Brain Death–Induced Impairment of Cardiac Contractile Performance Can Be Reversed by Explantation and May Not Preclude the Use of Hearts for Transplantation

Manuel Galíñanes and David J. Hearse

The shortage of suitable donor hearts for cardiac transplantation is exacerbated by the exclusion of those that exhibit contractile malfunction during the period after brain death but before excision. We have replicated the phenomenon of brain death–induced hemodynamic deterioration in the rat in vivo. After 60 minutes of brain death (defined as the absence of electrical activity in the brain), a variety of indicators of cardiac contractile function fell by approximately 50% (thus cardiac index fell from 21±2 to 11±1 ml/min per 100 g body weight). However, once excised and perfused ex vivo, the hearts recovered a level of cardiac function that was identical to that from control animals that had not been subjected to brain death. Similarly, when hearts were excised, stored (6 hours at 4°C), and reperfused ex vivo with blood, they also recovered a functional capability identical to that of normal hearts from animals that had not been subjected to brain death. Our results question whether hemodynamic instability in brain-dead individuals is necessarily an irreversible detrimental cardiac phenomenon and whether these hearts should be excluded from transplantation. (Circulation Research 1992;71:1213–1219)

KEY WORDS • brain death • myocardial protection • rat hearts • ischemia • reperfusion • heart transplantation

Cardiac transplantation is established as the treatment of choice for end-stage heart failure; however, shortage of donor hearts is an important limiting factor, and as many as 35% of patients awaiting transplantation may die before a suitable heart becomes available. Selection of a donor heart is subject to a number of exclusion criteria; a poll of 64 organ procurement organizations has shown that hemodynamic instability, age, and unfavorable electrocardiographic changes are the major causes for refusal of potential donors.

Brain death is well known to be associated with hemodynamic deterioration in both experimental preparations of patients. These changes may be accompanied by a switch from aerobic to anaerobic metabolism and are thought to constitute a contraindication to transplantation. If it were possible to reverse the hemodynamic deterioration, the potential pool of donor hearts might be considerably expanded. The consequences of brain death on cardiac function before and after the period of hypothermic organ storage that normally precedes cardiac transplantation has not previously been investigated. The aims of the present rat heart study were to assess the effects of brain death (induced by increasing intracranial pressure) on 1) hemodynamic function in vivo, 2) ex vivo contractile function immediately after excision, 3) the ability of the heart to withstand an extended period of hypothermic ischemic storage, and 4) the ability of the heart to recover contractile function after storage when reperfused ex vivo with warm blood.

Materials and Methods

Definition of Brain Death

For the present studies, brain death was defined as the absence of electrical activity in the brain as recorded by a silver disc electrode attached to the skull and a reference platinum needle electrode inserted in the skin of the cheek. The absence of electrical activity was associated with the presence of unreactive dilated pupils within the first 3 minutes after the induction of brain injury.

Surgical Procedures and Experimental Design

Induction of brain death and study groups. Rats (male Wistar, 300–350 g) were anesthetized with pentobarbital (60 mg/kg i.p.) and mechanically ventilated (through a tracheostomy) at a rate of 55 strokes per minute and a ventilation pressure of 12–14 mm Hg. Two groups were studied: 1) sham-operated control rats and 2) rats in which brain death was induced by the inflation of a balloon introduced into the subdural space through a craniotomy in the left parietal area (5 mm from the interparietal line). After insertion, the balloon was inflated with 300 μl saline...
Assessment of cardiac function in vivo. The femoral artery and vein were cannulated for recording arterial pressure; withdrawing blood for the analysis of blood gases, electrolytes, and pH; and administering heparin (1,000 units/kg) and blood transfusions. A microprobe for measuring body temperature was positioned in the aortic arch by cannulation of the left carotid artery. A cannula (Abbocath-T 22G) in the right atrium, introduced through the right external jugular vein, was used for continuous recording of the right atrial pressure (RAP) and for the injection of cold (4°C) saline (100 μl) for the assessment of cardiac output (CO) by a thermodilution computer (Hoyer Bremen). Blood transfusion was given as a 0.5 ml bolus when the RAP fell below the basal value (4.0 mm Hg); the RAP was maintained at this value to ensure that the preload was kept at similar levels in both groups throughout the study. Transfusion volumes of 0.5–1.5 ml were required. Heart rate (HR) and mean arterial pressure (MAP) were continuously recorded, and CO was measured 5 minutes before and 10 and 60 minutes after the induction of brain injury. Cardiac index (CI), stroke volume index (SVI), and systemic vascular resistance index (SVRI) were calculated from the CO and HR. At the end of the 60-minute period of brain death, blood samples were taken for the analysis of blood gases and pH and the determination of plasma concentrations of Na+, K+, and Ca++. In a parallel set of experiments, samples of blood and myocardial tissue were taken 3 and 60 minutes after the induction of brain death for the measurement of catecholamines in plasma and myocardium.

To study the effect of brain death on cardiac contractility, a polythene cannula (1 mm o.d.) was introduced into the left ventricle through the right carotid artery in control and brain-dead rats (n = 6 per group). Left ventricular developed pressure (LVDP), maximum rate of isovolumic left ventricular pressure development (LV dP/dtₘₐₓ), a highly sensitive index of acute changes in contractility,9,10 left ventricular end-diastolic pressure (LVEDP), and HR were then recorded for 60 minutes after the induction of brain death.

Preservation of the heart for long-term storage. After 60 minutes of brain death, the chest was opened, and the heart was excised and placed in cold (4°C) saline. The aorta was rapidly cannulated, and the St. Thomas’ Hospital cardioplegic solution containing (mmol/l) NaCl 130.0, KCl 16.0, MgCl₂ 16.0, CaCl₂ 1.2, and NaHCO₃ 10.0 at pH 7.8 was immediately infused at a constant pressure (45 mm Hg) for 2 minutes at 4°C. The hearts (n = 8 per group) were stored, immersed in the same cardioplegic solution, for 6 hours at 4°C. They were then reperfused as described below.

Ex vivo assessment of the recovery of cardiac function. A support rat was anesthetized with pentobarbital (60 mg/kg i.p.) and placed supine on a heated pad (37±0.5°C). It was allowed to breathe spontaneously a mixture of 95% O₂–5% CO₂, the flow of which was controlled so as to maintain arterial Po₂ and PCO₂ within their physiological ranges. Heparin (1,000 units/kg i.v.) was administered, and a femoral artery and vein were cannulated for the supply of arterial blood at 37°C to the perfused heart (previously stored for 6 hours at 4°C) and the return of venous blood to the support animal through a blood filter (pore size, 200 μm).

The stored hearts were reperfused for 40 minutes in the Langendorff mode11 with arterial blood from the support rat, delivered at a measured rate by means of a peristaltic pump. Perfusion pressure was monitored continuously and maintained at 60 mm Hg. The temperature of the blood was kept at 37.0°C by a thermostatically regulated heat exchange system. At the end of reperfusion, a balloon catheter attached to a pressure transducer was inserted into the left ventricle through the left atrium, and the balloon was progressively inflated to achieve stepwise increases of LVEDP (0, 4, 8, 12, 16, and 20 mm Hg). HR was maintained at 320 beats per minute by right atrial pacing.

In one group of experiments, after 1 and 60 minutes of brain death, hearts (n = 6 per group) from control and brain-dead rats were excised and taken for the immediate assessment of cardiac function without a period of storage. These hearts were perfused ex vivo with blood for 10 minutes, as described above. During this time, cardiac function was assessed as indicated earlier.

Expression of Results and Statistical Analysis

In vivo CI, measured as CO per 100 g body weight, is expressed as milliliters per minute per 100 g body weight; SVI, measured as (CI/HR) × 1,000, is expressed as microliters per beat per 100 g body weight; and SVRI, measured as (MAP–RAP)/CI, is expressed as millimeters of mercury per milliliter per minute per 100 g body weight. LV dP/dtₘₐₓ was expressed as millimeters of mercury per second. Concentrations of adrenaline and noradrenaline were measured in picomoles per milliliter in plasma and in picomoles per gram wet weight (adrenaline) and nanomoles per gram wet weight (noradrenaline) in tissue. All results are expressed as mean±SEM. Unpaired Student’s t test was used for comparison between two means. A difference was considered statistically significant at p<0.05.

Results

In Vivo Time Course for Hemodynamic Changes Arising as a Consequence of Brain Death

Figure 1 shows that rats in the control group were hemodynamically stable during the entire 60-minute study period. In contrast, those that were brain dead suffered major and sustained hemodynamic changes that could be divided into two distinct phases. The first, occurring during the first minute after inflation of the intracranial balloon, was characterized by a sudden increase in MAP (from 112±9 to 157±10 mm Hg) and HR (from 393±22 to 455±20 beats per minute). This response, also known as the Cushing reaction,12 was preceded in some cases by a very brief episode (a few seconds) of bradycardia and even asystole. During the second phase, which lasted for 1–5 minutes, there was a rapid and major fall in MAP to 54±4 mm Hg (i.e., to approximately 50% of the control value). Thereafter, MAP remained constant and depressed for the rest of the experiment. After the rapid fall in MAP, HR returned to within the physiological range.

RAP remained stable in control animals, whereas in brain-dead animals it tended to increase during the first 5 minutes after inflation of the balloon.
Figure 1 shows that brain death also causes a rapid deterioration of other indexes of cardiac function. Thus, within 10 minutes of the initiation of brain death, CI and SVI were reduced to approximately 50% of their preinjury control values. This deterioration was still present after 60 minutes of brain death. There were no significant changes in SVRI in either the control or the brain-dead groups.

Figure 3 shows that cardiac function, as assessed with an intraventricular catheter, was stable in control animals throughout the 60-minute period of study. As observed before with MAP, brain death caused a sudden but transient increase of the LVDP (from 150±4 to 249±20 mm Hg) during the first minute after the induction of brain death; this was followed by a rapid deterioration of approximately 50% of the basal value.

Cardiac contractility, as assessed by LV dp/dt max, also exhibited a sudden increase (from 6,317±204 to 10,500±610 mm Hg/sec) followed by a rapid deterioration of approximately 50% of the basal value. The initial changes in LV dp/dt max might be influenced, at least in part, by the coincident increase in HR (from 407±5 to 473±22 beats per minute); however, HR rapidly returned to control and thus could not explain the subsequent deterioration of myocardial contractility. Brain death also caused a sudden but transient decrease in LVEDP (from 7.5±1.5 to −7.3±5.5 mm Hg); however, this was restored rapidly to within the control range and was maintained for the rest of the experimental period.

Blood Gases, pH, and Electrolytes After Brain Death

Table 1 shows that brain death resulted in a compensated metabolic acidosis and a tendency toward mild hyperkalemia; however, the changes did not achieve a level of statistical significance.
Catecholamines in Plasma and Myocardium After Brain Death

Table 2 shows that the plasma and tissue concentrations of noradrenaline and adrenaline were similar in control and brain-dead rats after both 3 and 60 minutes of study.

Cardiac Function After Explantation and Ex Vivo Perfusion of Hearts

Figure 4 shows that left ventricular systolic and diastolic functions were identical in hearts from control rats and from brain-dead rats (both at 1 minute and 60 minutes after brain death) after excision (total denervation) and perfusion for 10 minutes as an isolated blood-perfusion preparation. Coronary flow was also similar in the three groups of hearts (2.6±0.1 ml/min for control rats, 2.6±0.1 ml/min for rats 1 minute after the induction of brain death, and 2.7±0.2 ml/min for rats 60 minutes after the induction of brain death).

Recovery of Cardiac Function After Long-Term Hypothermic Storage

Despite the deterioration of cardiac function observed in the brain-dead group before the 6-hour period of hypothermic storage, the recovery (Figure 5) of systolic and diastolic function after storage and reperfusion with blood for 40 minutes was similar in both study groups. The recovery of coronary flow was also similar in control hearts (3.2±0.2 ml/min) and in hearts obtained from brain-dead animals (3.2±0.2 ml/min). This represented an almost complete recovery of cardiac function after long-term hypothermic storage when compared with the function of explanted hearts not subjected to ischemia (Figure 4).

Discussion

We have shown that, although brain death results in rapid and major hemodynamic perturbations in vivo, these do not represent irreversible myocardial injury. Thus, excision and ex vivo blood perfusion of hearts from brain-dead animals result in a complete normalization of cardiac function. Furthermore, after excision,

| Table 1. Blood Gases, pH, Hematocrit, Hemoglobin, and Plasma Electrolytes in Sham-Operated Control Rats and in Brain-Dead Rats 60 Minutes After Induction of Injury |
|-------------------------------------------------|------------------|------------------|
| pH                                              | Control (n=6)    | Brain-dead (n=6) |
| Arterial CO₂ partial pressure (mm Hg)           | 7.44±0.02        | 7.36±0.05        |
| Arterial O₂ partial pressure (mm Hg)            | 31.4±2.3         | 27.7±4.0         |
| Hemoglobin (g/dl)                               | 98.5±5.8         | 93.4±9.1         |
| Hematocrit (%)                                  | 38±1             | 40±1             |
| O₂ saturation (%)                               | 12.5±0.3         | 13.3±0.3         |
| Base excess (mmol/l)                            | -1.8±0.9         | -6.1±3.0         |
| Bicarbonate (mmol/l)                            | 21.0±1.1         | 18.2±2.8         |
| Na⁺ (mmol/l)                                    | 97.6±0.7         | 95.3±2.0         |
| K⁺ (mmol/l)                                     | 147±2            | 147±1            |
| Ca²⁺ (mmol/l)                                   | 5.2±0.4          | 6.7±0.6          |
|                                                                                      | 1.33±0.02        | 1.28±0.02        |
Table 2. Plasma and Myocardial Content of Noradrenaline and Adrenaline in Sham-Operated Control Rats and in Brain-Dead Rats 3 and 60 Minutes After Induction of Injury

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Brain-dead (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 Minutes</td>
<td>60 Minutes</td>
</tr>
<tr>
<td>Plasma noradrenaline (pmol/ml)</td>
<td>12.6±5.3</td>
<td>7.4±2.1</td>
</tr>
<tr>
<td>Plasma adrenaline (pmol/ml)</td>
<td>7.6±2.4</td>
<td>7.8±2.7</td>
</tr>
<tr>
<td>Myocardial noradrenaline (nmol/g wet wt)</td>
<td>2.9±0.5</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Myocardial adrenaline (pmol/g wet wt)</td>
<td>0.12±0.07</td>
<td>0.14±0.06</td>
</tr>
</tbody>
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FIGURE 4. Graphs showing systolic and diastolic function in hearts from sham-operated control rats and brain-dead rats excised and immediately studied (without any period of hypothermic storage) 1 or 60 minutes after the induction of injury. LVDP, left ventricular (LV) developed pressure; LVEDP, LV end-diastolic pressure. Every point represents the mean of six measurements, and the bars represent the standard error of the mean.

FIGURE 5. Graphs showing recovery of systolic and diastolic function in hearts (n=8 per group) from sham-operated control rats and brain-dead rats. LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure. Hearts were subjected to 6 hours of hypothermic (4°C) global ischemia and 1 hour of reperfusion as isolated blood-perfused preparations.

an extended period of hypothermic storage, and reperfusion (such as occurs before and during cardiac transplantation), hearts from brain-dead animals had cardiac function similar to that in normal control animals. This surprising observation raises the potentially important clinical question: Is instability of cardiac function induced by brain death in a patient a valid criterion for exclusion of the heart as a donor organ?

Possible Cause of Brain Death–Induced Contractile Malfunction

Changes in preload, afterload, and heart rate. It is clearly important to determine whether the hemodynamic deterioration observed as a consequence of brain death is a direct consequence of impaired cardiac contractility or some other factor. Our results would suggest that the sustained deterioration of cardiac function induced by brain death is mainly attributable to a loss of contractile function. Whereas other potential contributory factors (such as preload, HR, and diastolic function) were altered soon after brain death, this was a transient phenomenon, and these indexes were rapidly normalized. Whether contractile and hemodynamic dysfunction is in some way a consequence of the early transient increase in contractility remains to be resolved. This early hyperactivity would certainly offer an explanation for the transient coincident decline in LVEDP. At present, however, we cannot exclude the possibility that some factors (such as catecholamines) that can affect the rate of isovolumic relaxation may account for some of the effects observed in the present study. Although we could not demonstrate any differ-
ences in tissue or plasma catecholamines 3 or 60 minutes after the induction of brain death, we cannot exclude the possibility of changes during the first 1–2 minutes, when major increases in contractile activity were observed. The apparently paradoxical occurrence of a simultaneous decrease in LVEDP and an increase in RAP may indicate a differential effect of brain death on right and left ventricular diastolic function; however, the nature of such a phenomenon remains to be defined.

Because of the technical difficulty of the present study, we did not assess the possible participation of changes in left ventricular afterload to the decline of cardiac contractile function observed during the first few minutes after brain death. However, 10 and 60 minutes after the induction of brain death, the indexes of SVR were not significantly affected; therefore, it is unlikely that this factor plays a role in the maintenance of the contractile dysfunction.

Changes in catecholamines and sympathetic tone. Clifton and colleagues14 have suggested that the cardiovascular changes that occur after severe head injury are mediated, at least in part, by an increase in sympathetic neural activity. Others15,16 have shown that, after elevation of intracranial pressure, sympathetic nervous activity may be greatly increased. Although we cannot rule out a possible involvement of catecholamines in the very early hemodynamic changes (observed in the first 2 minutes after brain injury), it would appear unlikely that alterations in sympathetic activity accounted for the profound disturbances of cardiac function observed in vivo during 60 minutes of brain death, since, as indicated earlier, we did not observe significant changes in catecholamine levels in cardiac tissue or plasma either 3 or 60 minutes after the induction of brain death.

Changes in plasma pH and ions. Other possible explanations for the functional changes might involve changes in pH, ions, or metabolism induced by brain death. Although we saw slight evidence of acidosis (possibly indicative of a switch from aerobic to anaerobic metabolism) and mild hyperkalemia, these conditions were undoubtedly insufficient to account for the very major impairment of contractile function.

Changes in hypothalamic–pituitary function. Novitzky and colleagues3 have speculated that hypothalamic–pituitary function may be modified by brain death and that this might be beneficially overcome by the administration of triiodothyronine, cortisol, and insulin5 in order to reverse the hemodynamic and metabolic consequences of brain death. However, Hall and colleagues27 have shown that thyroid-stimulating hormone, prolactin, and cortisol remain in the physiological range for at least 24 hours after the onset of brain death in human patients. Despite this, we believe that further work is warranted regarding the possibility that other neurohormonal factors may underlie brain death–induced perturbations of cardiac function.

Production of cardiodepressant factors. The presence of circulating cardiodepressant substances has been extensively investigated in different kinds of shock (i.e., hemorrhagic, traumatic, and endotoxic)18–22 in both humans and animals. However, the concept that shock induces the production of negative inotropic factors remains controversial partially because of the general inability to identify the exact nature (except for L-leucine19) of these factors. The water-soluble myocardial depressant factor,18 which is not released for 4–6 hours (and is not related to the hypotensive effect of sepsis), and the lipid-soluble cardiodepressant factor,20 which has been shown to be released within 2 hours after the onset of shock (and requires a severe hypotension and prolonged splanchnic hypoperfusion), are most unlikely to be responsible for the rapid changes in cardiac performance induced by brain death.

Production of opioids. It is known that endogenous opiate peptides, which are released into the general circulation in severe stress,23 are also elevated during circulatory shock of various etiology (i.e., hemorrhagic, traumatic, and spinal shock)23,25 and also during congestive heart failure.26 Because endogenous opioids inhibit catecholamine release from peripheral sympathetic nerves27 and adrenal medulla,28 and also have a direct depressant effect on myocardial function,29,30 it is possible that opioids might participate in the hemodynamic changes observed in the present studies. It has been reported by some investigators that the hemodynamic deterioration seen in shock24,31–34 and cardiac failure36,35 can be attenuated by the administration of opioid antagonists. However, other studies have failed to show cardiovascular improvement and/or increased survival after the administration of opioid antagonists.36–38 The role of endogenous opioids in the deterioration of cardiac function induced by brain death has not been studied and clearly warrants further investigation.

Brain Death and Hypothermic Tissue Preservation

Our results have also shown that despite brain death and the consequent impairment of contractile function for up to 1 hour before excision, our ability to reanimate hearts before or after extended periods of hypothermic storage is in no way compromised. This finding might at first sight appear to be at variance with the results of studies in which hearts from brain dead pigs were preserved for 24 hours by continuous hypothermic perfusion.39 These hearts recovered function less well than those from pigs that had not suffered brain injury. However, it is important to note that our study was designed to mimic the current clinical practice of storage for no more than 4–6 hours with preservation by single-dose cardioplegic infusion rather than continuous perfusion for long periods, as in the pig study.

Concluding Comments

The present studies are clearly limited in that they were undertaken in the rat with an ex vivo evaluation of functional recovery. Nevertheless, we believe that the findings are sufficient to argue that further studies should be undertaken with a view to questioning the need to exclude hearts for transplantation simply on the grounds of impaired hemodynamic characteristics before harvesting.

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References

2. Smart FW, Mobley RA, Holter VL, Sekela ME, Grinstead WC, Frazier OH, Van Buren CT, Young JB: Variability of organ utili-
zation criteria exacerbates the national shortage of heart donors.


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