Influence of Brain Death and Cardiac Preservation on Systolic and Diastolic Function and Coronary Circulation in the Cross-circulated Canine Heart

Gábor Szabó, M.D.,¹ Christian Sebening, M.D.,¹ Thilo Hackert,¹ Lutz Hoffmann,¹ Karin Sonnenberg,¹ Christian Hagl, M.D.,² Ursula Tochtermann, M.D.,¹ Christian F. Vahl, M.D.,¹ Siegfried Hagl, M.D.¹

¹Department of Cardiac Surgery, University of Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany
²Department of Cardiac Surgery, University of Hannover, Konstanty-Gutschow-Strasse 8, 30625 Hannover, Germany

Abstract. Previous studies have demonstrated hemodynamic instability and cardiac dysfunction in the brain-dead organ donor. It remains unclear if primary cardiac dysfunction is responsible for hemodynamic deterioration or decreased cardiac function is secondary to brain death-associated altered loading conditions. Therefore in the present study the effects of brain death on hemodynamics and cardiac function were analyzed in vivo in an open chest model and ex vivo in a cross-circulated heart preparation. In a second protocol, the impact of brain death-associated hemodynamic changes on postischemic graft function was investigated. Brain death was induced injecting saline in a subdural Foley catheter. Induction of brain death led to a hyperdynamic reaction followed by hemodynamic deterioration with a decrease of systemic vascular resistance and myocardial contractility. If the hearts were explanted and assessed ex vivo, no differences were found between control and brain-dead hearts. Furthermore, both control and brain-dead hearts showed full functional recovery after 4 hours of hypothermic ischemic storage. Despite hemodynamic deterioration in situ after brain death, there were no differences between the postischemic function of control and brain-dead hearts. These results indicate that myocardial dysfunction is not irreversible and may be secondary to altered loading conditions, and that the recovery of cardiac function after long-term hypothermic ischemic storage is not impaired by the hemodynamic changes observed in situ after brain death induction. These data may also indicate that potential donor hearts might not be excluded from transplantation on the basis of impaired hemodynamic characteristics, especially if they are evaluated by load-dependent parameters.

Perioperative donor heart performance is an important issue of cardiac transplantation. About 20% of potential donor hearts must be rejected on the basis of poor primary cardiac function or hemodynamic instability. On the other hand, the major cause of death after transplantation during the early postoperative period is graft failure, mostly in association with pulmonary hypertension [1]. Recent studies suggest that not pulmonary hypertension per se but preexistent myocardial damage [2] as a result of brain death or ischemia-reperfusion injury in association with pulmonary hypertension may cause graft dysfunction [3, 4]. A number of experimental and clinical studies report hemodynamic instability in the donor organism following brain death [3, 5–7], but it remains unclear which mechanisms lead to hemodynamic collapse in brain-dead organ donors. Catecholamine injury and hormone depletion are suggested to play a major role in cardiac dysfunction [3, 5]. In contrast, some studies demonstrated that neurohumoral factors may have only minor importance in brain death-associated cardiac dysfunction [8, 9]. Moreover, there is no evidence in the literature that the heart is the primary target of the detrimental effects of brain death resulting in hemodynamic instability or that myocardial dysfunction is secondary to changed loading conditions [6, 10, 11]. It remains also unclear if changes of cardiac function are reversible or irreversible. A further issue of controversial discussion is the impact of brain death-associated changes on the ischemic tolerance of the donor hearts and posttransplant graft function.

In the present study the effects of brain death on hemodynamics and cardiac function were analyzed in vivo in an open chest model and ex vivo in a cross-circulated heart preparation. The latter was used to exclude any load-dependent effect that may have an influence on cardiac function. In a second protocol, the impact of brain death-associated hemodynamic changes on postischemic graft function was investigated.

Materials and Methods

Preparation and Measurements

In Vivo Studies. Foxhound dogs (20–28 kg) were anesthetized with a bolus of pentobarbital (Nembutal; Abott) 12 mg/kg IV, paralyzed with pancuronium bromide (Pancuronium Organon) 0.1 mg/kg as a bolus and then 4 μg/kg/min IV, and endotracheally intubated. The level of anesthesia was maintained with piritramid (Dipidolor; Janssen) 1 mg/kg as a bolus and then 15 μg/kg/min IV. The dogs were ventilated with a mixture of N₂O and O₂ (40%/ 60%) at a frequency of 12 to 15/min and a tidal volume starting at 15 ml/kg/min. The settings were adjusted by maintaining arterial partial carbon dioxide pressure levels between 35 and 40 mmHg. The femoral artery and vein were cannulated for recording aortic
pressure (AoP) and taking blood samples for the analysis of blood gases, electrolytes, and pH. Basic intravenous volume substitution was carried out with Ringer's solution at 0.05 L/min/kg. According to the values of K⁺, HCO₃⁻ and base excess, substitution included administration of potassium chloride and sodium bicarbonate (8.4%). Rectal temperature and standard peripheral electrocardiograms (ECGs) were monitored continuously.

After lateral thoracotomy in the fourth intercostal space the pericardium was incised. A perivascular Micron electromagnetic flow probe was positioned at the proximal ascending aorta to measure aortic flow and cardiac output (CO). Stroke volume (SV) was calculated from the integrated flow signal. Left ventricular pressure (LVP) was measured by a Millar catheter tip manometer. Maximum rate of isovolumic left ventricular pressure development (dP/dt max) was assessed by an analogue differentiator on-line. The slope of the left ventricular pressure–volume relationships (Ees) was estimated by the single beat estimation method [6, 12] as a load-independent index of contractility. All hemodynamic parameters were registered on a Gould multichannel monitor unit and recorded on a PC computer for further off-line analysis.

**Ex Vivo Studies.** A support dog was anesthetized and mechanically ventilated as described above. Heparin (300 units/kg IV) was administered, and the left femoral artery and vein were cannulated for the supply of the perfused heart with arterial blood at 37°C and the return of venous blood to the support animal.

Donor hearts were isolated from brain-dead or control sham-operated animals after a 2-hour observation period in situ. The left subclavian artery and the left pulmonary artery of the donor hearts were cannulated for arterial blood perfusion and collecting coronary sinus effluent. The hearts were then excised and perfused through the aortic root in a retrograde manner with arterial blood from the support dog by a pressure-controlled roller pump. Perfusion pressure was kept constant at 80 mmHg. The hearts were immersed in a thermostatic waterbath. The temperature of the bath was kept at 37°C. A latex balloon was fixed on a 7F Millar catheter tip manometer with an internal lumen and placed in the left ventricle through an incision of the left atrium. The compliance of the balloon was negligible within a volume range of 0 to 50 ml. The mitral valve and the left atrium were closed with a 4-0 suture. The thebesian blood flow was vented via a 16F vent catheter. Left ventricular pressures were measured during isovolumic contraction at different balloon volumes and systolic and diastolic pressure–volume relations were constructed. Systolic function was evaluated by the maximal peak systolic pressure (LVSP), maximum rate of left ventricular pressure development (dP/dt), and the slope of the peak systolic pressure–volume relation (Ees). Diacolic function was assessed by negative maximum dP/dt, end-diastolic pressure (LVEDP) at a constant balloon volume of 16 ml, and the end-diastolic pressure–volume relation. All hemodynamic data were registered as described above. Coronary blood flow (CBF) was measured by an electromagnetic flowmeter, which was connected to the venous cannula. Coronary vascular resistance was estimated by dividing perfusion pressure and CBF. Myocardial oxygen consumption was calculated as the product of the CBF and the arteriovenous oxygen content difference. Arterial, venous, and myocardial lactate, glucose, and glycogen contents were measured with standard photometric methods.

**Experimental Protocol**

**In Vivo Studies.** In five animals brain death was induced by creating intracranial hypertension. A Foley catheter was introduced into the subdural space through a parietal burr hole in the skull. Rapid injection of 25 ml of saline inflated the balloon of the catheter, which produced an acute increase in intracranial pressure. Brain death occurred within a few minutes in all animals, and cerebellar herniation caused interruption of neurologic pathways between the midbrain and the spinal chord. Brain death was confirmed neuropathologically at the end of the experiments. The dogs were observed for 4 hours after brain death induction. Cardiocirculatory stability of the preparation was maintained by crystalloid volume substitution exclusively. No pressor or hormonal substances were applied during the experiments.

**Ex Vivo Studies.** Two groups of dogs (n = 6 for each group) were studied. In one group brain death was induced as described above; the other group with sham operation served as controls. After a 2-hour observation period in situ, the hearts were excised and immediately perfused by a support dog for ex vivo assessments. The ex vivo observation period was 2 hours.

**Postischemic Graft Function.** Sham-operated animals (n = 6) and animals with brain death (n = 6) were observed for 2 hours in situ as in the previous protocol. The hearts were then arrested with the standard Bretschneider's (HTK) solution over a period of 7 minutes. They were stored in the same solution for 4 hours at 4°C and then perfused parabiotically by a support dog for 2 hours as in the previous groups. The reperfusion time lasted 2 hours.

**Statistical Analysis**

All values were expressed as the mean ± standard error (SEM). The paired t-test was used to compare two means within the groups. One-way analysis of variance was used for comparison of the groups followed by the post-hoc Scheffe's test. A probability value less than 0.05 was considered statistically significant.

**Results**

**In Vivo Studies**

After brain death induction, hemodynamic parameters showed characteristic changes of the Cushing-type reaction (Fig. 1). The heart rate (HR) increased by 152 ± 14%. Transient and spontaneously reversible supraventricular and ventricular arrhythmias were observed in most of the animals. The LVP (150 ± 12%), myocardial contractility characterized by dP/dt max (432 ± 25%) and Ees (54 ± 12%), and systolic (148 ± 11%), diastolic (104 ± 10%), and mean AoP (106 ± 9%), and the SVR (15 ± 8%) increased significantly. The LVEDP tended to increase during the acute phase without reaching the level of statistical significance. The CO rose by 49 ± 5%.

The time course of the hemodynamic parameters after the acute phase is also shown in Figure 1. Within 15 minutes the CO, LVP, dP/dt max, and LVEDP reached baseline values. The HR showed a significant but slower decrease, and the values were equal to baseline levels after 120 minutes. The diastolic AoP and SVR decreased significantly about 10 minutes after brain death.