ATRIAL NATRIURETIC PEPTIDE RELEASE AT REST AND WITH EXERCISE AFTER CARDIAC TRANSPLANTATION WITH BICAVAL ANASTOMOSES

Bicaval anastomoses in orthotopic cardiac transplantation offer the advantage of preserving the right atrial geometry. To elucidate the impact of this anastomotic technique on atrial natriuretic peptide plasma levels at rest and with exercise, nine patients were submitted to a symptom-limited supine exercise test. Atrial natriuretic peptide plasma levels in samples obtained from the right atrium were elevated at rest (274.4 ± 60.4 pg/ml), at peak exercise (438.1 ± 71.7 pg/ml), and thereafter (328.1 ± 71.2 pg/ml) with respect to normal reference values of 21 ± 1 pg/ml at rest and 92 ± 14 at peak exercise. Renin, angiotensin, and aldosterone plasma levels were almost normal and did not indicate any pathologic processes in volume homeoostasis. Right-sided hemodynamic parameters were not correlated with atrial natriuretic peptide secretion. An adverse relationship between cold ischemic time of the donor organ and atrial natriuretic peptide release was found ($r = 0.88, p < 0.0008$), indicating that endocrine cardiocytes are sensitive to prolonged ischemia. Atrial natriuretic peptide release may thus be independent of the surgical approach, and other unique characteristics of the transplanted heart, such as denervation, are more likely to be responsible for elevated atrial natriuretic peptide plasma concentrations after orthotopic heart transplantation. (J THORAC CARDIOVASC SURG 1995; 110:1600-5)

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Atrial natriuretic peptide (ANP) is an important determinant in fluid and electrolyte homeostasis, regulating blood pressure and intravascular volume as a functional antagonist to vasopressin and the renin-angiotensin-aldosterone system.[1] ANP is synthesized and secreted by atrial cardiocytes, which are more frequent in the right atrium than in the left and are most abundant in the atrial appendage.[2] Excessive ANP plasma levels have been reported in patients with heart failure and also after standard cardiac transplantation.[1, 3-8] The stimulus for the disproportionate increase in plasma ANP level could be the elevated myocardial transmural pressure caused by the altered atrial anatomy and concomitantly changed hemodynamics associated with the composite right atrium.[1, 6, 9] This standard technique, originally described by Lower and Shumway,[10] comprises two atrial anastomoses, with a subsequent tissue increase of approximately 25% of the composite right atrium[8] and a larger than normal atrial diameter.[11] In a previous study,[11] we showed that bicaval anastomoses in heart transplantation, first introduced into clinical practice in 1989,[12] can normalize right atrial dimension compared with the conventional technique. The entire group from that study is included in this report. Bicaval anastomoses in cardiac transplantation provide a unique model of a normally configured right atrium with less secretory atrial tissue. Study of cardiac transplant recipients with bicaval instead of right atrial anastomoses may allow elucidation of the impact of the surgical approach on ANP plasma levels after cardiac transplantation.

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Methods

Patients. Nine consecutive patients with bicaval anastomoses were submitted to a graded, symptom-limited supine exercise test after routine endomyocardial biopsy

(EMB). The patients had been previously operated on according to a randomized protocol. Informed consent was obtained before the study from every patient. Demographic data of the studied subjects are depicted in Table I. The patients were free of rejection, as proved by EMB, and without clinical signs of congestive heart failure. All cardiac transplant recipients received a conventional triple-drug therapy consisting of cyclosporine (adjusted to a whole blood level of 250 to 300 ng/ml), azathioprine (1 to 2 mg/kg per day), and prednisone (0.2 mg/kg per day). Standard induction therapy with anti-T-lymphocyte globulin was done for 4 days. Prednisone was withdrawn as early as clinically possible.

Operative technique. The technique of bicaval anastomoses has been described in detail elsewhere.[12] In brief, the recipient is cannulated with separate caval cannulas as distally as possible with standard cardiopulmonary bypass and hypothermia of approximately 24° C. The heart is excised, leaving a 10 mm atrial cuff at the inferior vena cava and preserving the whole superior vena cava. Trimming is performed with respect to the donor heart size. Concerning the left atrium, only a small cuff combining the pulmonary venous ostia is left in place. The left atrial anastomosis is done with a continuous intraluminal suture of 3-0 Prolene (Ethicon, Inc., Somerville, N.J.). The superior vena cava is sutured with 5-0 Prolene interrupted sutures, and the inferior vena cava is anastomosed with a 4-0 Prolene running suture. The arterial anastomoses are performed conventionally with 3-0 Prolene sutures.

Exercise protocol and collection of blood samples. There was at least 1 hour of rest between EMB and the exercise testing on a supine exercise unit (Siemens 870 L; Siemens ag–Medizinische Technik, Hamburg, Germany). Workload was increased stepwise every 5 minutes by 25 W until either cardiopulmonary or physical exhaustion. Blood samples for hormone assays were obtained through a triple-lumen thermodilution pulmonary arterial catheter, which was inserted immediately after EMB. Blood samples were drawn from the right atrium at rest, at individual peak exercise, and 10 minutes later. They were collected in chilled tubes containing 10 mg ethylenediaminetetraacetic acid and were stored immediately on dry ice. After completion of the exercise test, the blood samples were centrifuged at 4° C for 20 minutes at 2600 rpm. The supernatant was stored at −80° C until hormone assay was done, according to the manufacturers' manual (Nichols Institute Diagnostics B.V., Wijchen, The Netherlands). Hemodynamic parameters were measured at each step in triplicate by the use of a Baxter REF 1 thermodilution computer (Baxter Healthcare Corp., Edwards Critical Care, Irvine, Calif.). The mean of three measurements was computed and used for subsequent evaluation.

Hormone assays

ANP. ANP was measured by a direct radioimmunoassay for human ANP (Nichols Institute Diagnostics). Free and bound hormone were separated by antibody–polyethylene glycol separation and centrifugation. After decantation of the supernatant, the pellet containing the bound fraction was counted. Quantitation was achieved by delayed addition of the iodinated tracer. Concentrations are expressed in picograms per milliliter after correction for dilution. The normal reference value for ANP at rest is 21 ± 1 pg/ml, as established in a recently published study.[3] The reference value for ANP secretion at exercise is 92 ± 14 pg/ml, established in the same study.[3] To our knowledge, there is no standard for postexercise ANP plasma level in healthy subjects.

Hormone levels were measured to exclude any influence on ANP levels resulting from disorders in the hormonal subsystems of the fluid homeostasis.

Antidiuretic hormone (Vasopressin). Arginine-vasopressin was measured with a double radioimmunoassay (Vasopressin Rapid RIA, RK-AR1, Bühlmann Labaratories Ltd., Schoenenbuch, Switzerland) by means of a modified method previously described by Glick and Kagan.[13] Plasma samples were extracted with ethanol. Values ranging from 0.8 to 6 pg/ml were considered normal.

Renin. Plasma renin concentration was measured with a commercially available radioimmunoassay detecting the active renin (Renin-IRMA Pasteur; Laboserv gmbh Diagnostica, Gießen, Germany). Supine normal values range from 10 to 30 pg/ml.

Aldosterone. Plasma aldosterone was measured by the use of a radioimmunoassay without previous extraction. This was achieved according to the concept of solid-phase specific antibody binding (Aldosterone-RIA, solid-phase radioimmunoassay, DPC; Biermann Diagnostics, Bad Nauheim, Germany). Supine normal values range from 12 to 125 pg/ml.

Statistical analysis. All values are expressed as the mean plus or minus the standard error of the mean, as an index of dispersion, unless otherwise specified. Linear regression was applied to determine the relationships between ANP plasma level and hemodynamic parameters. Statistical significance was analyzed with the Behrens-Fisher *t* test after evaluation of normal distribution according to the method of David.[14] A probability value less than 0.05 was considered significant.

Table I. *Patient characteristics*

	Bicaval (n = 9)
Age (yr)	43.6 ± 5.9
Height (cm)	180.2 ± 2.4
Weight (kg)	89.0 ± 5.0
Interval (mo)*	28.0 ± 4.6
Donor age (yr)	41.4 ± 5.8
Cold ischemic time (hr)	2.66 ± 0.29
Duration of heart failure (mo)†	25.0 ± 7.8
Cyclosporine blood level (ng/ml)	260 ± 66
Creatinine blood level (mg/dl)	1.5 ± 0.2

*Between heart transplantation and study.
†Before transplantation.

Results

Patient characteristics and work performance. Demographic data and the work performance of the studied subjects is compiled in Table I. The mean workload was 100 ± 33 W. Renal function, as reflected by creatinine blood level, and cyclosporine

Table II. *Hormonal response at rest, at peak exercise, and after exercise*

	Bicaval (n = 9)	*Reference value (supine)*
ANP		
Rest	274 ± 60.4	21 ± 1 pg/ml (ref. 3)*
Peak	438.1 ± 71.1	92 ± 14 pg/ml (ref. 3)*
After	338.1 ± 71.2	
ADH		
Rest	2.99 ± 0.73	0.8 pg/ml†
Peak	2.55 ± 0.67	6 pg/ml†
After	3.91 ± 0.82	
Renin		
Rest	20.44 ± 2.84	10 pg/ml†
Peak	25.78 ± 5.1	30 pg/ml†
After	23.44 ± 3.86	
Aldosterone		
Rest	125.4 ± 22.4	70 pg/ml†
Peak	164.8 ± 29.2	295 pg/ml†
After	148.1 ± 34.1	

*Significant, with $p < 0.05$.
†Not significant.

Table III. *Hemodynamic parameters at rest, at peak exercise, and after exercise*

	Bicaval
Right atrial pressure (mm Hg)	
Rest	6.4 ± 1.3
Peak	13.6 ± 1.8
After	8.2 ± 1.3
Mean pulmonary artery pressure (mm Hg)	
Rest	14.1 ± 1.5
Peak	31.7 ± 2.7
After	13.8 ± 1.5
Cardiac output (L/min)	
Rest	6.1 ± 0.5
Peak	11.6 ± 0.9
After	6.4 ± 0.61
Wedge pressure (mm Hg)	
Rest	8.6 ± 1.3
Peak	21.6 ± 7.8
After	8.1 ± 1.1
Pulmonary vein resistance ($dyn \cdot sec \cdot cm^{-5}$)	
Rest	74.7 ± 14.7
Peak	84.7 ± 10.4
After	98.8 ± 19.8

whole blood level were unobtrusive, with no noticeable variation from our standard transplant patients.

Hormonal response. The hormonal changes in blood obtained from the right atrium in response to exercise are presented in Table II. The mean ANP plasma level at rest was 274 ± 60.4 pg/ml; this was significantly elevated compared with the normal reference value of 21 ± 1 pg/ml ($p < 0.05$).[3] Furthermore, we obtained a value of 438.1 ± 71.7 pg/ml at peak workload. This is significantly elevated compared with the reference value of 92 ± 14 pg/ml in normal volunteers ($p < 0.05$).[3] After exercise, the ANP plasma level remained elevated at 338.1 ± 71.2 pg/ml. Because of a lack of normal reference values immediately after exercise, we compared this value with the normal standard of 21 ± 1 pg/ml and found it to be significantly elevated ($p < 0.05$). Vasopressin, renin, and aldosterone levels were in the normal range or only slightly elevated (Table II).

Hemodynamic data. Complete hemodynamic data are illustrated in Table III. Right atrial pressure increased 2.1-fold, from 6.4 ± 1.3 mm Hg at rest to 13.6 ± 1.8 mm Hg at exercise. After exercise, the right atrial pressure (8.2 ± 1.3 mm Hg) returned almost to the resting value. The mean pulmonary arterial pressure, on the other hand, increased from 14.1 ± 1.5 mm Hg to 31.7 ± 2.7 mm Hg at peak exercise, representing a 2.2-fold increase. However, cardiac output (6.1 ± 0.5 L/min) rose only to 11.6 ± 0.9 L/min at peak exercise. The wedge pressure increased from 8.6 ± 1.3 mm Hg to 21.6 ± 7.8 mm Hg at peak exercise. Pulmonary vascular resistance was uniformly low at rest, at peak exercise, and after exercise (Table III). We did not observe significant correlations by linear regression between ANP plasma levels and hemodynamic variables. An adverse correlation between cold ischemic time and ANP plasma levels obtained from the right atrium was found (Fig. 1).

Discussion

It is well established that ANP is predominantly synthesized and secreted by endocrine myocytes of the right atrium,[4, 5] but the mechanism of its release is still controversial.[15] Although ANP is secreted as pro-ANP 1-98, its entire biologic activity is mediated by the metabolite α-ANP,[15] which binds to cellular receptors identified in kidney, adrenal gland, endothelial cells, platelets, and brain.[1, 16, 17] The physiologic effect of ANP is the diminution of blood pressure by stimulation of natriuresis, with consequent volume reduction. This is achieved by an attenuated renal vascular tone with consecutive increased glomerular filtration and reduced osmolarity in the kidney.[18] Because renin secretion is inhibited, angiotensin and aldosterone plasma levels diminish, with a subsequent reduction of vascular tone in all vessels excluding the mesenteric vascular system.[19-22] A further known effect is the modulation of baroreceptor function, probably transmitted by central sympatholytics.[22, 23] These physiologic effects are important but limited mechanisms in the

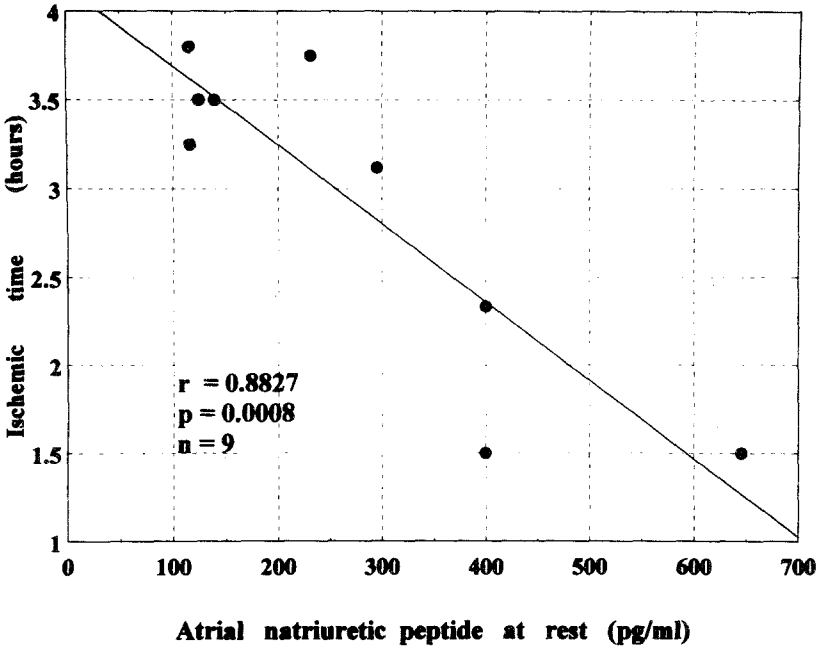

Fig. 1. Correlation between ANP levels obtained from the right atrium at rest and cold ischemic time of the donor organ.

response to the failing heart. Consistently, significantly increased ANP plasma levels have been reported not only in patients with cardiac failure but also, surprisingly, after orthotopic heart transplantation.[1, 6, 9]

ANP elevation after heart transplantation. The surgical approach itself has been addressed as one responsible factor for increased ANP plasma levels after transplantation.[1, 6, 9] Not only do the compound atria of donor and recipient show a cyclic torsion during the cardiac cycle, with increased transmural pressure,[24] they also result in atrial enlargement, as proved by echocardiography.[7, 11, 12, 24] A larger diameter may result in a higher transmural pressure, according to the law of Laplace, and thus be responsible for increased ANP secretion. A further important aspect may be the additional amount of atrial tissue associated with atrial anastomoses.[8] If ANP is reduced by right atrial appendectomy, as proven clinically in human beings[25] and experimentally in dogs,[26] it might be postulated that additional atrial tissue as an adjunct to altered atrial configuration increases ANP secretion after cardiac replacement. Because we showed in a previous study[11] that right atrial dimension is significantly reduced and normal after bicaval anastomoses, we believe that this patient population can serve as a model to study the impact of the surgical approach on ANP release after cardiac replacement.

On the basis of these observations, we hypothesized that patients with bicaval anastomoses and subsequent reduced atrial dimensions and tissue would have normal ANP plasma levels. Our findings do not support this assumption, however; we found elevated ANP concentrations in the studied group compared with normal reference values, as depicted in Table II (274 ± 60.4 pg/ml versus 21 ± 1 pg/ml, $p < 0.05$). The elevations of the ANP concentrations were comparable to those mentioned in patients after standard atrial heart transplantation.[1, 3, 6-8] Furthermore, a comparison of these data with additional data from patients with standard orthotopic heart transplantation ($n = 5$) who were also examined in this study clearly shows that patients with bicaval anastomoses do not have decreased ANP release (274 ± 60.4 pg/ml bicaval versus 226 ± 69.2 pg/ml atrial). The elevated ANP secretion after cardiac transplantation may therefore be caused by a more complex multifactorial process rather than simply a larger atrial tissue dimension or mass. Despite the lack of significant correlations between right atrial filling pressures and ANP plasma levels in this study, an association between right heart afterload and ANP secretion may still be possible, as

other investigators have proposed.[1, 6, 9] It cannot, however, be deduced from our data and limited patient number.

Total denervation and ANP release. This study gives clinical support to experimental findings in dogs[27] and rats[28] that totally denervated hearts are capable of excessive ANP secretion, because the use of bicaval anastomoses results in total denervation as a result of the total extirpation of the recipient's heart, in contrast to the standard technique in which innervated remnants of the right atrium remain. It has been proposed that the sympathetic system has a suppressive effect on ANP synthesis and that sympathetic denervation thus increases ANP release.[27] A hypersensitivity of ANP-synthesizing cells to circulating catecholamines may be a further possible mechanism.[7] Taking these and our results into account, one is tempted to assume that the altered innervation of the transplanted heart may play the major role in increased ANP secretion after heart replacement.

Cold ischemic time and ANP. Interestingly, a moderate and adverse correlation ($r = 0.88$) between ANP plasma level and cold ischemic time of the donor organ is evident (Fig. 1). Although we do not have a sufficient explanation at hand, this may indicate that ANP-secreting myocytes are sensitive to prolonged ischemic events. Further investigations including studies of myocardial contractile function are needed to elucidate the impact of ischemic time on different cardiac cell populations.

Study limitations. The most important limitation is the small sample size. Our results have to be interpreted carefully and need further investigations with larger patient numbers to fully elucidate the impact of the anastomotic technique on ANP secretion.

Conclusion. This study shows that the bicaval technique of cardiac transplantation, which, preserves the right atrial geometry, does not result in normal ANP release as long as 11.4 ± 2.3 months after operation. Whether the marked and excessive increase of ANP is related to denervation or to ischemic time remains to be evaluated in a larger series of patients.

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