgie cardiaque sous CEC. La concentration de CPN a diminué jusqu'à 48% de la valeur initiale au début de la CEC et est demeurée basse durant la procédure. Une diminution similaire fut observée au niveau des phosphatases alcalines, un enzyme qui s'est mesuré afin d'évaluer le degré d'hémodilution. Quand les données furent normalisées pour la dilution, aucune différence dans la concentration de CPM ne fut observée durant la CEC. De plus, aucun changement dans la concentration de CPN ne fut observé quand la protamine fut administrée afin de neutraliser l'héparine et aucun des 15 patients n'a démontré des effets secondaires lors de l'administration de la protamine. On conclut que la diminution du CPN lors de la CEC était due principalement à la dilution et non aux changements de la synthèse de la CPN ou son catabolisme. L'administration de protamine n'est pas associée aux changements significatifs dans le niveau de CPN aux patients chez qui on a renversé l'anticoagulation due à l'héparine sans incident.

Key words
ANAESTHESIA: cardiac;
BLOOD: coagulation, protamine;
ENZYMES: carboxypeptidase N;
POLYPEPTIDES: anaphylatoxin, kinins.

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Plasma carboxypeptidase N (CPN), has also been called kininase I and anaphylatoxin inactivator because it inactivates kinins and anaphylatoxins.[1-3] Partial CPN deficiency has been associated with the development of angioedema/urticaria due to impaired inactivation of anaphylatoxins generated during complement activation.[4] Anaphylatoxins and kinins have been implicated in the pathogenesis of complications occurring during extracorporeal circulation.[5,6] These peptides may be released during cardiopulmonary bypass (CPB) due to activation of the complement and kallikrein systems that can occur upon contact of plasma components with foreign surfaces in the extracorporeal circuit or with the heparin-protamine complex.[7] Protamine is a potent, partially competitive, inhibitor of human CPN *in vitro*.[8] Heparin can reverse the inhibition of CPN by protamine.[8] According to a recent report protamine may also inhibit CPN *in vivo*.[9]

Because of the importance of CPN in protecting the circulation from the deleterious systemic effects of anaphylatoxins and kinins, we investigated whether the concentration of CPN is altered during CPB in humans.

TABLE Changes in serum protein concentrations during cardiopulmonary bypass (CPB)

	Patient 1			*Patient 2*		
Change	*Before CPB*	*30 min in CPB*	*Change %*	*Before CPB*	*30 min in CPB*	*Change %*
Carboxypeptidase N (mOD · min^{-1} · 30 μl^{-1})	24.5	13.0	−47	20.0	10.0	−50
Alkaline phosphatase (NU · dl^{-1})	42.0	21.0	−50	56.0	34.0	−39
Total protein (g · dl^{-1})	6.5	3.6	−45	6.3	3.8	−40
Albumin (g · dl^{-1})	3.1	1.8	−42	3.2	2.0	−37
α_1-globulin (g · dl^{-1})	0.3	0.1	−67	0.2	0.1	−50
α_2-globulin (g · dl^{-1})	1.0	0.5	−50	0.8	0.5	−37
β-globulin (g · dl^{-1})	1.1	0.8	−27	1.4	0.8	−43

Methods

The study protocol was approved by our Institutional Review Board and informed consent was obtained from all patients. Fifteen patients ASA physical status III–IV, aged 46–85 yr, scheduled to have cardiac surgery utilizing CPB, were studied. All patients were premedicated with morphine and scopolamine. Induction and maintenance of anaesthesia were standardized using weight-related dosages of sufentanil. Vecuronium bromide was used for neuromuscular blockade. Cardiopulmonary bypass was performed with a membrane oxygenator (Sarns 7000) with moderate hypothermia (rectal temperature of 27° to 29° C) and a flow of 2.2–2.4 L · min^{-1} · m^{-2}. The extracorporeal circuit was primed with 2,000 ml of isotonic crystalloid solution containing 1.15% albumin. Before vascular cannulation, anticoagulation was achieved with 300 U · kg^{-1} of heparin (from beef lung; The Upjohn Company, Kalamazoo, MI), supplemented as needed to maintain an activated clotting time of at least 480 s during CPB. Protamine sulfate (LyphoMed Inc., Rosemont, IL), 3 mg · kg^{-1} was injected over a ten-minute period after CPB.

Blood samples for CPN and alkaline phosphatase measurements were drawn from the radial artery or central venous catheter before induction of anaesthesia, ten minutes after induction, ten minutes after heparin, 1, 10 and 30 min after the start of CPB, and ten minutes after the end of protamine infusion. Alkaline phosphatase was measured to assess the degree of haemodilution. In some patients blood was drawn simultaneously from the radial artery and central venous circulation to study whether there was an arterial venous difference in the concentration of CPN. Blood samples obtained before CPB and after protamine administration were drawn into heparinized syringes. In addition, venous blood samples were obtained from 16 normal healthy volunteers 25 to 66 yrs of age. All samples were centrifuged and the plasma was divided into aliquots, frozen rapidly and stored at −70° C until assayed.

The activity of CPN in plasma was assayed with furylacryloyl (FA)-Ala-Lys substrate by measuring the decrease in absorbance at 336 nm (37° C) in a recording spectrophotometer and expressed in millioptical density units (mOD) · min^{-1} · 30 μl^{-1} of plasma.[10] The intra-assay coefficient of variation was 5.4% and the sensitivity 0.4 mOD · min^{-1} · 30 μl^{-1} of plasma. The activity of alkaline phosphatase in plasma was measured by hydrolysis of p-nitrophenylphosphate in a photometer at 410 nm and expressed in normalized units (NU) · dl^{-1}.[11] In two patients the concentrations of serum total protein, albumin and globulins were established by electrophoresis in agarose gel using the Beckman Paragon system,[12] before and after the start of CPB.

All data are presented as the mean ± SEM. Analysis of variance for repeated measures and the Bonferroni pairwise procedure were used to compare baseline with subsequent values.[13] Differences were considered to be significant at $P < 0.05$.

Results

The concentration of CPN in plasma decreased to about 48% of the initial value upon initiation of CPB (from 23.3 ± 1.2 to 11.1 ± 0.9 mOD · min^{-1} · 30 μl^{-1}) and remained constant throughout the procedure (Figure 1). A similar decrease was observed in the level of alkaline phosphatase (from 62.6 ± 4.1 to 33.0 ± 4.0 NU · dl^{-1}) (Figure 1). In order to correct the level of CPN in each sample for the effect of plasma dilution, we multiplied the enzyme activity by the factor: (alkaline phosphatase) baseline/(alkaline phosphatase) sample. When the data were normalized for dilution, no difference in the concentration of CPN was observed after the patients were placed on CPB (not shown). Furthermore, no changes in the concentration of CPN were observed when protamine was given to neutralize heparin after CPB (Figure 1). None of the 15 patients experienced any of the reported side-effects of protamine administration.

Measurements of serum protein, albumin and globulins revealed obvious dilution during CPB (Table). The

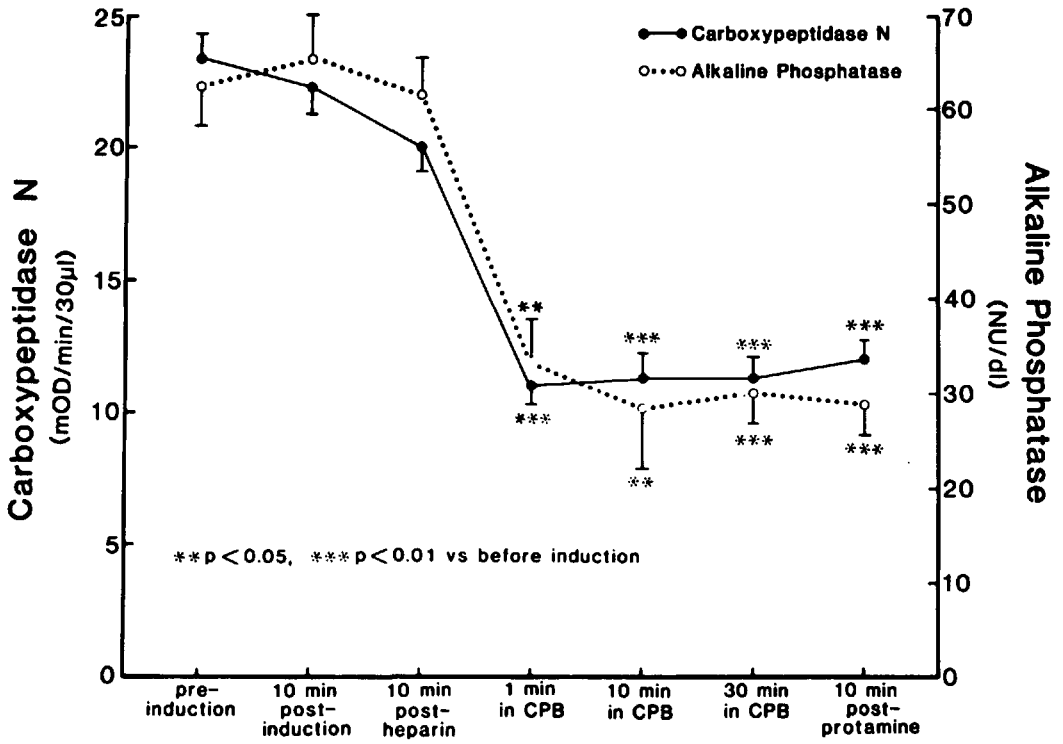

FIGURE 1 Carboxypeptidase N and alkaline phosphatase concentrations in plasma of patients undergoing CPB (mean ± SEM). *P* values were obtained with the Bonferroni pairwise procedure.

concentration of CPN in the plasma samples collected prior to induction of anaesthesia (23.3 ± 1.2 mOD · min^{-1} · 30 μl^{-1}) was not different from the level of CPN established in 16 normal volunteers during the period the experiments were conducted (21.7 ± 1.1 mOD · min^{-1} · 30 μl^{-1}).

The plasma concentration of CPN in these patients was unaffected by anaesthesia (Figure 1) and it was similar in blood drawn simultaneously from the radial artery and the central venous circulation either before or during CPB (arterial = 18.7 ± 1.6 mOD · min^{-1} · 30 μl^{-1}, venous = 20.2 ± 1.2; n = 9).

Discussion

The plasma concentration of CPN decreased upon initiation of CPB and remained low throughout the procedure. Based on a similar decrease seen in alkaline phosphatase and other plasma proteins we conclude that the decrease in CPN was due primarily to dilution. No changes in the concentration of CPN were observed after protamine administration in these patients who did not react to protamine.

Several complications have been associated with CPB surgery including the postperfusion lung syndrome characterized by interstitial oedema and vascular damage,[14,15] the prolonged bleeding time unexplained by haemodilution or inadequate reversal of heparin,[16–18] and the protamine reversal syndrome.[19–21] These have been attributed to activation of the complement system, coagulation factor XII, and the kallikrein-kinin system, and to thromboxane release.

Complement activation with release of anaphylatoxins (C3a, C4a, and C5a) may occur during CPB due to the action of blood oxygenators[22,23] and after CPB by interaction with the protamine-heparin complex[20,24] (Figure 2). Anaphylatoxins are potent vasoactive peptides which also liberate histamine, contract smooth muscles, increase vascular permeability and activate neutrophils to release inflammatory mediators such as thromboxanes.[25–27] Thromboxane has been implicated as a primary mediator of pulmonary hypertension and bronchoconstriction induced by protamine in some patients.[27]

Blood oxygenators and polyanions (heparin) can also activate factor XII which, in turn, activates both the complement system and plasma kallikrein.[6] Plasma kallikrein acting upon high molecular weight kininogen releases bradykinin. Bradykinin produces vasodilatation, bronchoconstriction and increases vascular permeability[28] (Figure 2).

The activity of anaphylatoxins and bradykinin is regulated in human plasma by a carboxypeptidase. The human plasma enzyme, CPN (EC 3.4.17.3), cleaves the C-

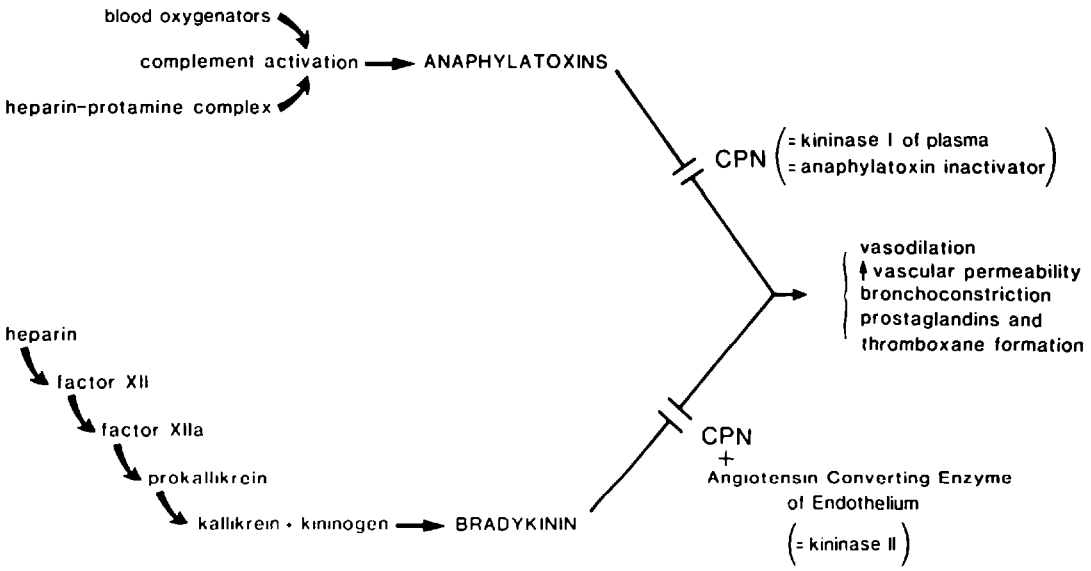

FIGURE 2 Cascade of events leading to the release of anaphylatoxins and bradykinin during and after CPB. CPN = carboxypeptidase N.

terminal lysine or arginine from a variety of peptides and proteins.[1,2,29] Because it was originally discovered as an enzyme which inactivates bradykinin by the release of the C-terminal arginine, it was first called kininase I.[2] Carboxypeptidase N is also called anaphylatoxin inactivator because it similarly inactivates these complement-derived peptides by cleaving the C-terminal arginine residue.[3] Carboxypeptidase N is considered to be an enzyme important for sustenance of life as no subject completely lacking the blood enzyme has ever been found. A major inactivator of bradykinin *in vivo* is angiotensin converting enzyme (kininase II) on vascular endothelial cells.[2,30] In conditions, such as CPB, in which the lung endothelial surface is excluded from the circulation,[31] CPN becomes more important as a kininase. This would also be the case in patients receiving inhibitors of angiotensin converting enzyme (e.g., captopril, enalapril) as therapy for hypertension or congestive heart failure.

In a previous study on the effect of CPB on anaphylatoxin inactivator (CPN) it was found that after normalization of the data for haemodilution, the level of anaphylatoxin inactivator decreased to 20% of the normal value during CPB.[32] Although this is contrary to our findings, differences in the materials used for extracorporeal circulation may explain this result. Carboxypeptidase N, a large 280 kDa macromolecule, is a tetramer that contains two low molecular weight (50 kDa) active subunits and two high molecular weight (83 kDa) glycosylated subunits that keep the enzyme in the circulation. If the CPN tetramer dissociates by the action of oxygenator or filter membranes, the low molecular weight subunits containing the active center could exit the circulation.[29] In addition, the method of correcting for haemodilution (which was not stated in the previous study) could have contributed to the difference.

Protamine given to neutralize heparin after extracorporeal circulation can trigger, episodically, undesirable haemodynamic responses. Some patients react mainly with pulmonary vasoconstriction, bronchoconstriction and systemic hypotension,[27,33] while others develop haemodynamic deterioration that resembles anaphylaxis.[33–35] Diabetic patients receiving protamine-containing insulin may react to protamine with true (IgE-mediated) anaphylaxis.[36,37] It has been suggested that complement activation by the protamine-heparin complex is the underlying mechanism of the adverse reactions, particularly in patients with no history of exposure to protamine.[19–22,24,27] In addition, as shown previously, protamine is a potent *in vitro* inhibitor of CPN.[8] In the present study we found that protamine given slowly over a 10-min period had no effect on CPN activity (measured *in vitro*), or the cardiovascular status of the patients. The apparent lack of inhibition of CPN by protamine might have been due to its complexing with circulating heparin. Heparin and CPN may compete for protamine *in vivo* because the addition of heparin abolishes the binding and inhibition of CPN by protamine *in vitro*.[8]

The incidence of protamine reactions has been reported to be 0.06–1%.[38,39] This low overall incidence of protamine reaction is consistent with the lack of adverse effects in our prospective study restricted to 15 patients.

It is hard to predict with certainty whether the decrease

in CPN concentration after CPB is sufficient to contribute to possible deleterious effects. In a majority of patients, this level of CPN is probably sufficient to inactivate harmful mediators generated during the procedure. This is based on the fact that a patient with the lowest level of CPN ever reported (21% of normal) had recurring bouts of angioedema associated with this deficiency.[4] Other family members with levels of about 25–60% of normal did not exhibit any symptoms attributable to the low CPN levels. Thus, a 50% reduction in CPN during CPB may not be sufficient to cause significant symptoms. However, in patients who might have a lower than normal level of CPN (either genetically determined or due to disease), a further 50% reduction could have dire consequences. In addition, in patients who produce abnormally high levels of anaphylatoxins and kinins during CPB and/or after protamine administration (which could partially inhibit CPN), a 50% reduction in CPN concentration could seriously impair their ability to inactivate these mediators.

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