Increased Circulating Bradykinin During Hypothermia and Cardiopulmonary Bypass in Children

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SUMMARY To determine whether cold could activate the kallikrein-kinin system in vivo as it does in vitro, the circulating systemic concentrations of bradykinin were serially measured in 10 children with congenital diseases of the heart undergoing corrective cardiac surgery. Bradykinin was measured by radioimmunoassay in blood samples obtained before, during and after profound hypothermia (to 18°C) and cardiopulmonary bypass. The circulating concentrations of bradykinin increased significantly as body temperature decreased during surface cooling. The increase in circulating bradykinin was associated with a decrease in the circulating level of bradykininogen, the precursor of bradykinin. With the onset of cardiopulmonary bypass and hence, removal of the lung and pulmonary converting enzyme from the circulation, there was a further rise in the already elevated concentrations of bradykinin. This is the first in vivo demonstration that hypothermia leads to an increase in the circulating concentrations of bradykinin.

COLD HAS BEEN ASSOCIATED with activation of the kallikrein-kinin system in vitro. Kallikrein, when activated, releases bradykinin from its circulating precursor, bradykininogen. Bradykinin is a vasoactive nonapeptide that produces vasodilatation and increases vascular permeability and is degraded by converting enzyme, an enzyme located on the luminal surface of vascular endothelial cells. Normally, the effects of bradykinin are short-lived because it is almost totally degraded in a single passage through the lung by pulmonary converting enzyme.

During corrective cardiac surgery in infants and small children, the patient is cooled to below 20°C and the lungs and, hence, pulmonary converting enzyme is removed from the circulation by cardiopulmonary bypass (CPB). We hypothesized that the hypothermia would result in the generation of bradykinin in vivo while the removal of the major site of converting enzyme, the lungs, by CPB would result in a delay in its degradation. Our study shows for the first time that 1) hypothermia increases circulating bradykinin in vivo; and 2) in hypothermic patients, removing pulmonary converting enzyme by CPB further increases the levels of circulating bradykinin.

Materials and Methods

Ten children ranging in age from 1 week to 16 months scheduled for deep hypothermic circulatory arrest for correction of congenital cardiac lesions were studied in accordance with ethical standards of the Committee on Human Investigation of our institution. Informed consent from the parents or guardians was obtained. Before and after CPB, blood samples (2 ml) were obtained from arterial cannulas (radial or femoral) as body temperature changed. During CPB, blood samples were obtained from the venous lines draining blood from the right atria to the bypass oxygenator. The circulating levels of bradykinin and bradykininogen were determined from these samples.

In all patients, a disposable Temptrol bubble oxygenator (Bentley) with a roller pump was used. A clear prime containing 1 liter of 2.5% dextrose water in Ringer's lactate and 50 g of salt-poor albumin or a blood prime containing 500 ml of ACD whole blood, 150 ml of 2.5% dextrose water in ½ normal saline, 2000 units of heparin, 15 mEq of sodium bicarbonate, 600 mg of calcium chloride, and 12.5 g of salt-poor albumin was perfused at approximately 200–210 ml/kg/min for children under 6 months of age and 2.3 l/min/m² for children over 6 months of age.

The average duration of prebypass cooling (the interval between the induction of anesthesia and CPB during which arterial and central venous catheters were inserted and surface cooling took place) was 100 ± 30 minutes, bypass cooling was 6 ± 2 minutes, hypothermic circulatory arrest was 66 ± 24 minutes and postbypass rewarming was 42 ± 16 minutes.

Bradykinin in the blood samples was extracted by the methods of Mashford and Roberts using several modifications described in detail elsewhere. In brief, blood kininase was immediately inactivated by drawing 1 ml of blood into cold plastic syringes containing 0.05 ml of 0.05 M phenanthroline in 95% ethanol. Within 20 seconds, bradykinin was separated from plasma proteins by denaturation and precipitation of the proteins by adding blood to tubes containing 4 volumes of 95% ethanol at room temperature. The tubes were then frozen in dry ice until further processing. Lipids were removed from the ethanol suspension with diethyl ether. The ethanol suspension was evaporated under reduced pressure then reconstituted in assay buffer. This was stored at −70°C until assayed.

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Radioimmunoassay of bradykinin was performed by the methods of Goodfriend and Odya and have been previously described in detail. In brief, diluted specimens were incubated with phosphate buffer containing lysozyme and phenanthroline, $^{125}$I-tyr-5-bradykinin as the trace and antibradykinin antibody at a final dilution of 1:20,000. After overnight incubation at $4^\circ$C, bound pep tide was separated from free peptide by precipitation with saturated ammonium sulfate solution and then counted in a well-type gamma counter. At each dilution, the assay was done in duplicate. The standard deviation of replicates from 10 standard curves was 0.24%. The sensitivity of the assay, defined as the lowest concentration of standard that produced a bound fraction significantly different from no bradykinin, was 20 pg/ml. Over the range of 10–300 pg/ml, the mean coefficient of variation was 7.20% (SD 0.95%). The normal circulating concentrations of bradykinin determined in 10 children was 1.0 ± 0.1 ng/ml. The anti-bradykinin antibody used was kindly supplied by Drs. Odya and Goodfriend and fully cross-reacted with active forms of kinin but cross-reacted by less than 0.01% with the des-arg$^9$-bradykinin or des-phe$^6$-arg$^9$-bradykinin metabolites.

Cirulating levels of bradykininogen, an alpha-2-macroglobulin, were determined by the methods of Diniz and Carvalho in nine of the patients. Bradykininogen was estimated by assaying the amount of bradykinin liberated from denatured plasma by an excess of trypsin. Heparinized blood samples were centrifuged and plasma was separated at 37°C in polypropylene tubes. Plasma was denatured by heating with dilute acetic acid at 90–96°C. The denatured plasma was adjusted to pH 7.8 by the addition of sodium hydroxide and Tris buffer. The plasma was incubated with crystalline trypsin for 30 minutes at 37°C and the reaction stopped by the addition of absolute alcohol at boiling temperature. After heating for 10 minutes at 70°C, the suspension was evaporated under reduced pressure at 35°C. The dried residue was reconstituted in assay buffer and bradykinin was radioimmunoassayed as described above. Plasma protein concentration was determined by refractometry.

Data are expressed as mean ± SEM and were analyzed using the $t$ test and regression analysis.

**Results**

The relation of circulating bradykininogen to body temperature in a representative patient is shown in figure 1. The prebypass cooling phase, which took 85 minutes, the body temperature decreased from 37°C to 26°C and the circulating level of bradykinin doubled from 1.1 to 2.1 ng/ml. With the removal of the lungs from the circulation during CPB and, hence, the removal of pulmonary converting enzyme, there was a dramatic increase in the circulating concentration of bradykinin from 2.1 to 5.8 ng/ml. During the bypass cooling phase, which took 6 minutes, bradykinin continued to rise as body temperature decreased. During the period of circulatory arrest lasting 50 minutes, corrective surgery took place. At the end of this period, the apparatus was reconnceted and bypass rewarming took place. At the beginning of bypass rewarming, there was a markedly elevated bradykinin concentration of 9.9 ng/ml, which probably represented a washout of the accumulated bradykinin. During bypass rewarming, which took 50 minutes, bradykinin progressively decreased as the body temperature and pulmonary blood flow slowly increased. After bypass (postoperatively), bradykinin returned to normal.

Figure 2 shows the relationship of circulating bradykininogen to body temperature in all 10 patients. The least-squares regression line for ungrouped data was calculated from the data. There was a significant rise in circulating bradykininogen (from 2.2 ± 0.2 ng/ml to 2.2 ± 0.2 ng/ml) during the prebypass cooling when body temperature decreased from 38°C to 26°C ($p < 0.01$). Bradykininogen increased significantly to 8.3 ± 1.2 ng/ml as body temperature rapidly fell during bypass cooling ($p < 0.01$). After circulatory arrest during bypass rewarming, bradykininogen decreased progressively from 9.2 ± 0.8 ng/ml to 2.7 ± 0.4 ng/ml as body temperature increased ($p < 0.01$). Although there was a wide range of bradykininogen concentrations during rewarming, in each patient there was a progressive fall in bradykinin. At the end of surgery, with the circulation intact and body temperature normal, bradykininogen returned to normal (1.2 ± 0.1 ng/ml).

The levels of bradykininogen in plasma before, during and after CPB are shown in figure 3. Each time point is compared with the preceding time point using the $t$ test for paired data. There is a significant decrease in the circulating level of bradykininogen from 9.9 to 8.5 ng/g protein by paired analysis ($p < 0.01$) during the prebypass cooling phase, with a further decrease to 7.6 ng/g with the onset of CPB ($p < 0.05$). As body temperature rapidly decreased during bypass cooling, circulating bradykininogen decreased further ($p < 0.05$). The change in bradykininogen levels during bypass rewarming was not significant. With the return of the circulation to normal, there was a significant increase ($p < 0.01$) in
circulating bradykininogen to 8.6 ± 0.4 μg/mg protein.

Figure 4 shows the relation of circulating concentrations of bradykinin to body temperatures grouped into quartile ranges during prebypass cooling. There was a significant increase in circulating bradykinin concentrations at body temperatures less than 35°C (p < 0.01) and further significant increases when body temperatures were less than 31.5°C (p < 0.01).

The concentrations of bradykinin in the priming fluid of the bypass apparatus and the circulating concentrations just before CPB are compared in figure 5. There is a significantly lower concentration of bradykinin in the priming fluid than in the circulation just before CPB (p < 0.01).

In four patients, simultaneous samples of blood entering and leaving the bypass apparatus were obtained for measurement of bradykininogen and bradykinin concentrations. There was no significant difference in the blood concentration of bradykinin entering (7.3 ± 2.4 ng/ml) and in blood leaving (6.1 ± 2.8 ng/ml) the bypass apparatus. There was no significant difference in the bradykininogen levels in these simultaneously drawn samples.
**Discussion**

This is the first in vivo demonstration of a significant rise in circulating concentrations of bradykinin in hypothermic patients. With previously available assay techniques, the in vitro activation of kallikrein by cold and, hence, an elevation of bradykinin, could be shown only after prolonged (usually overnight) storage at lower (usually 0°C) temperatures.\(^1\)\(^2\) Using a more sensitive technique of radioimmunoassay, we have demonstrated a significant rise in circulating bradykinin when body temperature was less than 35°C, a smaller reduction in temperature than those previously used.

When the pulmonary circulation and, hence, pulmonary converting enzyme, were bypassed, there was a further increase \((p < 0.01)\) in the already elevated level of circulating bradykinin. It has been reported that the plasma protein fractions used as priming fluid in the bypass apparatus can contain high levels of bradykinin.\(^10\) In order to ascertain that the initial rise in circulating bradykinin during the bypass cooling was due to the removal of pulmonary converting enzyme from the circulation and not to the addition of bradykinin from the priming fluid, we compared the concentrations of bradykinin in the priming fluid of the bypass apparatus and circulating concentrations just before CPB (fig. 5). The results indicate that the priming fluid was not the source of increased bradykinin and suggest that in the hypothermic patient, the initial rise in circulating bradykinin during bypass cooling was probably due to the removal of the major site of bradykinin clearance, the lungs, from the circulation.

Bradykinin is degraded by both converting enzyme (kininase II) and circulating kininases (kininase I). During CPB, when the major site of converting enzyme, the lung, is removed, extrapulmonary kininase activity becomes important in determining the level of circulating bradykinin. Nagaoka and Katori\(^11\) estimated that in adult patients with body temperatures of 33–36°C, extrapulmonary kininase activity during CPB was reduced by one half. Favre et al.,\(^12\) however, showed that converting enzyme activity at extrapulmonary sites when body temperature was 25–30°C was sufficient to form angiotensin II in quantities proportional to plasma renin activity. Although our study does not allow us to assess the extrapulmonary kininase activity, the observation that bradykinin levels continued to rise during bypass cooling suggests that extrapulmonary kininase activity was decreased or that bradykinin generation vastly exceeded bradykinin degradation.

In contrast to the studies of Nagaoka and Katori,\(^11\) we did not find a higher concentration of bradykinin and a lower concentration of bradykininogen in the blood leaving than in the blood entering the pump. Thus, we could find no evidence to indicate that events occurring within the bypass apparatus could account for the elevated concentrations of bradykinin in our patients. Our findings that elevated levels of bradykinin occur in these patients before the onset of CPB suggest that the generation of bradykinin is most likely due to the activation of kallikrein by cold.

Bradykinin has the properties of increasing vascular permeability to proteins and water and producing vasodilation. Chen\(^13\) reported that during surface cooling down to 24–25°C in dogs, hematocrits increased and plasma volumes decreased. These changes were measured under clinical conditions that are similar to those in our study, i.e., prebypass cooling, when we measured a twofold rise in the circulating level of bradykinin in the arterial circulation. In support of the notion that an elevated level of bradykinin produces an increase in vascular permeability and vasodilation during cardiac surgery, Nagaoka and co-workers\(^14\) showed an increase in hematocrit and a decrease in systemic vascular resistance in adult patients during CPB when bradykinin levels increased. When a kallikrein inhibitor, Trasylol, was given, no rise in hematocrit or fall in systemic vascular resistance could be shown. This suggests that the reported tendency for plasma proteins to fall, hematocrits to rise and water retention to occur in infants and children after deep hypothermic cardiac surgery\(^16\) could be attributed to the elevated circulating levels of bradykinin during hypothermia and cardiopulmonary bypass.

This study establishes that blood concentrations of bradykinin are elevated during hypothermic cardiopulmonary bypass; the elevated kinin levels have direct implications for fluid balance and blood pressure regulation in the postoperative period. The mechanisms by which kinin levels become elevated are not known but may include 1) the direct effect of cold on kallikrein activation, 2) the inhibition of kallikrein inhibitor activity and 3) cold-induced release of tissue factors, e.g., factor VII.

In summary, we have shown that cold increased the circulating level of bradykinin in vivo and that removing the lungs and, hence, pulmonary converting enzyme, from the circulation by cardiopulmonary bypass further increased circulating bradykinin. We
speculate that an elevated circulating level of bradykinin could produce circulatory instability and alter vascular permeability in children undergoing hypothermic cardiopulmonary bypass and circulatory arrest for corrective open heart surgery.

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