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Mechanisms of Transplant Right Ventricular Dysfunction

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Objective

Right ventricular (RV) dysfunction remains the leading cause of early mortality after cardiac transplantation. The effect of brain death and subsequent hypothermic cardioplegic arrest and storage on subsequent post-transplant right ventricular function was examined.

Summary Background Data

Right ventricular dysfunction in the donor heart usually is attributed to failure of the donor right ventricle to adapt to the sudden increase in afterload (pulmonary vascular resistance) in the recipient. Strategies to improve ventricular mechanics in the postoperative period are aimed at reducing pulmonary vascular resistance with vasodilators or augmenting right ventricular contractility with inotropic agents. Events occurring in the donor heart (brain death, hypothermic cardioplegic arrest, and storage) also may be directly related to post-transplant RV dysfunction.

Methods

A canine model of brain death and orthotopic cardiac transplantation was used. A dynamic pressure-volume analysis of RV mechanics was performed using micromanometers and sonomicrometric dimension transducers. Systolic function was assessed by measurement of preload recruitable stroke work (PRSW). Brain death was induced in 17 dogs by inflation of an intracranial balloon. Right ventricular function then was assessed serially to 6 hours (PRSW). Right ventricular adrenergic β receptor density and function was sampled at control and after 6 hours of brain death. The effect of cardioplegic arrest and hypothermic storage was assessed in a second group of 17 dogs, using the same instrumentation and method of RV analysis.

Results

A significant decrease in right ventricular PRSW occurred after brain death, with the average decrease being 37% $\pm$ 10.4% from the control. The RV myocardial β adrenergic receptor density did not significantly change (253 $\pm$ 34 fmol/ng control *vs.* 336 $\pm$ 54 fmol/ng after brain death). The adenylyl cyclase activity of the RV β receptor was assessed and was not altered by brain death. Orthotopic transplantation after cardioplegic arrest and hypothermic storage significantly decreased RV PRSW from 23.6 $\pm$ 2.0 $\times$ 10^3 erg to 13.5 $\pm$ 1.4 $\times$ 10^3 erg.

Conclusions

These data indicate that the donor right ventricle is exposed to factors significantly detrimental to its mechanical performance well before facing an increased afterload in the recipient. Strategies to reduce RV dysfunction associated with brain death and hypothermic storage could positively impact post-transplant survival.

The leading cause of early mortality after cardiac transplantation is donor graft dysfunction. The overall operative mortality after orthotopic heart transplantation has remained unchanged at 10% for the past 5 years,[1] despite efforts to improve myocardial protection with the University of Wisconsin solution, the addition of metabolic precursors to cardioplegia, and strategies to minimize reperfusion injury of the transplanted heart. Clinically, donor right ventricular (RV) dysfunction remains more of a problem than left ventricular dysfunction, and usually is present to some degree after almost all orthotopic cardiac transplant procedures. It has been attributed to failure of the donor right ventricle to adapt to the sudden increase in afterload (increased pulmonary vascular resistance), which is present in the recipient as a consequence of long-standing LV dysfunction and congestive heart failure.[2]

Hemodynamic events in the donor, as a consequence of brain death, have been shown to have an adverse effect on cardiac function.[3–6] These changes occur even in controlled experimental conditions in the absence of hypovolemic shock or cardiac arrest.[7] Studies in potential clinical donors and in brain-dead animals have shown that brain death can have major histopathologic and functional effects on the myocardium.[8] Because of the wide variation in peripheral vascular resistance over the course of brain death, limited data are available that demonstrate the direct effect of brain death on myocardial contractility using load-independent assessment of ventricular function. Observations on specific RV functional changes associated with brain death are even more lacking. Although brain death-induced ventricular dysfunction in the donor would intuitively be related to post-transplant dysfunction in the recipient, limited data are available on this direct relationship,[9] with most observations limited to LV changes. Furthermore, little is known about the changes in β-adrenergic receptor density and function after brain death.

After being subjected to the deleterious effects of brain death in the donor, the transplanted right ventricle then undergoes a period of global ischemia, protected by hypothermic cardioplegic arrest. The right ventricle classically is believed to tolerate global ischemia better than the left ventricle because of lower energy requirements and wall stress. However, the transplanted right ventricle is immediately faced with an increased afterload after reperfusion and separation from cardiopulmonary bypass while recovering from the period of global ischemia required for the harvest, transport, and implantation. Intrinsic RV dysfunction and altered contractility associated with the period of cold preservation and subsequent orthotopic transplantation has not been characterized and would be a direct determinant of post-transplant RV dysfunction and the ability to adapt to increased pulmonary vascular resistance.

The purpose of this investigation was twofold. In one group of animals, the effect of brain death on intrinsic RV function was examined using a canine model and a load-independent pressure-volume assessment of RV mechanics. Right ventricular myocardial β-adrenergic receptor function also was studied in this group of animals as a possible mechanism of dysfunction. In a second group of animals, the effect of global ischemia with hypothermic cardioplegic arrest and orthotopic transplantation on subsequent RV function was studied, using similar analysis of ventricular mechanics.

METHODS

Experimental Brain Death Group

Anesthesia and Monitoring

Seventeen adult mongrel dogs (23–31 kg) were anesthetized with 5 mg/kg of intravenous thiopental and 20 mg/kg of intramuscular ketamine, supplemented as needed until brain death was induced. The animals were intubated and ventilated with a tidal volume of 15 mL/kg at an inspired oxygen concentration of 100%. The arterial pH, pO_2, and pCO_2 and the hematocrit and serum potassium levels were measured at hourly intervals. Metabolic acidosis was normalized with intravenous sodium bicarbonate, and the serum potassium was maintained between 4.0 and 5.0 mmol/L. An esophageal temperature probe was placed and body temperature was maintained between 36 C and 37 C throughout the experiments. The urinary bladder was catheterized to record urine output and electrocardiogram monitoring was performed from three limb electrodes. Electroencephalogram monitoring was recorded after needle electrodes were applied bilaterally to the parieto-occipital skull area and with a reference electrode in the cheek.

Study Design and Preparation

A median sternotomy and anterior pericardiotomy were performed to expose the heart. A transonic flow meter (Transonic Systems, Inc., Ithaca, NY) was applied around the main pulmonary artery to measure RV output. Hemispheric ultrasonic dimension transducers (1.5 mm outer diameter, Vernitron, Bedford, OH) were positioned across the base-apex major axis, anteroposterior minor axis of the left ventricle, and across the septal-free wall minor axis diameters of both the right and left ven-

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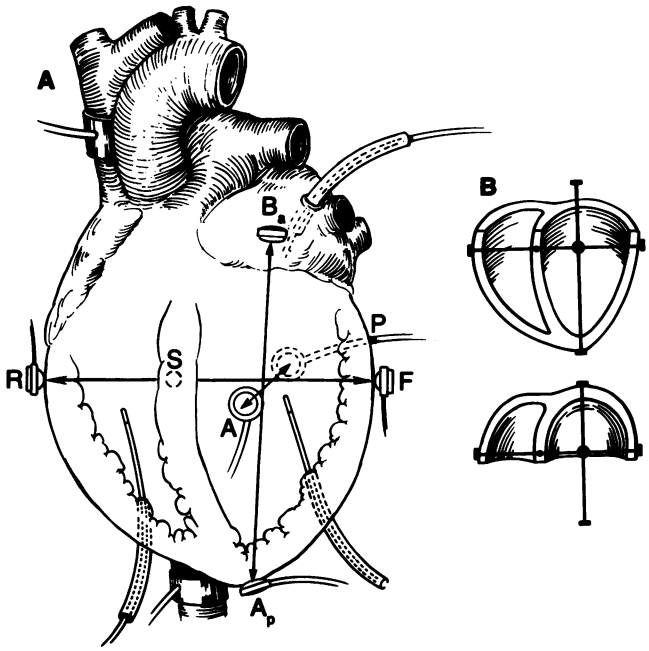

Figure 1. Model of biventricular assessment of ventricular function showing instrumentation of dimension tranducers to measure LV anterioposterior diameter, LV septal-free wall diameter, RV septal free-wall diameter, and LV base-apex diameter.

tricles to measure left and RV cavitary volumes. Millar pressure catheters (Millar Instruments, Houston, TX) were placed in the left and right ventricle, left atrium, and pulmonary artery for continuous pressure recording of left and RV pressure, end-diastolic right and left ventricular pressure, left atrial pressure, and pulmonary artery pressure (Fig. 1). Dynamic RV volume was measured according to the ellipsoidal shell subtraction method.[10] Systolic biventricular function and dimensional analysis was assessed by calculating global stroke work, and preload was measured as end-diastolic diameter or chamber volume. The linear relationship between stroke work and end-diastolic volume was quantified during vena caval occlusion.[11] The slope (preload recruitable stroke work [PRSW]) and x-intercept (volume) of this linear regression represent load-independent indices of right and LV systolic function and contractility (Fig. 2). Direct measurements of right and left ventricular filling pressure was taken at the end of diastole after the a-wave and was designated right and left ventricular end-diastolic pressure. The mean left atrial pressure was maintained at 6 to 10 mm Hg by infusion of lactated Ringer's solution.

Induction, Diagnosis, and Validation of Brain Death

Brain death was induced by an intracranial pressure rise accomplished by inflation of subdurally placed 16-F Foley balloon with 15 to 18 mL of saline infused over a 2- to 3-minute period. Anesthetic agents were discontinued after brain death was induced. Brain death was determined when cornea and pupillary reflexes became absent and spontaneous respiration ceased for 3 minutes (disconnection from the ventilator). Electroencephalogram changes were recorded, and the cessation of neuroelectrical brain activity by electroencephalogram monitoring was defined as the recorded unchanged oscillatory noise curve without high amplitude waves and spikes.

At termination of the experiment, a craniotomy was performed, and the cerebrum with the cerebellum and brain stem were extracted en bloc. The specimens were preserved for 7 to 14 days to perform a subsequent macroscopic-neuropathologic examination.

Data Acquisition and Analysis

Baseline hemodynamic and ventricular functional data were collected before and 15, 45, 90, 120, 240, 360,

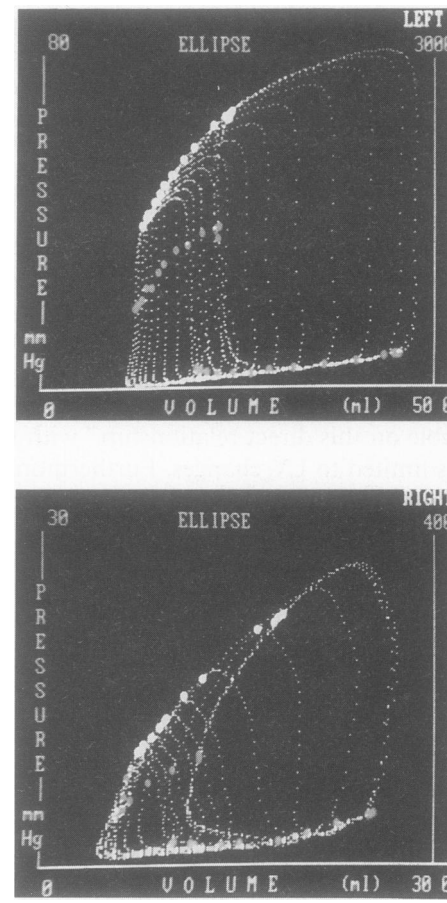

Figure 2. Pressure-volume loops (stroke work) plotted for each ventricle over consecutive cardiac cycles during occlusion of both venae cavae. x axis = intracavitary ventricular volume; y axis = intracavitary pressure. The slope of the linear regression of stroke work for each cardiac cycle is PRSW (preload recruitable stroke work).

and 420 minutes after brain death was induced. Functional and hemodynamic data were digitized on-line, collected, and stored on a microprocessor (PDP 11/23, Digital Equipment Corp., Maynard, MA). Pressure data and cardiac output were analyzed with software developed in our laboratory. All data were digitized at 500 Hz and filtered by a 50-Hz low-pass filter stored on magnetic media and analyzed on a Zenith 386/20 personal computer (Zenith Data Systems Corp., St. Joseph, MO).

Myocardial Biopsies

Two RV transmural excisional biopsies (100–120 mg) from the RV free wall were taken before and 6 to 7 hours after the induction of brain death. The biopsies were instantaneously frozen in liquid chlorodifluoromethane and stored in liquid nitrogen for analysis of the adrenergic receptor density and function. Four other canines were chosen as experimental animals but did not undergo brain death, and served as controls. Biopsies were taken from the heart in the control animals after instrumentation with pressure and dimension transducers was completed and at the end of an observation interval of 6 hours. Biopsies in the control group were processed as described previously, and β-adrenergic receptors were analyzed.

β-Adrenergic System

Myocardial membranes were prepared in the following manner: whole myocardial tissue samples were homogenized in 5 mL of ice-cold lysis buffer (5 mmol/L tris-HCl [pH 7.4], 5 mL edetic acid, leupeptin [10 μg/mL], and aprotinine 20 μg/mL). Nuclei and cellular debris were sedimented at 500 rpm for 15 minutes. The supernatant was then filtered and the membranes were pelleted by centrifugation at 40,000 rpm for 15 minutes. The membranes were washed with 5 mL of binding buffer (75 mmol/L tris-HCl, 12.5 mmol/L $MgCl_2$, and 2 mmol/L edetic acid) and resuspended in fresh binding buffer. Ligand binding assays were done in duplicate on membranes in 400 μl of binding buffer with saturating concentrations of the β-adrenergic receptor radioligand [^{125}I]-cyanopindolol, as previously described.[12]

Adenylate cyclase activity was determined under basal conditions, in the presence of progressively higher concentrations of isoproterenol (1×10^{-9} to 1×10^{-4} mol/L) or in the presence of 10 mmol/L sodium fluoride (NaF). Incubation was for 10 minutes at 37 C, reactions were terminated by the addition of 1 mL of ice-cold 0.4 mmol/L adenosine triphosphate (ATP), 0.3 mmol/L cyclic adenosine monophosphate (cAMP), and [H^3] cAMP (50,000 cpm/mL). Alpha-[P^{32}] ATP was isolated and quantitated as previously described.[13] Basal- and isoproterenol-stimulated cyclase activities for each membrane preparation were normalized as a percent of the activity achieved with 10 mmol/L sodium fluoride, which maximally activates stimulatory G-protein directly.

Statistical Analysis

Statistical analysis of data taken before and after brain death was performed with a standard two-tailed paired Students' t test. Baseline values and follow-up data were compared on an IBM (Cary, NC) personal computer using Stat View II (Abacus Concepts, Inc., Berkeley, CA). The results are expressed as mean and standard error of the mean (±SEM). A difference was considered statistically significant at $p \leq 0.05$.

Orthotopic Transplantation Group

Donor Preparation

Ten adult mongrel dogs underwent standard orthotopic transplantation using the atrioplasty technique described by Lower and Shumway.[14] The donors were prepared and anesthetized as described previously, and underwent biventricular instrumentation with pressure micromanometers, dimension transducers, and ultrasonic flow probes. Baseline data were obtained during transient venal caval occlusions. The data were digitized, stored, and analyzed as previously described in the experimental brain death group. The micromanometers and flow probes were removed from the heart before the animal was heparinized (350 units/kg). The ultrasonic crystal dimension transducers were disconnected from the sonomicrometer and the leads were wrapped together and protected. The heart was then removed as 1 L of St. Thomas's cardioplegia at 4 C was infused into the aortic root after cross-clamping the ascending aorta. The superior and inferior venae cavae, left and right pulmonary veins, pulmonary artery, and aorta were sequentially divided, and the heart was removed and immediately immersed in 4 C normal saline.

Recipient Preparation

The recipient animal received immunosuppressive therapy consisting of oral cyclosporine (10 mg/kg), azathioprine (2 mg/kg) and intravenous methylprednisolone (Solu-Medrol, Upjohn, Kalamazoo, MI; 25 mg/kg) 2 hours before induction of general anesthesia. The recipient animal was prepared for surgery in a manner identical to the donor, without placement of pressure, dimension, and flow transducers. Systemic anticoagulation was achieved with intravenous heparin (350 units/kg), and the animal was cannulated for cardiopulmonary bypass. The right femoral artery was exposed, and a 16-F arterial cannula was inserted and secured. Venous drainage was accomplished with bicaval cannulation

placed through extrapericardial segments of the inferior and superior venae cavae.

The recipient was placed on cardiopulmonary bypass using a membrane oxygenater, the circuit being primed with 1000 mL of crystalloid solution. Flow rates were kept at 80 to 100 mL/minute/kg and mean arterial blood pressure was maintained at 60 to 80 mm Hg. Blood was sampled regularly for arterial blood gas analysis and serum electrolyte determinations.

After initiating cardiopulmonary bypass, the recipient's aorta was cross-clamped, snares were tightened around the caval cannulae, and the cardiectomy was performed at the level of the atrioventricular groove with the great vessels divided just above the valves. The donor heart was then placed in the pericardial cavity, and all four anastomoses were completed using continuous 4.0 monofilament suture, anastomosing sequentially the left atrium, right atrium, aorta, and pulmonary artery. Deairing was accomplished before removal of the cross-clamp, using standard techniques. The flow probes and micromanometers were reapplied. The heart usually needed one direct current cardioversion and resumed a junctional rhythm. Atrial pacing wires were applied, and the heart was paced and cardiopulmonary bypass was discontinued after 25 minutes of reperfusion without requiring inotropic support.

Statistical Analysis

The experiment was designed to permit analysis between the two groups, with each animal acting as its own control. Statistical analysis was performed on a personal computer. To test significance of changes from pretransplant levels, a paired Students' t test was employed. Results are expressed as mean ± standard error of the mean, and statistical significance was considered when $p \leq 0.05$.

Experimental Approval and Animal Rights

The experimental set-up and procedures conformed to the guidelines established by the American Physiological Society and the National Institutes of Health (Guide for the Care and Use of Laboratory Animals, National Institutes of Health publication, revised 1985). The experiments were approved by the Duke University Institutional Animal Care and Use Committee (DUIACUC Registry #A477-93-10R3).

RESULTS

Hemodynamic Changes

Inflation of the subdurally placed balloon produced an intracranial pressure increase, global brain and brain stem ischemia, brain herniation, and compression of the mid brain and medulla oblongata, which interrupted neurologic pathways and intracranial blood supply. The Cushing reflex, characterized by a rise in systolic and diastolic blood pressures and bradycardia, was triggered in all 17 animals. In addition to bradycardia, other initial electrocardiographic changes included junctional escape beats and complete atrioventricular dissociation. This phenomenon was brief and, within 30 to 90 seconds of balloon inflation, was followed by a progressive tachycardia in combination with continued hypertension. Furthermore, cardiac output also was increased during this period. At the peak of this phenomenon, systolic blood pressure rose from a baseline value of 129 mm Hg (±4.6) to 402 mm Hg (±15.5), whereas diastolic blood pressure rose from 87 mm Hg (±4.0) to 246 mm Hg (±12.3). In 12 of the animals, systolic blood pressure increased to greater than 500 mm Hg, which actually was beyond the range of the recording device, whereas diastolic blood pressure ranged from 300 mm Hg to 360 mm Hg. Right ventricular systolic pressure increased from 30 mm Hg to almost 150 mm Hg. Furthermore, cardiac output rose to values of greater than 10 L/minute. The dominant arrhythmias during this peak period were supraventricular tachycardia and third-degre atrioventricular block. Furthermore, 50% of the animals developed acute severe ST-segment depression, all of which resolved spontaneously. The entire hyperdynamic response lasted anywhere from 8 to 20 minutes (mean = 12.8 ± 1.2 minutes) before heart rate and blood pressure declined to baseline values or below. Cardiac output remained elevated. The cardiovascular and hemodynamic changes occurring after brain death are summarized in Table 1. Diabetes insipidus occurred in all but one animal, with the urine output after brain death averaging 12.2 ± 0.8 mL/kg/hour.

Right Ventricular Function After Brain Death

Very high linear relations ($r > 0.95$) were obtained between calculated right and left ventricular volume and pressure-volume loops (stroke work) during transient vena caval occlusion before and after brain death. Baseline right ventricular PRSW ranged from 11 erg × 10^3 to 34 erg × 10^3 (mean = 22 ± 1.3 erg × 10^3), whereas baseline left ventricular PRSW ranged from 48 erg × 10^3 to 107 erg × 10^3 (mean = 75 ± 3.9 erg × 10^3). There was a significant decrease in biventricular PRSW values after brain death. The average decrease in right ventricular PRSW was 37% (±10.4), and in left ventricular PRSW, 22% (±7.3). These PRSW changes are summarized in Table 2. The percentage changes from baseline biventricular stroke work are demonstrated in Figure 3, whereas

Table 1. HEMODYNAMIC CHANGES AFTER BRAIN DEATH

Time (min)	0	120	240	360	420
HR (beats/min)	116 (3)	126 * (3)	121 (3)	123 (6)	104 (9)
MBP (mm Hg)	101 (4)	75 † (5)	62 † (5)	47 † (6)	31 † (9)
SYS (mm Hg)	129 (5)	95 † (5)	85 † (5)	79 † (4)	77 † (5)
DIA (mm Hg)	87 (4)	65 † (5)	50 † (4)	46 † (3)	44 † (3)
CO (mL/min)	1451 (87)	1439 (118)	1671 (177)	2049 * (234)	2058 * (303)
LVEDP (mm Hg)	6.3 (0.4)	7.3 (0.7)	9.0‡ (1.1)	9.4† (.05)	9.8† (0.3)
RVEDP (mm Hg)	1.2 (0.3)	1.4 (0.5)	2.2‡ (0.6)	3.0† (0.6)	3.7† (0.3)
SVR (dyne·sec·cm^{-5})	5752 (384)	4602 * (570)	3370 † (493)	1934 † (321)	1198 † (413)
PVR (dyne·sec·cm^{-5})	387 (37)	290 (38)	283 * (35)	287 * (29)	261 * (43)

HR = heart rate; MBP = mean arterial blood pressure; SYS = systolic; DIA = diastolic; CO = cardiac output; LVEDP = left ventricular end-diastolic pressure; RVEDP = right ventricular end-diastolic pressure; SVR = systemic vascular resistance; PVR = pulmonary vascular resistance.
* $p < 0.05$.
† $p < 0.001$.
‡ $p < 0.01$.
SEM in parentheses.

the changes in indices of RV function, slope (PRSW), and x-intercept (volume) occurring after brain death are displayed in Figure 4.

The β-Adrenergic Receptors

After brain death, β-adrenergic receptor density increased insignificantly from 282 fM/mg (±42) to 568 fM/mg (±173) in the right ventricle, and significantly from 291 fM/mg (±64) to 353 fM/mg (±56) in the left ventricle. No significant change in the β-adrenergic receptor density was observed in the control group (Fig. 5). There was an insignificant increase in unstimulated biventricular adenylate cyclase activity ($p = 0.1$) (Fig. 6). However, isoproterenol-stimulated adenylate cyclase activity increased significantly from 31.4% (±2.0) to 34.1% (±1.7) in the right ventricle and from 31.8% (±1.3) to 40.8% (±1.3) in the left ventricle. No significant change was observed in the control animals (Fig. 7). Increased adenylate cyclase activity was evaluated for all the prepared right and left ventricular biopsy membranes collectively. To achieve a 50% maximum isoproterenol-stimulated adenylate cyclase response (EC_{50}), the concentration of isoproterenol required was reduced from 295 nmol/L to 194 nmol/L for the right ventricle, and from 278 nmol/L to 185 nmol/L for the left ventricle following brain death ($p = NS$).

RV Function After Transplantation

Despite using contemporary techniques of myocardial preservation with hypothermic cardioplegic arrest and storage, orthotopic transplantation was shown to reduce RV contractility as measured by right ventricular PRSW by 43%. Total ischemic time was limited to 85.1 ± 2.8 minutes from harvest of the heart in the donor (non—

Table 2. CONTRACTILITY CHANGES AND PRELOAD-INDEPENDENT STROKE WORK AFTER BRAIN DEATH*

Time (min)	0	120	240	360	420
LV PRSW (erg 10^3)	75.0 (3)	58.1† (2)	56.5† (3)	61.9† (6)	56.7† (7)
RV PRSW (erg 10^3)	22.0 (5)	14.1† (1)	14.8† (1)	13.6† (1.3)	12.9† (3)
LV dP/dt (mm Hg/second)	1611 (93)	1180 ‡ (39)	1229 ‡ (45)	1230 ‡ (82)	1285 ‡ (294)
RV dP/dt (mm Hg/second)	401 (18)	310 § (10)	314 § (12)	359 § (22)	337 § (67)

* Left and right ventricular (LV/RV) PRSW changes (preload-independent systolic stroke work) and dP/dt (first derivative of left and right ventricular pressure) before (0) and after induction of brain death.
† $p < 0.001$.
‡ $p < 0.01$.
§ $p < 0.05$.
SEM in parentheses.

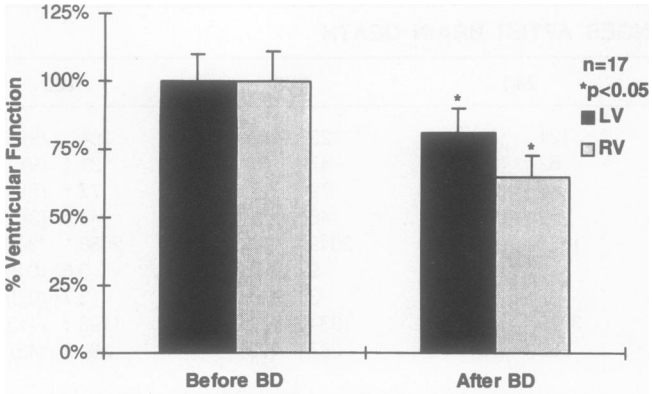

Figure 3. Cardiac dysfunction after brain death.

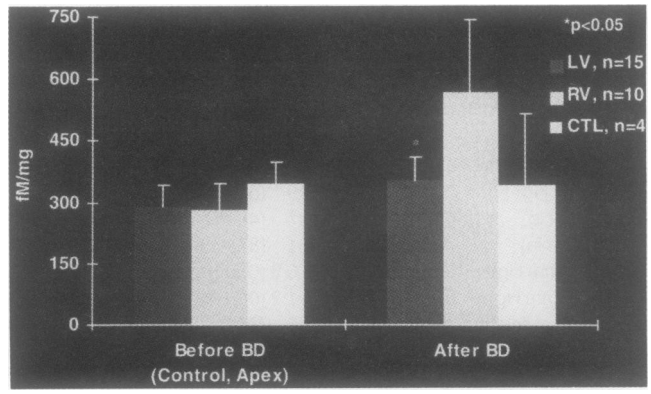

Figure 5. Beta-adrenoreceptor density before and after brain death.

brain-dead animal) to reperfusion in the recipient animal. Total cardiopulmonary bypass time was limited to 82 ± 6.6 minutes. A decrease in right ventricular PRSW was associated with the harvest, storage, and implantation with the control index of 23.6 ± 1.8 erg × 10^3 dropping to 13.5 ± 1.4 erg × 10^3 after weaning from cardiopulmonary bypass (Fig. 8). A similar decrease in left ventricular contractility was not seen (pretransplant left ventricular PRSW 66.2 ± 3.5, and post-transplant left ventricular PRSW 66.5 ± 2.6). This would indicate that myocardial preservation was not the explanation for the decrease in RV function associated with the transplant.

DISCUSSION

The influence of brain death on cardiac function, metabolism, and structure has been studied experimentally for more than 10 years. In 1984, Novitzky introduced an experimental baboon brain death model and described changes in LV function as assessed by dP/dt, histopathologic changes consisting of subendocardial necrosis, and a conversion from aerobic to anaerobic metabolism in the myocardium associated with hormonal alterations related to pituitary injury and dysfunction.[5] Other experimental models of brain death were subsequently established, with these studies showing varying degrees of the detrimental effect of brain death on cardiac function, myocardial metabolism, and peripheral hemodynamics.[15–17] In a clinical study of 172 donor hearts, Darracott-Cancovic investigated the association of myocardial damage in the donor with the recipient survival after cardiac transplantation.[9] The mortality rate of patients receiving hearts with impaired myocardial function before transplantation was 44%, compared with 6% for recipients of hearts with normal ventricular function.

The present study applied a highly sensitive and previously validated model of assessing global ventricular function in the setting of acute brain death established by sudden elevation of intracranial pressure. This allowed assessment of changes in intrinsic myocardial mechanics and function independent from the severe changes in peripheral loading conditions, which accompany the cate-

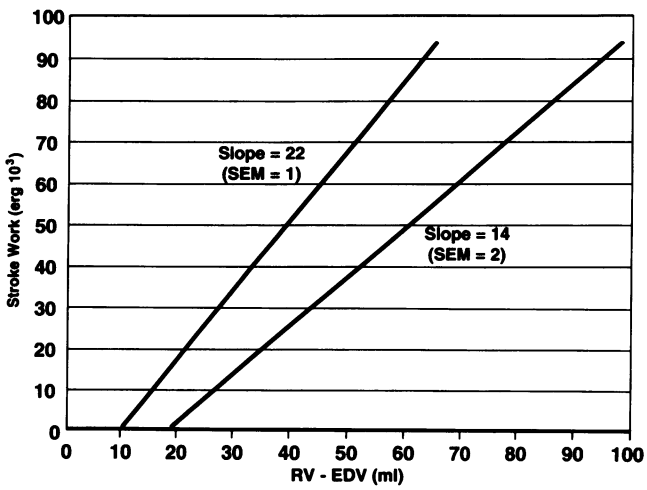

Figure 4. Decrease in slope of the linear relationship between RV stroke work and RV end-diastolic volume, reflecting a decrease in contractility.

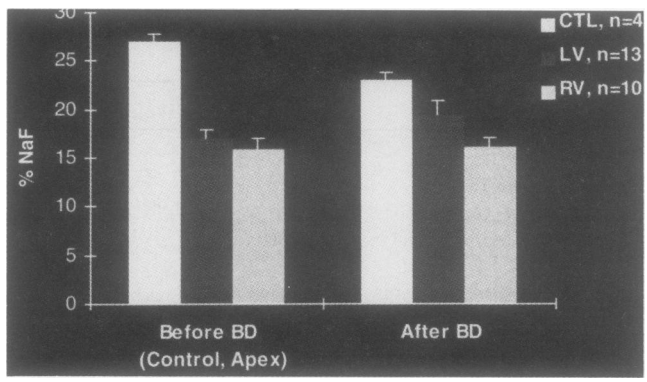

Figure 6. Beta-adrenoreceptor function after brain death basal adenylate cyclase (AC) activity.

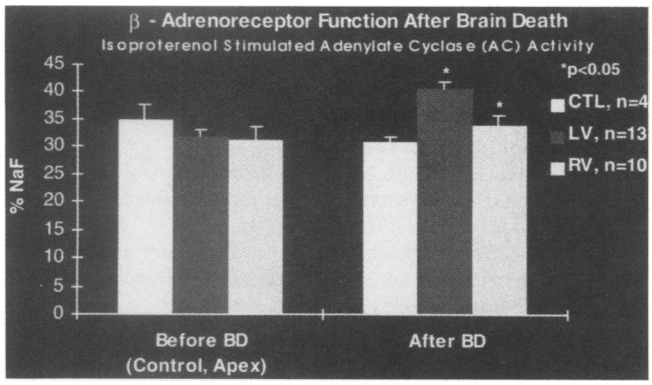

Figure 7. Effect of isoproterenol stimulation on adenylate cyclase activity before and after brain death.

cholamine storm of brain death (elevated peripheral and pulmonary vascular resistance), and the later loss of vasomotor tone and neurogenic control, resulting in decreased peripheral and pulmonary vascular resistance. The experiments outlined in this report document that brain death has a significant impact on cardiac function in the organ donor, and presumably influences subsequent ventricular performance after implantation in the recipient. After 6 hours of brain death, ventricular systolic function and contractility, expressed by the linear relationship of load-independent recruitable stroke work (PRSW), and cavitary volume were significantly decreased. This decrease in PRSW represents an objective loss of myocardial function of 37% for the right ventricle and 22% for the left ventricle. The effects of brain death on myocardial performance were assessed over this extended period of time (up to 7 hours), and no inotropic or vasoactive medications were applied while the measurements were being made. Under these experimental conditions, using indices of RV function not influenced by loading conditions, the intrinsic contractility of the ventricle during brain death could be directly assessed.

The etiology and mechanism of the deterioration in RV (and LV) function associated with the onset of brain death as produced in the study would appear to be related to the severe hyperdynamic and hypertensive state associated with the catecholamine release that accompanies acute increase in intracranial pressure. Previous investigators have associated the catecholamine increase occurring after brain death with myocardial injuries, ischemic events, myocardial infarctions, and hemodynamic instability, and death.[18,19] The molecular basis for catecholamine-mediated cardiotoxicity currently is unclear and appears to be multifaceted.[20]

The deterioration in ventricular mechanics and function after brain death was not related to dysfunction of the β-adrenergic receptor system, as demonstrated in the assessment of this myocardial receptor before and after brain death. In fact, an up-regulation of the myocardial β-adrenergic reception was found that consisted of increased β-adrenergic receptor density, increased isoproterenol-stimulated adenylate cyclase activity, and increased sensitivity to isoproterenol.

Predominant injury to right and left ventricular function associated with brain death appears to have occurred acutely during the hyperdynamic response, when systemic systolic blood pressure increased to approximately 500 mm Hg and the pulmonary artery systolic pressure increased to almost 150 mm Hg. These were associated with a fourfold increase in pulmonary vascular resistance and a twofold increase in peripheral vascular resistance. On a cellular level, the acute mechanical injury associated with such degrees of hypertension could result in sarcomeres becoming stretched beyond their normal working range. The disruption of the normal relationship of actin and myosin could result in reduction in the number of cross-bridge interactions and account for the subsequent decrease in mechanical function of the ventricle as assessed by the relationship between stroke work and end-diastolic volume.

To assess the effect of cardiac harvest with hypothermic cardioplegic preservation, a relatively short period of storage and subsequent orthotopic transplantation with cardiopulmonary bypass were assessed using the sensitive load-independent analysis of ventricular function based on on-line pressure-volume relationships. This aspect of the study was performed using non—brain-dead donors to separate the influence of brain death from the transplant operation on subsequent RV function in the recipient after orthotopic implantation. The results demonstrated that right ventricular PRSW dropped significantly from 23.6 ± 1.8 erg $\times 10^3$ in the donor animal, to 13.5 ± 1.4 erg $\times 10^3$ after transplantation and separation from cardiopulmonary bypass in the recipient animal. This drop of more than 40% was seen despite using contemporary techniques of cold cardioplegic arrest and storage, which have been shown to preserve biventricular function in isolated heart models using normal hearts

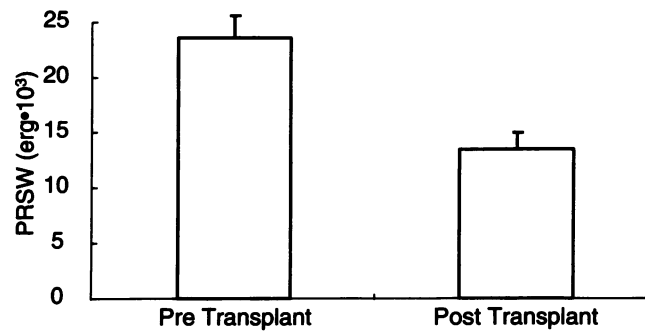

Figure 8. A decrease in PRSW, index of contractility, was seen after transplant in the experimental model for the RV.

subjected to 2 hours of global ischemia.[21] Indeed, a similar decrease in left ventricular PRSW was not observed in the same group of animals using the same technique of myocardial preservation. The explanation for this decrease in ventricular function associated with the transplant procedure is not clear. Recently, attention has been directed to the geometric distortion of the transplanted atria and atrioventricular valves as a result of the biatrial anastomosis technique originally introduced by Shumway and Lower. This is believed to result in varying degrees of valvular insufficiency and altered filling and efficiency of the ventricles caused by an abnormal anatomic configuration. For that reason, investigators have studied the bicaval and pulmonary venous anastomosis technique, and preliminary results have shown improvement in the degree of atrioventricular valvular regurgitation in a clinical review.[22] It is not known whether the bicaval and pulmonary venous anastomotic technique rather than orthotopic cardiac transplantation would change the altered contractility of the transplanted right ventricle by preserving the normal geometric configuration.

This study investigated the role of pretransplant brain death and the required period of "protected" global ischemia required for harvest and transplant as determinants of post-transplant RV dysfunction in the recipient. A significant biventricular dysfunction was shown to develop after induction of acute brain death, with the decrease in contractility of the right ventricle being more predominant than in the left. The mechanism of this altered systolic function is not related to down-regulation (decreased receptor density) or uncoupling (decreased function) of the myocardial β-adrenergic receptor system. The acute mechanical injury to the myocardium from nonphysiologic extremes in pressure and wall stress probably are related to subsequent patterns of ventricular injury. These experiments also demonstrate that the transplant procedure itself probably is a contributor to post-transplant ventricular dysfunction due to altered geometry of the right ventricle secondary to the atrioplasty anastomotic technique of implantation of the donor heart. Further studies are needed to examine how preventing acute mechanical injury to the ventricle during the catecholamine release and hyperdynamic phase of brain death could ameliorate subsequent post-transplant dysfunction. The concept of the bicaval and pulmonary venous anastomosis in implanting the donor heart also may prevent the post-transplant RV dysfunction that is commonly seen clinically and has been documented experimentally.

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References

1. Kaye MP. The registry of the International Society of Heart and Lung Transplantation: tenth official report—1993. J Heart Lung Transplant 1993; 12:541–548.
2. Kirklin JK, Naftel DC, McGiffin DC, et al. Analysis of morbid events and risk factors for death after cardiac transplantation. Am J Cell Cardiol 1988; 11:917–924.
3. Greenhoot JN, Reichenbach DD. Cardiac injury and subarachnoid hemorrhage: a clinical, pathological, and physiological condition. J Neurosurg 1969; 30:521–531.
4. Novitzky D, Cooper DKC, Reichart B. Hemodynamic and metabolic responses to hormonal therapy in brain dead potential organ donors. Transplantation 1987; 43:852–854.
5. Novitzky D, Wicomb WN, Cooper DKC, et al. Electrocardiographic, hemodynamic and endocrine changes occuring during experimental brain death in the Chacna baboon. J Heart Transplant 1984; 20:29–32.
6. D'Amico TA, Buchanan SA, Lucke JC, et al. The preservation of cardiac function after brain death: a myocardial pressure-dimension analysis. Surg Forum 1990; 41:277–279.
7. Meyers CN, D'Amico TA, Peterseim DJ, et al. Effects of triiodothyronine and vasopressin on cardiac function and myocardial blood flow after brain death. J Heart Lung Transplant 1993; 12: 68–80.
8. Kolin A, Norris JS. Myocardial damage from acute cerebral lesions. Stroke 1984; 15:1990–1993.
9. Darracott-Cancovic S, Stovin PGI, Wheeldon D, et al. Effect of donor heart damage on survival after transplantation. Eur J Cardiothoracic Surg 1989; 3:525–532.
10. Feneley M, Elbeery J, Gaynor JW, et al. Ellipsoidal shell subtraction model of right ventricular volume. Circ Res 1990; 67:1427–1436.
11. Glower DD, Spratt JA, Kabas JS, et al. Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. Circulation 1985; 71:994–1009.
12. Milano CA, Allen LF, Rockman HA, et al. Enhanced myocardial function in transgenic mice overexpressing the β_2-adrenergic receptor. Science 1994; 264:582–586.
13. Salomon Y, Lodos C, Rodbell M. A highly sensitive adenylate cyclase assay. Anal Biochem 1974; 58:541–548.
14. Lower RR, Shumway NE. Studies on orthotopic transplantation of the canine heart. Surg Forum 11:18–22.
15. Blaine EM, Tallman R, Frolicher D, et al. Vasopressin supplementation in a porcine model of brain-dead potential organ donors. Transplantation 1984; 38:459–464.
16. Huber TX, D'Alecy LG. A simplified organ donor model produced by permanent complete central nervous sytem ischemia in dogs. J Crit Care 1991; 6:12–18.
17. Galiñanes M, Hearse DJ. Brain deah-induced impairment of cardiac contractile performance can be reversed by explantation and may not preculde the use of hearts for transplantation. Circ Res 1992; 71:1213–1219.
18. Ferrans VJ, Hibbs RG, Weilbaecher DG, et al. A histochemical and electron microscopic study of epinephrine-induced myocardial mecrosis. J Mol Cell Cardiol 1970; 1:11–22.
19. Todd GL, Baroldi G, Pieper GM, et al. Experimental catecholamine-induced myocardial necrosis: I, morphology, quantification, and regional distribution of acute contraction band lesions. J Mol Cell Cardiol 1985; 17:317–338.
20. Bristow MR, Kantrowitz NE, Ginsburg R, et al. Beta-adrenergic function in heart muscle disease and heart failure. J Mol Cell Cardiol 1985; 17:41–52.
21. Elbeery JR, Lucke JC, Rankin JS, et al. Analysis of myocardial

function in orthotopic cardiac allografts after storage in UW solution. J Heart Lung Transplant 1991; 10:527–530.
22. Blanche C. Valenza M, Czer LS, et al. Orthotopic heart transplantation with bicaval and pulmonary venous anastomosis. Ann Thorac Surg 1994; 58:1505–1509.

Discussion

DR. W. RANDOLPH CHITWOOD, JR. (Greenville, North Carolina): Dr. Jurkiewicz, Dr. Copeland, Members, and Guests. I would like to congratulate Dr. Van Trigt on a well-designed and well-presented experimental study that attempts to elucidate some of the mechanisms relating to right heart failure after orthotopic cardiac transplantation.

It is well known that recipient pulmonary vascular resistance may offer a formidable afterload and, thus, strain to a heart that is just emerging from hibernation after a harvest that may have occurred many hours earlier.

The intent of this study was to assess factors, other than acute increases in afterload, as determinants of early right ventricular dysfunction after orthotopic cardiac transplantation. Dr. Van Trigt used sonomicrometric cardiac dimension analysis to assess preload recruitable stroke work, which is a load independent determinant of systolic function.

In this study, brain death was shown to have a significant and negative effect on right ventricular contractility. Right ventricular dysfunction was more pronounced than comparative left ventricular systolic impairment. In the cohort without brain death, but transplanted, right ventricular preload recruitable stroke work was reduced as well. Although not defined clearly, this deleterious effect resulted presumably from geometric distortion associated with the biatrial anastomosis.

The reviewer is concerned that there is not an explanation for a potential common mechanism of dysfunction between these two separate models.

My first question: As right ventricular adrenergic receptor density and responsiveness were not affected by brain death, was there any alteration in beta sites in the transplanted group?

Secondly, what would be the effect of combining the two experimental groups so that RV function was studied after transplanting a heart taken after being subjected to several hours of brain death?

Thirdly, could there be a genetic mechanism that switches adrenergic receptors or other effector sites either on or off in response to brain death?

Lastly, have you examined the effect of brain death on right ventricular diastolic function in these models?

Dr. Van Trigt, congratulations on your election to fellowship in the Southern Surgical Association and for the presentation of this provocative and clinically applicable experimental study. I wish to thank the Association for the privilege of discussing this important paper.

DR. WILLIAM L. HOLMAN (Birmingham, Alabama): Dr. Jurkiewicz and Dr. Copeland, Members, and Guests. This paper is important because it gets at one of the most vexing problems in cardiac transplantation—acute failure of the donor right ventricle.

In clinical practice, it is interesting that, although the right heart often struggles for the first few hours or days after implantation, its function then improves and, ultimately, the right ventricle preforms well in the vast majority of patients. Presumably, this recovery is due to an acute decrease in pulmonary vascular resistance, as well as an improvement in right ventricular contractility. Accelerating the improvement in right ventricular function or avoiding right ventricular dysfunction altogether, would obviously be a major advance.

I have two questions, Dr. Van Trigt.

First, did you record the temperature in the right ventricular freewall during the transplant procedure? Was topical hypothermia used as the heart was implanted in the recipient?

Second, did you separately examine the changes in the septal-right ventricular freewall dimension as a source for change in right ventricular performance after brain death or after the transplant operation?

As you know, abnormal septal motion is a common echocardiographic finding after transplantation, as well as after other cardiac operations.

I thank the Association for the privilege of discussing this paper. And, Dr. Van Trigt, I thank you for providing me with a copy of your group's excellent manuscript.

DR. ERLE H. AUSTIN III (Louisville, Kentucky): Dr. Jurkiewicz, Dr. Copeland, Members, and Guests. I also would like to congratulate the authors on what I think is an elegant study dealing with a very important aspect of cardiac transplantation.

Over the past 20 years, we have seen significant improvements in mid- and long-term survival after heart transplantation, but little improvement has occurred with early mortality, which, as Dr. Van Trigt has indicated, is primarily related to the function of the donor heart, especially the right ventricle.

For those of us involved in heart transplantation, there is nothing more frustrating than performing a cardiac transplant and discovering that the donor heart does not have the ability to sustain the recipient circulation.

In a canine model, Dr. Van Trigt and colleagues have demonstrated a major effect of brain death on contractility of the donor heart. I, for one, was amazed to see that the acute onset of brain death was associated with systemic systolic pressures of 400 mm of mercury and pulmonary pressures of 150 mm of mercury—a major workload that must place the donor heart under undue stress.

In this study, brain death alone resulted in a 37% decrease in right ventricular contractility, as measured by preload recruitable stroke work, today's state-of-the-art measurement for ventricular contractility.

In another group of animals, the process of harvesting, preservation and orthotopic implantation resulted in a 43% increase in right ventricular contractility. And by my calculations, by the time the donor heart is implanted, the right ventricle has lost almost 65% of its pre-braindeath contractility. In view of this information, it is amazing that we do as well as we do in clinical heart transplantation.

Nevertheless, the authors have developed a model that beautifully characterizes the effects of brain death as well as standard techniques of harvesting, preservation and implantation on right ventricular contractility.