Extracorporeal Circulation Influence on Plasma Atrial Natriuretic Peptide Concentration in Cardiac Surgery Patients*

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An attempt was made to clarify the change of plasma human atrial natriuretic peptide (hANP) concentration before, during, and after cardiac surgery in heart failure with extracorporeal circulation. Plasma hANP concentration did not significantly decrease during total aortic cross clamping (ACC) with complete clamping of the superior and inferior vena cavae. This finding may be explained by the suppression of endopeptidase activities and the response of hANP receptors due to the low body temperature. Plasma hANP concentration strongly increased from 56.6 ± 8.4 to 208.9 ± 40.7 pg/ml (n = 5) by the release of total ACC. This strong increase of hANP in the plasma may occur due to the rapid increase of atrial pressure from zero to 12.5 mm Hg caused by releasing the total ACC. The molecular form of plasma hANP obtained after the release of total ACC was alpha-hANP alone, which was estimated by gel permeation chromatography and reverse HPLC.

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\[ hANP = \text{human atrial natriuretic peptide; ACC = aortic cross clamping; ECC = extracorporeal circulation; CPB = cardiopulmonary bypass; RIA = radioimmunoassay; RF-HPLC = reverse phase high performance liquid chromatography; GPC = gel permeation chromatography } \]

It has been well known that plasma atrial natriuretic peptide is secreted from the atrium of the heart.\(^1\) Plasma hANP concentration is known to increase in congestive heart failure patients,\(^2,3\) and hANP may be secreted not only from the atrium but also from the left ventricle in such patients.\(^4\) Plasma hANP shows a rapid turn-over in normal subjects,\(^5,6\) and hANP secretion is mainly controlled by the atrial stretch of pressure due to the change in endogenous hANP concentration in the plasma when no blood fills the heart.

The present study attempted to clarify the change in hANP concentration in plasma during total aortic cross clamping, and after the acute release of ACC in cardiac surgery using extracorporeal circulation (ECC).

**Patients and Methods**

**Patients**

Five heart disease patients who received surgery (three men and two women), whose ages ranged from 36 to 58 with a mean average of 48.2 ± 7.4 years (mean ± SD), were studied. Aortic valve replacement was performed on three patients who suffered from aortic regurgitation and mitral stenosis, mitral valve replacement was performed on one patient with mitral stenosis and tricuspid regurgitation, and one patient had removal of a left atrial myxoma.

**Methods of Operation and ECC**

After induction with morphine sulfate, anesthesia was maintained with Fentanyl and pancuronium. All patients had continuous monitoring by ECC, radial arterial pressure, central venous pressure, and left atrial pressure during surgery.

After a median sternotomy and pericardiotomy were performed, heparin was administered, and an arterial inflow catheter from the cardiopulmonary bypass pump was inserted into the ascending aorta. In each of the five patients, two Sarns F venous catheters were placed through the right atrium into the superior and inferior venae cavae, respectively, with tourniquets. A membrane oxygenator and Gambio roller pump were used for the CPB circuit, which was primed with 2 L of lactate Ringer's solution with 600 ml of whole blood. A Holofiber dialyzer united in the CPB circuit was used to prevent abnormal hemodilution. After satisfactory partial ECC was demonstrated, total ECC was obtained, without constriciting the cavae around the cannulae, using tape snares.

Both the superior and inferior vena cava were clamped, and then the ascending aorta was cross-clamped, and chilled cardioplegic solution was infused into either the ascending aorta or directly into the coronary ostia. The endpoint was core cooling to a rectal temperature of between 25° and 27°C. Myocardial protection was effected by multidose cardioplegia and topical hypothermia to a myocardial temperature of 15°C. After the surgery was completed, the patients were rewarmed and ECC was discontinued.

**Collection of Blood Samples**

Blood samples were obtained as follows: (1) before surgery but after the induction of anesthesia; (2) five minutes after starting partial ECC; (3) five minutes after starting total ECC; (4) 30 minutes after carrying out ACC; (5) 5 minutes after releasing ACC; (6) 45 minutes after releasing total ACC; (7) just before discontinuing ECC; and (8) 30 minutes after discontinuing ACC. The blood samples of periods (2) and (8) were obtained from the central vein catheter, and those of periods (3) through (7) were taken from the...
The arterial line of the CPB circuit into ice-chilled tubes containing 6 mg of EDTA-2K.

Radioimmunoassay

The concentration of hANP in plasma and urine was determined by radioimmunoassay as described previously. The assay buffer for RIA was 0.01 M phosphate buffer, pH 7.4, containing 0.14 M NaCl, 0.01 M sodium azide, and 11 mg/ml of heat-inactivated human serum albumin. This assay buffer was used to dissolve all reagents. The incubation mixture consisted of 0.1 ml of sample (varying amounts of plasma or urine and an appropriate amount of the assay buffer), 0.1 ml of anti-hANP serum (immunized with human alpha-hANP conjugated bovine thyroglobulin), and 0.1 ml of the assay buffer. After allowing the mixture to stand for 20 hours at 4°C, 0.05 ml of [125I] labeled human alpha-hANP (specific activity of 74 TBq/mmoll, 120 pg/ml) was added, followed by further incubation for 24 hours at 4°C. The bound and free ligands were separated by adding 0.5 ml of the assay buffer containing 10 μl of goat antirabbit gamma-globulin, 1 μl of normal rabbit serum, and 5 percent polyethylene glycol (mean Mr 7500), followed by incubation for 20 minutes at 4°C and centrifugation for 30 minutes at 3,000 rpm. The supernatant was discarded and the radioactivity in the sediment was counted. The amount in the sample was read from a standard curve obtained from an assay of alpha-hANP standard using the same procedure as above. Immunologic cross-reactivity of the antiserum with rat alpha-hANP (1-28), alpha-hANP (7-28), alpha-hANP (5-27), [Met0]alpha alpha-hANP, and beta-hANP was 100 percent, while cross-reactivity with rat alpha-hANP (5-25) and bovine thyroglobulin was less than 0.01 percent in molar ratios of these substances. About 30 to 40 percent of the total radioactivity of [125I] alpha-hANP was bound in the absence of unlabeled alpha-hANP. The minimum detectable quantity of hANP was 0.3 pg with 90 percent confidence, and the 50 percent-binding intercept of the standard curve was 3.6±0.3 pg/tube (mean ± SD, n=10). Serial dilutions of plasma and urine with the assay buffer gave curves parallel to the standard curves of authentic alpha-hANP as shown in Figure 1. The recovery of externally-added authentic alpha-hANP was 96 percent in plasma and 100 percent in urine. Gel permeation chromatography of plasma and urine samples gave one main active peak corresponding to authentic alpha-hANP, and no peak at the position of the void volume. Nonspecific binding of [125I] alpha-hANP was 2.7±0.4 percent (n=15) in plasma, 2.6±0.3 percent (n=15) in urine, and 2.7±0.4 percent (n=15) in the assay buffer, indicating that interference by substances in plasma and urine was not significant. The intraassay and interassay coefficients of variation (n=10) were 7.8 and 6.1 percent, respectively.

Reverse Phase High Performance Liquid Chromatography

The RP-HPLC was run on an octadeckylsilica column eluted with a linear gradient acetonitrile (15-60 percent) in 0.09 percent trifluoroacetic acid for over one hour. The flow rate was 0.7 ml/min and the fraction volume was 0.7 ml. The 0.3 ml plasma was adjusted to pH 3 with HC1. All of the acidified sample was applied to a cartridge (Sep-Pak C18), and the absorbed peptides were eluted with 50 percent methanol in 0.1 M acetic acid. The eluate was evaporated under a nitrogen gas stream and subjected to RP-HPLC. The elution positions of peptides were monitored by ultraviolet absorbance at 280 nm or by RIA.

Gel Permeation Chromatography

The GPC was performed using a column (2 x 45 cm). A 0.2 ml sample of plasma was diluted with HC1 and adjusted to 1 ml and pH 3. The acidified samples were directly charged onto the column and eluted with 0.5 M acetic acid, as previously reported. The elution positions were calculated by a peptide molecular weight calibration kit and authentic alpha- and beta-hANP.

Statistical analysis was performed using the paired Student's t-test.

RESULTS

As shown in Figure 1, an average of the rectal temperature and body temperature measured just after ECC decreased to 25°C at 30 minutes after carrying out ACC, and it recovered to a normal temperature by 45 minutes after the release of total ECC. An average of both the right and left atrial pressures decreased during surgery, and it reached zero pressure after total ACC with clamping of both the inferior and superior vena cavae. Atrial pressure recovered soon after the release of total ACC and closed the atrium, and it reached a slightly higher level after the operations.

The mean average plasma hANP concentration of the patients before surgery was 127.6±23.4 pg/ml (n=5, mean ± SEM). As shown in Figure 2, the plasma hANP concentration slightly but significantly decreased to 73.8±10.0 pg/ml at the start of total ECC. However, the concentration did not significantly change during total ACC. After the release of ECC, the concentration strongly increased to 208.9±40.7 pg/ml.
from 99.4±22.8 pg/ml at five and 45 minutes, respectively. Plasma hANP concentration still maintained a high level 30 minutes after the release of ECC. Details of data obtained from these patients are shown in Table 1. Atrial pressure right after release of ACC still showed zero, suggesting the atmospheric pressure, since the atrium was not yet closed at this time. The hANP concentration in the plasma increased with coronary re-perfusion when the heart and whole body temperature increased compared to stage 4.

A blood sample was obtained for the determination of hANP molecular forms at 45 minutes after the release of ACC. Alpha- and gamma-hANP peaks were noted in the plasma hANP chromatograms estimated by GPC and RP-HPLC, as shown in Figure 3.

**DISCUSSION**

Most patients examined showed high plasma hANP concentrations before surgery due to cardiac disease. During surgery, the mean average plasma hANP concentration decreased, but this decrement was not statistically significant even though during complete ECC. Surprisingly, the plasma hANP concentration did not degrade in the body while both atriums and ventricles were completely empty and no blood came from the coronary sinus. There are two possibilities to explain this result. One is that some organ(s) secretes hANP besides the heart, and the other is that the degradation of hANP in the plasma is extremely delayed. No organ except the brain has been reported to secrete hANP. The hANP content in the brain is much less than that in the atrium, and that in cerebrospinal fluid is also much less than in plasma.

In addition, hANP may not pass through the blood-CSF barrier. Thus, the possibility that organs other than the atrium secrete hANP to maintain the hANP level in the plasma is questionable. The second possibility is considerable. The half-life of hANP in plasma is only about two minutes at 37°C. However, the body temperature of these patients in the condition of total ACC was lowered to approximately 25°C and the heart was cooled to 15°C. Under these temperatures, endopeptidase activity in organs may decrease, and the biologic and catalytic receptors for hANP may be inactive. Thus, it is possible to explain prolonged hANP half-life in plasma during total ACC as a prolongation of hANP degradation time. Hodsmann et al. reported that the plasma hANP concentration slightly increased with a head-up tilt due to the small increase in right atrial pressure.

![Figure 2. Change of plasma atrial natriuretic peptide concentration during and after cardiac surgery. Vertical line indicates mean ± SEM.](image)

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**Table 1—Clinical Data of Patients Undergoing Cardiac Operation**

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Mean ± SEM: 10.2±36.6±127.6±4.4±31.8±82.9±25.6±73.8±23.8±56.6±

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Mean ± SEM: 28.8±99.4±10.4±32.2±208.8±12.4±35.8±176.4±10.0±35.6±196.6±

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*AP, atrial pressure (mm Hg); BT, body temperature (°C). Numbers indicate same categories as explained in Figure 1.*
Raine et al. found a linearly positive correlation between the plasma hANP concentration and mean RAP. In the present study, no correlation was found between the plasma hANP concentration and RAP in the patients, and the plasma hANP concentration strongly increased after the release of total ACC. This suggests that a rapid increase of RAP enhances hANP secretion from the atrium.

In conclusion, plasma hANP in patients during cardiac surgery was only slightly degraded, possibly due to the low body temperature in addition to no hANP secretion from the heart, and a rapid increase of RAP may enhance hANP secretion from the atrium. The finding of a strong increase in plasma alpha-hANP after the release of total ACC may be induced by a rapid increase of RAP.

REFERENCES