Variable Effects of Explosive or Gradual Increase of Intracranial Pressure on Myocardial Structure and Function

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Background. Studies done in potential donors for heart transplantation and in experimental animals have suggested that brain death can have major histopathological and functional effects on the myocardium.

Methods and Results. We developed experimental models of brain death using dogs to study the hemodynamic and catecholamine changes, the extent of myocardial structural damage, and the recovery potential of donor hearts obtained from brain-dead donors. Brain death was caused by increasing the intracranial pressure (ICP) suddenly or gradually by injecting saline in an epidural Foley catheter. In a first series of experiments, dogs given a sudden rise in ICP (n=5) showed a hyperdynamic response and a 1,000-fold increase in the level of epinephrine after brain death. Histology revealed 93±2% of the myocardium to be severely ischemic. Dogs given a gradual rise in ICP (n=6) showed a lesser hyperdynamic response, almost 200-fold increase in the level of epinephrine after brain death, and mild ischemic damage to the myocardium (23±1%). In a second series, hearts obtained from brain-dead and non–brain-dead donors were transplanted in recipients, and the weaning and recovery potential were studied. All four recipients with hearts from non–brain-dead donors were weaned with good functional recovery. Also, all four recipients with hearts from brain-dead dogs given a gradual rise in ICP were weaned with only moderate functional recovery. However, only two of four recipients with hearts from donors given a sudden rise in ICP were weaned and showed poor functional recovery.

Conclusions. Our results indicate that a sudden rise in ICP can cause irreversible myocardial damage.

KEY WORDS • brain death • infarction, focal • donor potential

Donor hearts for transplantation are often from brain-dead patients who have suffered extensive central nervous system damage caused by subarachnoidal hemorrhage or traumatic brain injury. Most centers accept the clinical diagnosis of irreversible loss of brain stem function as brain death, which enables the heart to be excised while it is still beating within the donor. Sometimes hearts that appear to be macroscopically satisfactory indicate poor function and require inotropic support or retransplant. Data from the International Heart Transplant Registry show that 25% of all recipient deaths worldwide after transplantation were due to “cardiac failure” that was unrelated to acute rejection or infection.

There are a number of factors that could affect the posttransplant cardiac mortality, among them blood group matching, storage and transport of the donor heart, subsequent ischemic period, and the cause of donor death. Hearts obtained from donors who had suffered shorter periods of brain injury and with a subsequent shorter period of ischemia during transplantation were assessed as being good throughout transplantation and showed good functional recovery.

Studies in potential clinical donors and in experimental animals have suggested that brain death can have major histopathological and functional effects on the myocardium. Anatomopathological studies of the failed hearts very often reveal focal damage of the myocardium. These cardiac lesions are very typical, with petechial hemorrhage in the subendocardium, contraction bands, and coagulative myocytolysis. Such lesions were also found in patients dying from acute cerebral lesions in experimental situations on catecholamine administration or when brain death was induced by acutely increasing the intracranial pressure. These typical lesions are believed to be caused by a catecholamine excess that occurs during the pro-
cess of brain death and are thought to be related to a cytosolic calcium overload.\textsuperscript{12}

We developed experimental models to induce brain death by increasing the intracranial pressure (ICP) suddenly or gradually. The objectives of the experiments were 1) to study how a sudden or gradual increase in ICP causing traumatic brain injury affects the catecholamine levels, hemodynamics, and myocardial structure and 2) to study the weaning and donor potential of hearts obtained from brain-dead and non–brain-dead dogs after transplantation in recipient dogs.

Methods

Anesthesia and Monitoring

Eleven dogs (weight, 28±4 kg) received 0.25 ml/kg i.m. Hypnorm (10 mg/ml fluanisone plus 0.2 mg/ml fentanyl) as premedication. Anesthesia was induced with 10–20 mg/kg i.v. Nembutal (sodium pentobarbital). After endotracheal intubation, the dogs were artificially ventilated with a mixture of 30% oxygen in air. The minute volume ventilation was adapted to maintain an end-tidal P\textsubscript{CO\textsubscript{2}} of 30–40 mm Hg. A neurological examination was performed to test the brain stem reflexes (cornea and pupil light reflex). Cannulas were inserted to monitor peripheral arterial blood pressure (AP) and left ventricular pressure (LVP) and its first derivative (dP/dt). A Swan-Ganz catheter was inserted to measure the cardiac output (CO) at regular intervals. The EEG and ECG were recorded continuously. Arterial blood samples were taken at regular intervals to determine blood gases, the total and cardiac fractions of lactic dehydrogenase (LDH 1 and 2), creatine kinase (CK), and levels of circulating catecholamines epinephrine, norepinephrine (NE), and dopamine (DM). After baseline measurements, brain death was induced. The criteria for establishing brain death were 1) cerebral perfusion pressure (CPP=mean AP–ICP) ≤0 mm Hg, 2) isoelectric EEG, and 3) absent brain stem reflexes.

Biochemical Analysis

Plasma catecholamine levels were determined as follows: 5 ml of arterial blood was mixed with 100 μl of solution (9 g EGTA, 6 g glutathione, 100 ml distilled water at pH 7). This was centrifuged at 2,500 rpm for 5 minutes; the plasma was removed and stored at −30°C to prevent breakdown of catecholamines until assayed using high-performance liquid chromatography. Similarly, for the enzyme levels, 5 ml of arterial blood was mixed with 10 μl of heparin (5,000 U/ml, Novo Nordisk) and centrifuged, and the plasma was stored at −30°C until assayed using commercially available kits.

Histology

At the end of the experiment, all hearts were perfused in situ with 2% glutaraldehyde buffered to pH 7.4 with 0.1 M Sörensen phosphate. After excision of the heart, the atria were separated from the ventricles. The left ventricle was cut into five 1–1.5-cm-thick slices parallel to the apex. Toluidine blue–stained semithin (2-μm) sections and hematoxylin and eosin–stained semithick (6-μm) sections were used for the assessment of myocardial structural damage in the epicardium and the endocardium. Using a 10-mm grid with 100 squares, a semiquantitative analysis of the ischemic damage and microinfarctions was made per section. This was expressed as percentage of the entire area that was analyzed.

Experimental Protocol 1: Effects of ICP Changes on Hemodynamics, Catecholamine Levels, and Myocardial Structure

To study how a sudden or gradual increase in ICP that causes traumatic brain injury affects the catecholamine levels, hemodynamics, and myocardial structure, brain death was induced as follows: A burr hole was drilled in the two parietal bones 1 cm from the sagittal suture. In one hole, an epidural Foley catheter was inserted. A small incision was made in the dura mater of the second hole, through which a Martel catheter was introduced into the central part of the lateral ventricle for ICP measurements. The ICP was continuously recorded, and CPP (CPP=mean AP−ICP) was calculated at regular intervals. The ICP was increased rapidly or gradually by injecting saline in the Foley catheter to inflate the balloon of the catheter until brain death occurred.

Group 1A: Explosive increase in ICP. The ICP was raised acutely in five dogs by injecting 4-mI boluses of saline every hour in the Foley catheter until brain death occurred. Baseline arterial blood samples were taken for catecholamine, enzyme, and blood gas analysis. Later, blood samples were taken for the catecholamine levels at 1 minute, 2 minutes, 5 minutes, and 10 minutes after the injection of each bolus of saline. Enzyme levels and blood gases were determined every hour after the initial baseline samples. After brain death, the dogs were monitored until hemodynamic collapse. Finally, the hearts were perfused in situ with glutaraldehyde and excised for histological analysis.

Group 1B: Gradual increase in ICP. In six dogs, an infusion pump was used to infuse saline at a rate of 4 ml/hr in the Foley catheter to raise the ICP gradually until brain death occurred. Baseline arterial blood samples were taken for catecholamine, enzyme, and blood gas analysis. Later, blood samples were taken every hour until the CPP was 50 mm Hg. When the CPP was <50 mm Hg, samples for catecholamines were taken every 5 minutes; when the CPP was <20 mm Hg, samples were taken every minute until the CPP became negative. (The half-life of catecholamines is 2–3 minutes. The sampling time was therefore adjusted according to the changes in CPP and/or other hemodynamic parameters so as not to miss a possible catecholamine burst.) Samples for blood gases and enzymes were taken every hour after the baseline samples. After brain death, the dogs were monitored for up to 4 hours. Finally, the hearts were perfused in situ with glutaraldehyde and excised for histological analysis.

Experimental Protocol 2: Donor Heart Potential

To study the weaning and donor potential of hearts obtained from brain-dead and non–brain-dead dogs after transplantation in recipient dogs, 24 dogs (12 donors and 12 recipients) weighing 30±4 kg were used.

Group 2A. This group (n=4) contained non–brain-dead donors.


<table>
<thead>
<tr>
<th>Table 1: Protocol I: Hemodynamic Parameters: Baseline Values and After Brain Death</th>
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<tbody>
<tr>
<td>HR</td>
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<tr>
<td>C</td>
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<tr>
<td>------</td>
</tr>
<tr>
<td>66±10</td>
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<tr>
<td>Group 1A</td>
</tr>
<tr>
<td>79±17</td>
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<tr>
<td>Group 1B</td>
</tr>
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</table>

Average values±SD.
HR, heart rate (beats per minute); MAP, mean arterial pressure (mm Hg); ICP, intracranial pressure (mm Hg); dP/dt, first derivative of left ventricular pressure; CO, cardiac output (l/min); T°C, body temperature degrees Celsius; C, baseline measurements; BD, after brain death. *p<0.05.

**Group 2B.** This group (n=4) contained brain-dead donors. Donors suffered a gradual increase in ICP (saline infused at a rate of 10 ml/hr).

**Group 2C.** This group (n=4) contained brain-dead donors. Donors suffered a sudden increase in ICP (single bolus of 10 ml saline).

**Procedures**

The procedure for the induction of brain death in groups B and C was as described above (protocol 1). The sampling of arterial blood for enzyme and catecholamine levels for group 2B was the same as group 1B above, and for group 2C it was the same as group 1A above. For group 2A, samples were taken under baseline conditions and then every 5 minutes until excision of the heart. The hemodynamic parameters were monitored. A transmural left ventricular needle biopsy (Tru-Cut biopsy needle, Travenol Laboratories) was taken and fixed in glutaraldehyde after brain death was established.

The following standard procedure was carried out in all groups: 1) Within 1 hour after brain death, donor hearts were excised after receiving 11 of National Institutes of Health cardioplegia. 2) The hearts were stored in cold Ringer's solution (4°C) with a total ischemia period of 4 hours. 3) Orthotopic transplantation in a recipient was carried out with reperfusion time (on cardiopulmonary bypass) of 1 hour. 4) Support therapy was kept to a minimum (isoprenaline hydrochloride used to maintain the heart rate at 100 beats per minute; 0.5 g calcium chloride, 250 mg solumedrol, 20 mg xylcocaine, and 0.8 M bicarbonate as required). 5) Cardiac function was measured for 1 hour after weaning from bypass. The left atrial pressure was maintained at 10 mm Hg; further ECG, AP, LVP, and dP/dt were monitored, and CO was measured every 10 minutes. 6) Finally, the hearts were perfused and fixed in situ with glutaraldehyde and excised for histology.

**Statistics**

Values are given as mean±SD. The Mann-Whitney U test was used for two-tailed comparisons. A value of p<0.05 was considered statistically significant. The Kruskal-Wallis test was used to compare the three groups.13

**Results**

**Effect of Brain Death on Hemodynamics, Catecholamine Release, and Myocardial Structure: Protocol**

**Hemodynamics.** The baseline measurements were not significantly different (p>0.05) in groups 1A and 1B (see Table 1). The heart rate, mean arterial pressure (MAP), ICP, CO, and dP/dt values for both groups were within the normal range for dogs. After epidural insertion of the Foley catheter (volume, 1.5–2 ml), there was a slight increase in the ICP in both groups (group 1A: 18±9 mm Hg; group 1B: 23±9 mm Hg), with no changes observed in the other parameters. The ICP returned to baseline values in both groups within 5–10 minutes. When the increase in ICP commenced, the hemodynamics changed at a different rate in the two groups. In both groups, the degree of blood pressure response appeared to be directly related to the intensity of the stimulus, that is, the height to which the ICP was raised (see Table 1). The classical Cushing response was followed by a hyperdynamic state (increased CO, tachycardia, hypertension, and hyperpyrexia) when the dogs were brain dead. The body temperature increased by almost 2°C in both groups (see Table 1).

**Explosive increase in ICP (group 1A).** The hemodynamic profile of a typical experiment from this group where repeated bolus injections were made is shown in Figure 1A. After the first bolus of 4 ml, there was an increase in the ICP to a peak value of 82 mm Hg. Later, the ICP dropped sharply and settled at a new steady-state level. Apart from the heart rate, which showed a transient drop, there was practically no change in the other hemodynamic parameters. After 1 hour, another bolus of 4 ml was injected. Once again, the ICP raised instantly to 130 mm Hg, then decreased and settled at another steady-state level. A third injection produced qualitatively the same effect, i.e., a sudden increase followed by a rapid decline to a new steady-state level. Brain death was considered to occur when the CPP was ≤0 mm Hg. At this stage, a neurological deficit could be detected. In the example illustrated in Figure 1A, brain death occurred after the third bolus, which increased the ICP to a very high level (>250 mm Hg). In addition, the MAP increased and after a brief time lag reached its peak, which was higher than the ICP peak. This, too, dropped sharply and within 1 hour after brain death was equal to 56 mm Hg, less than 50% of the baseline value. The heart rate peak occurred shortly after the MAP peak. There was a tremendous increase in the hemodynamic parameters after brain death. The dP/dt increased to 12,000 mm Hg/sec (almost sixfold increase compared with the baseline value). The CO increased to three times its baseline value (see Table 1 for average values±SD for group 1A). Very similar changes were observed in all other dogs from this group. One dog of the five required two boluses (8 ml), one of five needed a third bolus (12 ml), and three of five required a fourth bolus (16 ml) before brain death occurred. The average volume required to induce brain death was 13.6±3.6 ml. The hyperdynamic state was observed in all dogs and
FIGURE 1. Panel A: Graphs show hemodynamic changes that occurred during brain death in a typical experiment from group IA, in which intracranial pressure (ICP, mm Hg) was raised suddenly. After baseline measurements, a single bolus of 4 ml of saline was injected at time 0 minutes, 60 minutes, and 120 minutes. The hyperdynamic response occurred after brain death. Horizontal line above the top panel indicates the period during which brain death became apparent. Only 1 hour after brain death, there was a tremendous drop in hemodynamic parameters. MAP, mean arterial pressure (mm Hg); HR, heart rate (beats per minute); A, epinephrine level (ng/ml-1); CO, cardiac output (l/min-1); dP/dt, first derivative of the left ventricular pressure (mm Hg/sec-1); BS, baseline values; FC, time when Foley catheter was introduced. Panel B: Light microscopy showing a typical example from group IA (i.e., dogs given a sudden rise in ICP); myocytes with irreversible ischemic damage and subsequent changes with a progression to focal necrosis are shown. The cytoplasm of the ischemic cells has a granular appearance and is lightly stained because of loss of contractile material; the mitochondria are swollen, and there is clumping of chromatin at the periphery of the nucleus. The arrow points to a cell with intracellular edema: the contractile material has almost disappeared, and the cell is about to burst open. Immediately below the swollen cell, a focal infarction with disintegrated cell membranes of a small group of myocytes can also be seen (Toluidine blue staining; magnification, ×416).

maintained for a variable period of time in this group. One hour (60±21 minutes) after brain death, MAP dropped to 65±20 mm Hg (from 206±70 mm Hg at the time of brain death). The heart rate, dP/dt, and CO also decreased rapidly to much lower values within the hour. This state of collapse was observed in all five dogs.

Gradual rise in ICP (group 1B). A different pattern of changes was observed in this group when the ICP was raised by a slow saline infusion. The hemodynamic profile of a typical experiment from this group is shown in Figure 2A. After starting the constant infusion at 4 ml/hr, there was a steady rise in the ICP that reached a value of 145 mm Hg in the example shown. The infusion pump was stopped when brain death was established (CPP≤0 mm Hg, negative brain stem reflexes and an isoelectric EEG). When the ICP approached 100 mm Hg, a transient drop occurred in the MAP. At this point, there was also a drop in heart rate. The pump was stopped when brain death was established. The ICP dropped slightly and then remained approximately constant. After the point where CPP was equal to 0 mm Hg, MAP increased, and after a brief time lag reached its peak, which was higher than the ICP peak. MAP dropped gradually and 2.5 hours after brain death was equal to 86 mm Hg. The heart rate peak occurred shortly after the MAP peak. There was an increase in the hemodynamic parameters after brain death. The dP/dt increased to 3,500 mm Hg/sec (twofold increase compared with baseline value). CO increased to almost three times its baseline value (see Table 1 for average values±SD for group 1B). The average volume required to induce brain death in this group was 10.1±1.5 ml. The ICP reached 130±39 mm Hg at the point of brain death (150±22 minutes after starting the infusion). The
maximum values of MAP and dP/dt observed in this group were significantly less \( (p<0.05) \) than those observed in group 1A. The dogs were monitored for variable periods after brain death. In this group almost 3 hours (170±25 minutes) after brain death, MAP was 87±25 mm Hg and heart rate was 113±6 beats per minute. Although a gradual decrease in the hemodynamic parameters was observed, this group remained relatively stable during the period that the dogs were monitored and did not show a tendency toward hemodynamic collapse.

**ECG**

Both groups had a baseline ECG with normal sinus rhythm. During the hyperdynamic state, there was sinus tachycardia with ventricular extrasystoles. In some cases, ST-T changes and supraventricular tachycardia were observed. The arrhythmias were observed after brain death occurred. The tachycardia persisted to the termination of the experiment, when there was a pronounced decline in the MAP. In group 1B, where the ICP was raised gradually, sinus tachycardia was observed; however, the incidence of other arrhythmias was much less.

**Neurological Deficit**

Initially, a normal EEG pattern was observed, and the brain stem reflexes were present in all dogs in both groups. When the CPP was <50 mm Hg (the ischemic threshold for normal brain), a decrease in the electrical activity was observed, manifested as a gradual flattening of the signal. Some dogs showed unilateral mydriasis when the CPP was in the low positive range (15–0 mm Hg). Temporally, the neurological deficit (bilateral mydriasis and absence of cornea reflex) corresponded well with flattening of the EEG and the lack of cerebral perfusion (CPP≤0 mm Hg): This is when brain death was established. Almost simultaneously, there was initiation of the hyperdynamic state. The same response was observed in both groups, although the degree of

*Figure 2. Panel A: Graphs show hemodynamic changes that occurred during brain death in a typical experiment from group 1B, in which intracranial pressure (ICP, mm Hg) was raised gradually. After baseline measurements, the infusion pump was started at time 0 minutes. The pump was stopped when brain death was established. Hyperdynamic response occurred after brain death. Vertical lines on the top and bottom panels and horizontal line above the top panel indicate the period during which brain death became apparent. In this particular experiment, 2.5 hours after brain death there was only a small change in hemodynamic parameters. MAP, mean arterial pressure (mm Hg); HR, heart rate (beats per minute); A, epinephrine level (ng/ml−1); CO, cardiac output (l/min); dP/dt, first derivative of the left ventricular pressure (mm Hg/min); BS, baseline values; FC, time when Foley catheter was introduced. Panel B: Light microscopy showing a typical example from group 1B (i.e., dogs given a gradual rise in ICP); normal myocytes and myocytes with some ischemic damage are shown. The arrow points to the swollen mitochondria as seen in the myocytes with mild ischemic damage (Toluidine blue staining; magnification, ×416).*
hemodynamic response was greater in group 1A. Figures 1A and 2A show the brain death zone, where a gradual change occurred with the flattening of the EEG and disappearance of the brain stem reflexes.

Biochemistry

**Catecholamines.** The levels of catecholamine before and after brain death are given in Table 2. Both groups had comparable levels at baseline, but there was a very big difference in the levels of circulating catecholamines detected after brain death.

**Group 1A: Explosive rise in ICP.** Every time the CPP dropped below the ischemic threshold (after each shot, with a sudden rise in ICP), an increase in the circulating catecholamines was observed (see Figure 1). One to 2 minutes after each injection, there was a rise in the catecholamine levels, which decreased greatly after 5 minutes and was almost equal to the baseline values after 10 minutes. The highest catecholamine level (see Figure 1A, top panel) was detected after the bolus that raised the ICP to a value high enough to cause brain death. In this particular experiment, a 1,200-fold increase in the epinephrine level was noted. The average values for this group were: epinephrine, 750-fold; NE, 400-fold versus control levels (see Table 2). These were detected 1–2 minutes after the last bolus that induced brain death. These levels had fallen to very low values at 60±21 minutes after brain death. No substantial increases were observed for DM.

**Group 1B: Gradual rise in ICP.** No increases in the circulating catecholamine levels were detected until the ICP was >50 mm Hg. Steadily increasing levels were noted when 50 mm Hg>CPP>0 mm Hg. Once again, highest levels were noted (average: epinephrine, 175-fold; NE, 40-fold control levels) when brain death occurred. These levels were significantly less than those observed in group 1A (p<0.05). At the end of the experiment, the levels had dropped to baseline values. No substantial increases were observed for DM. The results from a typical experiment of group 1B, with a 100-fold increase in the epinephrine levels that occurred when there was brain death, are shown in Figure 2A.

**Enzymes.** In both groups, the LDH levels remained in the normal range (80–240 units/l) throughout the experiment. The CK control values were within the normal range (48–240 units/l) but increased after burr holes were drilled in the skull (group 1A: 380±120; group 1B: 1,096±1,140). All animals maintained this elevation until the end of the experiment (group 1A: 400±273; group 1B: 1,109±1,204) with no significant changes after brain death.

**Histology**

Light microscopy revealed normal myocardium and also varying degree of focal ischemic damage: There was altered staining of the cytoplasm with granular appearance (which could be due to loss of contractile material). Higher magnification revealed myofibrils of varying degree, clarification of the nucleus, swollen mitochondria, and microinfarctions. No infiltration by inflammatory cells was observed, nor was fibrous tissue formation observed. In group 1A, 93% of the entire area analyzed revealed ischemic damage (severe myo- cytosilation with swollen mitochondria and necrotic nuclei; see Figure 1B). Intracellular edema leading to microinfarctions was also observed, with 1% of the entire area analyzed showing focal infarctions. In group 1B, on the other hand, normal myocytes were observed, as was disseminated (focal) ischemic damage, which was milder (see Figure 2B) and significantly less than in group 1A (p<0.05). Up to 23% of the myocardial area analyzed revealed some degree of myocytolysis, with 100 times less focal infarctions compared with group 1A (see Table 2).

**Protocol 2: Effect of Brain Death on the Donor Heart Potential**

The baseline parameters were not significantly different in the three groups (p>0.05, see Table 3). The hearts were excised within 55±7 minutes after brain death was established. Total ischemia time was 240±20 minutes. After transplantation, the dogs were maintained on cardiopulmonary bypass for 1 hour and received minimum drug support. The functional parameters (MAP,
CO) were monitored for 1 hour after weaning from bypass to determine the recovery potential.

Non–Brain-Dead Donors: Group 2A

The hearts were excised after cardioplegic arrest within 1 hour of baseline measurements. The catecholamine levels remained almost at baseline concentrations, and the hemodynamic status was stable. The non–brain-dead donors showed the best functional outcome of the three. All four dogs could be weaned from bypass and showed acceptable functional recovery. Histological analysis of the preischemic biopsy (which was taken before administration of the cardioplegic solution) revealed the structure of normal myocardium. However, at the end of the experiments, 33±5% of the myocardium analyzed revealed mild ischemic damage. (See Tables 4 and 5.)

Brain-Dead Donors Given an Explosive Rise in ICP: Group 2C

This group behaved in a similar way as group 1A in protocol 1. One to 2 minutes after the bolus injection, there was an explosive increase in the circulating catecholamine levels (average: epinephrine, 1,200-fold; NE, 450-fold versus control values). No substantial increases were observed for DM. Almost simultaneously, the EEG became flat, and the brain stem reflexes and the CPP became negative. The hemodynamics had risen to a similar level as in group 1A after brain death (see Tables 1 and 3), and there was also a greater incidence of arrhythmias. The hearts were excised after cardioplegic arrest within 1 hour of brain death. Only two of the four dogs were weaned and showed moderate functional recovery (see Table 5). Histological analysis of the preischemic biopsy revealed evidence of ischemic damage and also focal infarction. This damage had increased to 90±5% (see Table 4) by the end of the experiment, with 1.6% of the myocardium showing focal infarctions.

Significant differences were observed between groups 2B and 2C (p<0.05) regarding the levels of circulating epinephrine and norepinephrine after brain death. The same was true for the histological damage, MAP, ICP, and dP/dt. These results show the same trend as groups 1A and 1B. Furthermore, there was significant difference (p<0.05) in the donor potential (weanability and functional recovery considered together, nonweaned dogs were considered to have zero functional recovery) among the three groups: between groups 2A and 2B, groups 2A and 2C, and also groups 2B and 2C (see Table 4).

Discussion

Brain-dead patients eventually undergo asystolic cardiac arrest.1 Donor hearts that have not undergone cardiac arrest are preferred by most centers where heart transplantation occurs. However, in view of the scarcity in donor heart availability and in situations where cardiac arrest is not due to a gradual functional deterioration, hearts that have undergone cardiac arrest are used for transplantation. Ideally, if a brain-dead patient is to be considered as a potential donor, then the heart must be excised before the function begins to deteriorate.

Sometimes periods up to 24 hours are required before full donor heart recovery occurs after transplantation. It is possible that acute graft failure could be due to the myocardial damage that occurred during the agonal brain death period.11 Catecholamines have been implicated in the myocardial damage that is observed after brain death. However, little is known about the relation

### Table 4. Protocol 2: Catecholamine Concentrations and Myocardial Damage

<table>
<thead>
<tr>
<th></th>
<th>Epinephrine (ng/ml)</th>
<th>Norepinephrine (ng/ml)</th>
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<tr>
<td></td>
<td>C</td>
<td>BD</td>
</tr>
<tr>
<td>Group 2A</td>
<td>0.03±0.01</td>
<td>...</td>
</tr>
<tr>
<td>Group 2B</td>
<td>0.03±0.01</td>
<td>11.7±5.2*</td>
</tr>
<tr>
<td>Group 2C</td>
<td>0.03±0.01</td>
<td>36.9±17.2*</td>
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</table>

Average values±SD.

C, baseline measurements; BD, after brain death; ischemia (%), percentage ischemic damage of the myocardium; infarction (%), percentage focal or microinfarctions. *p<0.05.

### Table 5. Protocol 2: Weaning Potential and Functional Recovery (Over 1 Hour) for the Three Groups After Transplantation

<table>
<thead>
<tr>
<th></th>
<th>Weaning potential</th>
<th>MAP (mm Hg)</th>
<th>CO (l/min)</th>
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<tbody>
<tr>
<td>Group 2A</td>
<td>4/4</td>
<td>54±5*</td>
<td>2.4±0.2*</td>
</tr>
<tr>
<td>Group 2B</td>
<td>4/4</td>
<td>44±4*</td>
<td>1.9±0.1*</td>
</tr>
<tr>
<td>Group 2C</td>
<td>2/4</td>
<td>24±1*</td>
<td>1.0±0.1*</td>
</tr>
</tbody>
</table>

Average values±SD.

MAP, mean arterial pressure (mm Hg); CO, cardiac output (l/min). *p<0.05.
between the extent of brain injury, catecholamine levels, and the severity of myocardial damage. Hamill et al.\textsuperscript{14} reported that in 33 patients with traumatic brain injury, the levels of circulating catecholamines appeared to be excellent endogenous and readily quantifiable markers that reflected the extent of brain injury and predicted the likelihood of patient recovery. The cardiac repercussions, however, were not investigated. It is unclear whether the sympathetic response reflects a diffuse axonal injury or specific lesions (brain stem and/or other).\textsuperscript{14} Many studies using experimental models of brain death have also reported tremendous increases in the catecholamine levels and typical cardiac and pulmonary lesions.\textsuperscript{15–18} All of these studies, however, used acute increases in the ICP to induce brain death. Evidence from clinical situations shows that patients dying of acute intracranial lesions large enough to produce acute increases in ICP show transmural scattered foci of myocardial injury. These cardiac lesions were not seen in patients with slowly progressive intracranial tumors or in patients dying of noncerebral causes.\textsuperscript{5} Also, patients dying within a few hours after the sudden increase in ICP did not reveal such damage. From autopsy studies of patients dying at various periods after sudden increases in ICP, it appears that focal myocardial damage requires at least 6 hours to develop, and 8–12% of the patients dying from acute cerebral lesions show such cardiac damage.\textsuperscript{5}

Our data show that dogs given a sudden rise in ICP demonstrated a hyperdynamic state of greater degree when the ICP was equal to or more than MAP. This coincided with the huge peaks of catecholamines, which presumably caused the greater myocardial structural damage observed (93±2%). Furthermore, all five dogs showed a tendency toward hemodynamic collapse within 1 hour (60±21 minutes) after brain death. Two of the four recipient dogs (receiving hearts from donors given a sudden increase in ICP) could not be weaned from bypass. These donors had also produced very high catecholamine peaks after brain death (see Table 5). Histological analysis of the preschismic biopsy revealed evidence of damage and also focal infarctions. After 4 hours of total ischemia (240±20 minutes), up to 90% severe ischemic damage was observed. On the other hand, dogs given a gradual increase in the ICP did not present tremendous increases in hemodynamics or catecholamine levels and remained stable for longer periods after brain death. There was, however, a significant percentage of mild ischemic damage (23±1%) to the myocardium. Furthermore, all four dogs receiving hearts from donors given a gradual increase in the ICP were weaned. The preschismic biopsy did show some mild damage to the myocardium. At the end of the experiment, there was considerably more damage to the myocardium (up to 66%) compared with group 1B, in which brain death was induced by gradually increasing the ICP. It appears that the 4-hour ischemia period must contribute toward increasing the ischemic damage observed. This is further supported by the observations made in the group with non–brain-dead donors (group 2A). In this group, the preschismic biopsy was perfectly normal; however, at the end of the experiment there was up to 33% mild ischemic damage to the myocardium.

A gradually expanding mass perhaps allows the brain mass to accommodate, allowing less distortion and perhaps less ischemia. Brain tissue is known to behave mechanically as viscoelastic material rather than as a truly elastic material, demonstrating the phenomenon of pressure relaxation.\textsuperscript{19} Furthermore, evidence for the low elastic properties of the brain is the rapidity with which brain stem displacement and transtentorial herniation occur during acute expansion of a mass.\textsuperscript{20} We also noticed that the systemic pressure did not change until the ICP was ≥100 mm Hg, perhaps because of cerebral ischemia and hypoxia and exhaustion of autoregulation.\textsuperscript{9} The sympathetic storm that is known to occur in the agonal period of brain death appears to be a common response to brain injury, be it mechanical, ischemic, electrical, or tissue laceration.\textsuperscript{10} The origin of circulating catecholamines is attributed to discharge of sympathetic nerve endings and to release from the adrenal medulla.\textsuperscript{21}

Our experimental models of brain death could have led to a release of catecholamines by distortion and compression of brain mass, vascular compression, and/or architectural distortion. Low levels of injury allow for a transient increase in ICP and other hemodynamic variables and then reverse as compensatory processes or autoregulation becomes effective. With the injury severity increasing, the autoregulation is progressively impaired.\textsuperscript{10} Our experiments showed that when the sympathetic storm is built up slowly, the hemodynamic changes were not so intense and acute. The sympathoadrenergic system appears to have a graded ability to respond to stress.\textsuperscript{10} A continuous and prolonged expansion of an epidural supratentorial balloon leads to progressive cerebrospinal ischemia beginning in the cerebrum and progressing to the pons, medulla oblongata, and spinal cord in an orderly rostrocaudal fashion.\textsuperscript{21} In our experimental setup, although a graded sympathoadrenergic response was observed that gave rise to a graded hyperdynamic response, the general pattern of events was the same. From the work of Schrader et al.\textsuperscript{21,22} in which radioactive microspheres were used to study the tissue blood flow before and during the Cushing response, and from our own experimental work, we were able to develop a scheme to summarize the events and consequences involved in the process of brain death (see Figure 3). When the ischemic threshold of the brain was overcome, we observed a decrease in heart rate, MAP, and CO (this has been attributed to a vagal activation). By this time, the entire cerebrum is ischemic. With further progression of the ischemia, (i.e., as it approaches the brain stem), there is mixed vagal and sympathetic stimulation when the ischemia reaches the pons. This gives rise to the Cushing response (thought to be a potential life saver\textsuperscript{14}), which is a triad of bradycardia, hypertension, and irregular breathing pattern (the irregular breathing pattern could not be observed in our case because the dogs were artificially ventilated). As the ischemia progresses to the lower end of the medulla oblongata, thus enveloping the entire brain stem, the vagal cardio-motor nucleus becomes ischemic, whereby there is only sympathetic activation. Here, we observed the hyperdynamic state. At this point, all the criteria used by us to establish brain death were satisfied effectively at the point where brain death occurred. This probably also explains why the peak heart rate was observed after the peak MAP. As the ischemia progresses along the
spinal cord, there is progressive paralysis of the spinal sympathetic pathways, whereby there is sympathetic deactivation with a decrease in the hemodynamic parameters (the cardiovascular collapse observed by us in group 1A). Perhaps the same would be observed in group 1B had we monitored those dogs long enough.

The myocardial structural damage thought to have been caused by catecholamines is described in clinical and experimental situations. This is described variously as myocyteolysis, contraction band necrosis, focal coagulative necrosis, subendocardial petechial hemorrhage, edema formation, and interstitial mononuclear cell infiltration. Histological analysis from our experiments showed loss of myofibrillar material in varying degree, swelling of the cytoplasm, swelling of mitochondria, and sometimes focal necrosis. The damage ranged from mild disruption of the contractile elements (as seen in dogs given a gradual rise in ICP), which most probably is reversible, to greater loss of contractile material with intracellular edema and focal necrosis (as seen in dogs given a sudden rise in ICP). These findings were reflected in the donor potential of hearts that had sustained such damage. Furthermore, almost invariably, the subendocardium showed greater evidence of damage.

The pathogenesis of catecholamine cardiotoxicity is attributed to relative hypoxia that occurs because of the direct stimulation of the myocardium, the coronary microcirculatory effect, membrane permeability alteration, catecholamine oxidation products (adrenochrome), and other contributory pathogenetic factors. Although catecholamines have been used clinically for treatment of pump failure, excessive administration or release exceeding physiological doses may deplete the energy reserves of cardiac muscle cells. This leads to complex biochemical and subsequent structural changes that continue from reversibility on to necrosis. In some pathological states such as congestive heart failure and pheochromocytoma, there is an elevation of circulating catecholamines. In congestive heart failure, this might represent a complex dysfunction of sympathetic neural regulation rather than merely a reflex compensatory response. There is, however, a marked patient-to-patient variation, because arterial NE levels are within normal limits in one third of patients with severe congestive heart failure but are markedly elevated in others. The extent of plasma NE elevation correlates directly with the severity of left ventricular dysfunction and cardiac mortality. In patients harboring a functional pheochromocytoma, the catecholamine cardiomyopathy manifests itself as active focal myocarditis and/or increased myocardial fibrosis caused by healed infarction. In these patients, cardiovascular disorders are often the predominant cause of death. The myocardial structural damage observed by us bears a direct correlation with the catecholamine levels found.

The process of brain death is complex, with a multifactorial interaction. In intensive care units (ICUs), the ICP is not always monitored, and sometimes the profile of hemodynamic changes that might have occurred in the brain-dead donor during the agonal period is lacking. There is also no information about the catecholamine levels or the hormonal depletion of insulin, cortisol, T3, T4, and antidiuretic hormone (ADH), all of which might affect the viability of the grafted heart. The
occurrence of diabetes insipidus caused by ADH de- ple- tion is long known, and a substitution with Minrin or some other ADH analogue is a standard therapy in most ICUs.27 Although little is known over the levels of cortisol and insulin, it is thought that their concentra- tions remain low normal over longer periods.28 Novitzky et al29,30 have described the decrease in the T3 and T4 levels after brain death. They suggested that thyroid hormone depletion is the major cause of hemodynamic instability and that a hormonal substitution will improve donor potential. However, the evidence is insuffi- cient.31,32 In any event, it is important to realize that the early myocardial dysfunction could be due to the extent of cerebral damage or due to inadequate supportive measures after brain death. Our experimental studies show: 1) Irreversible myocardial damage could be caused by a sudden increase in ICP. 2) Cardiac lesions can occur very quickly after brain death when the ICP is raised suddenly. 3) When the sympathetic storm is built up slowly, the hemodynamic changes are not so intense and acute, nor is the structural damage greater. 4) The greater is myocardial damage, the greater are catechol- amine concentrations. Furthermore, the 4-hour isch- emia period (which is the accepted ischemia time, at present) also appears to contribute toward the eventual myocardial damage that is observed. 5) The intensity of the stimulus (that is, the height to which the ICP is raised) could predict the catecholamine levels, the height of the hyperdynamic response, and the donor potential. It is possible that such cardiac damage may contribute to the early failure of some transplants and obscure or complicate the histological manifestations of rejection in others.

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