Evidence of Functional Myocardial Ischemia Associated With Myocardial Dysfunction in Brain-Dead Pigs

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Background—Cardiac dysfunction after brain death has been documented, but its mechanisms remain unclear. Myocardial ischemia has been suggested as a possible cause. The aim of the present study was to investigate the existence of an imbalance between myocardial oxygen delivery and demand as a possible cause of myocardial dysfunction in brain-dead pigs.

Methods and Results—Interstitial myocardial lactate and adenosine concentrations were assessed with cardiac microdialysis in 2 groups of animals: brain-dead pigs (n=7) and brain-dead pigs treated with labetalol (10±3 mg/kg) (n=7).

Heart rate (HR), left ventricular (LV) dP/dt\text{max}, rate-pressure product (RPP), cardiac output (CO), and left anterior descending coronary artery blood flow (QLAD) were continuously monitored. Brain-dead pigs exhibited a transient significant increase in HR, LV dP/dt\text{max}, RPP, and CO and a limited increase in QLAD. This resulted in functional myocardial ischemia attested to by the significantly increased adenosine and lactate microdialysate concentrations. In brain-dead pigs treated with labetalol, there was a moderate increase in HR, QLAD, and adenosine microdialysate concentrations; LV dP/dt\text{max}, RPP, CO, and myocardial lactate concentrations remained stable, confirming the preservation of aerobic metabolism.

Conclusions—Brain death was associated with an increase in myocardial interstitial adenosine and lactate concentrations, as well as with myocardial dysfunction; all were attenuated by labetalol, suggesting an imbalance between oxygen consumption and oxygen delivery as a possible cause of myocardial dysfunction after brain death.

Key Words: adenosine ■ ischemia ■ nervous system, sympathetic ■ transplantation

Improvements in immunosuppression have permitted the expansion of heart transplantation programs. Nevertheless, a shortage of donor organs and posttransplantation cardiac graft dysfunction have caused experimental and clinical research to be focused on the cardiovascular functional changes that occur during brain death.1–4 At the beginning of the 1900s,5 Cushing documented cardiovascular dysfunction after brain death, but its mechanisms remain poorly understood. Highly increased plasma catecholamines that originate from the adrenal glands1,2 and cardiac sympathetic nerve endings,4,6 as well as observations of smooth muscle contraction bands in the media of coronary arteries of brain-dead baboons,7 have suggested a possible ischemic mechanism as leading to the myocardial dysfunction associated with brain death.8

We recently demonstrated that labetalol, a mixed α/-β-adrenergic receptor antagonist, attenuated the cardiovascular abnormalities induced by brain death, confirming the deleterious effects of cardiac sympathetic activation associated with brain death on myocardial function.9 One of the possible mechanisms underlying the beneficial effects of labetalol in experimental brain death could be related to a preserved balance between myocardial oxygen consumption and oxygen delivery. To confirm the hypothesis of a possible relationship among brain death–induced uncontrolled activation of the cardiac sympathetic nervous system, myocardial metabolism disturbances, and myocardial dysfunction, we investigated the consequences of early therapy with α/-β-adrenergic blockade with labetalol on myocardial metabolism and function. Changes in the physiological determinants of myocardial oxygen demand (heart rate [HR], left ventricular [LV] dP/dt, cardiac output [CO]) and in oxygen supply (coronary blood flow [CBF]), as well as an estimate of myocardial oxygen demand-supply balance (interstitial myocardial adenosine and lactate using in vivo cardiac microdialysis technique4), were studied in a model of experimental brain death in pigs.

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Circulation is available at http://www.circulationaha.org

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Methods

Anesthesia and Monitoring
Domestic 4- to 6-month-old pigs weighing 25 to 40 kg were chosen as experimental animals. All animals were housed and treated in accordance with accepted practices for humane laboratory animal care. This study was approved by our institutional animal investigation committee. The animals were fasted overnight. They were premedicated with 10 mg/kg ketamine (Panpharma) and 0.2 mg/kg diazepam (Lafon-Ratiopharm) via intramuscular injection. Anesthesia was induced with sodium thiopental (Nesdonal; Rhône-Poulenc Rorer) (initial dose 5 mg/kg, maintained via continuous infusion at a rate of 100 to 150 mg/h). The trachea was intubated with a cuffed endotracheal tube, and the lungs were mechanically ventilated at a minute volume of 150 mL/kg. Adjustments were made to maintain the PaCO₂ in the range of 35 to 42 mm Hg. The ECG was monitored continuously. A median sternotomy was performed, and a micrometer-tipped catheter (Millar Mikro-Tip; Millar Instruments, Inc) was passed into the LV via the apex to measure LV pressure and the diastolic blood pressures. The right external jugular vein was inserted via the right common carotid artery to measure systolic coronary artery (LAD) to measure CBF (QLAD). An arterial catheter was passed into the subdural space, and the balloon was rapidly distended with 20 mL/kg of 6% hydroxyethylstarch (Elohes; Bio-sedra Pharma) infused intravenously over 40 minutes, and hemody-

Cardiac Microdialysis
Two concentric flexible probes (Medicorp) (membrane length 12 mm, outside diameter 300 µm) assembled with a 50 000-Da molecular weight cutoff polyacrylonitrile membrane (AN69; Hospal) were successively implanted into the free wall of the LV through a fine guiding needle. Once the needle traversed the myocardium, it was removed, and the probe was gently pulled until the dialysis membrane was completely embedded within the myocardium. Probe recovery performances, as determined in vitro, were 56±2% for lactate and 76±2% for adenosine. Microdialysis probes were perfused at 2 µL/min with Ringer’s solution (147 mmol Na⁺, 4.0 mmol K⁺, and 2.3 mmol Ca²⁺, degassed under vacuum) using a microinjection pump (Harvard Apparatus). The effluent was collected in plastic Eppendorf microtubes kept on ice during the collection period with 20-minute sampling intervals and then frozen immediately to −80°C and stored for later analysis.

Induction of Brain Death
Experimental brain death was obtained by producing an acute increase in intracranial pressure that resulted in the immediate interruption of cerebral blood flow. Briefly, a burr hole was drilled in the right frontoparietal region. A Foley catheter was introduced into the subdural space, and the balloon was rapidly distended with normal saline (20±10 mL) until electroencephalographic activity completely disappeared.

Experimental Design
Animals were randomly divided into 2 groups of 7: group 1 consisted of brain-dead animals (BD), and group 2 consisted of brain-dead animals treated with labetalol (BD+Lab). A 3-hour equilibration period with 20-minute microdialysis sampling intervals was observed after probe implantation. Brain death was then induced (T0), and sample collection over 20-minute intervals was continued. The first microdialysis sample after brain death was collected after 20 minutes (T20), and the last was collected after 3 hours (T180). Hemodynamic monitoring was performed before and 5 minutes (T5) after brain death induction and then every 20 minutes (from T20 to T180). At the third hour (T180), a volume expansion protocol was performed with 20 mL/kg of 6% hydroxyethylstarch (Elohes; Bio-sedra Pharma) infused intravenously over 40 minutes, and hemody-

Laboratory Procedures
Arterial and venous blood gas tensions, pH, and hemoglobin were measured with an ABL II automated blood gas analyzer (Radiometer A/S). Microdialysate lactate concentration was analyzed using enzymatic methods (kit 256773; Boehringer Mannheim GmbH Diagnostica). Microdialysate adenosine was analyzed by HPLC. Separation of adenosine was achieved with a Symmetry C18 column (Symmetry; Waters) and a gradient of 1% (pH 5.3) to 25% (pH 5.58) methanol in 1 mmol/L KH₃PO₄, as mobile phase. Adenosine was detected at 254 nm.

Statistical Analysis
Statistical analysis was performed with StatView IV software (Abacus Concepts). Results are expressed as the mean±SEM. Intragroup and between-group comparisons were achieved using ANOVA with repeated measures, followed by Fisher’s PLSD test when statistical significance was detected, or nonparametric test when appropriate. Correlations were examined using the least squares method. The criterion of significance was P<0.05.

Results
Hemodynamic data for both groups are presented in Figures 1 to 5, and biochemical data are presented in the Table. No significant differences were observed between the 2 groups before brain death.

Hemodynamic Changes After Brain Death in BD and BD+Lab Pigs
After the induction of brain death, BD pigs exhibited a transient significant increase in HR (from 90±5 to 170±12 bpm, P<0.001; Figure 1), LV dP/dtmax (from 1700±210 to 5050±450 mm Hg/s, P<0.001; Figure 2), RPP (from 10 926±1173 to 34 260±2110 beats · mm Hg · min⁻¹, P<0.001; Figure 3), and CO (from 2.5±0.3 to 3.4±0.6 L/min, P<0.001; Figure 4), achieved as of the fifth minute. A
concomitant, significant, but somewhat limited increase in CBF (from 43 ± 6 to 71 ± 8 mL/min, P < 0.05) was also observed (Figure 5). After this classic “autonomic storm,” these parameters returned to baseline values within 40 minutes.

Within the same experimental period, BD + Lab pigs exhibited a completely different hemodynamic profile. Induction of brain death resulted in a moderate increase in HR (from 88 ± 7 to 100 ± 8 bpm, P > 0.05; Figure 1) and CBF (from 42 ± 5 to 56 ± 6 mL/min, P > 0.05; Figure 5), whereas the other hemodynamic parameters remained stable.

Significant changes occurred after volume expansion in both groups. BD pigs exhibited an impaired hemodynamic response with decreased LV dP/dt values and only a moderate increase in CO, whereas BD + Lab pigs showed an adapted hemodynamic response as attested to by increased LV dP/dt and CO values (P < 0.05). A moderate and similar increase in CBF was observed in both groups.

Changes in Myocardial Interstitial Adenosine and Lactate Concentrations After Brain Death in BD and BD + Lab Pigs

As expected, after an initial increase and a rapid decrease in dialysate adenosine and lactate content due to probe implantation, adenosine and lactate dialysate concentrations remained stable during the entire equilibration period in both groups (data not shown).

After brain death induction, a 6-fold increase in adenosine dialysate concentrations (from 0.19 ± 0.03 to 1.29 ± 0.08 µmol/L, P < 0.05) was observed in BD pigs (Table). Subsequently, adenosine levels rapidly decreased, reaching baseline values after 1 hour. During the same period, BD + Lab pigs presented a significant increase in adenosine values (from 0.19 ± 0.03 to 0.63 ± 0.16 µmol/L, P < 0.05). However, compared with BD pigs, adenosine interstitial concentrations were significantly lower in BD + Lab pigs (P < 0.05).

In BD pigs, lactate microdialysate concentrations increased (from 0.60 ± 0.05 to 1.05 ± 0.08 µmol/L, P < 0.05; Table). These levels returned to baseline values after 1 hour of brain death. On the other hand, lactate microdialysate concentrations did not significantly increase in BD + Lab pigs.

After volume expansion, a significant increase in adenosine levels was observed in both groups (BD from 0.22 ± 0.07 to 0.87 ± 0.5 µmol/L, BD + Lab from 0.22 ± 0.04 to 0.75 ± 0.37 µmol/L, P < 0.05). Similarly, a significant increase in lactate dialysate concentrations was observed in BD pigs (0.72 ± 0.12 to 1.24 ± 0.15 µmol/L, P < 0.05), whereas this increase was not statistically significant in BD + Lab pigs (0.70 ± 0.09 to 0.85 ± 0.06 µmol/L, P > 0.05) (Table).

### Time Course of Myocardial Interstitial Adenosine and Lactate Concentrations in 7 BD Pigs and 7 BD + Lab Pigs

<table>
<thead>
<tr>
<th>Time</th>
<th>Adenosine, µmol/L</th>
<th>Lactate, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.19 ± 0.03</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>T0 to T20</td>
<td>1.29 ± 0.08*</td>
<td>1.05 ± 0.08</td>
</tr>
<tr>
<td>T20 to T40</td>
<td>0.51 ± 0.06*</td>
<td>0.91 ± 0.13*</td>
</tr>
<tr>
<td>T40 to T60</td>
<td>0.32 ± 0.04</td>
<td>0.8 ± 0.12</td>
</tr>
<tr>
<td>T60 to T80</td>
<td>0.22 ± 0.03</td>
<td>0.73 ± 0.12</td>
</tr>
<tr>
<td>T80 to T100</td>
<td>0.19 ± 0.03</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td>T100 to T120</td>
<td>0.20 ± 0.04</td>
<td>0.73 ± 0.09</td>
</tr>
<tr>
<td>T160 to T180</td>
<td>0.22 ± 0.07</td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td>Volume Expansion</td>
<td>0.87 ± 0.15†</td>
<td>1.24 ± 0.15†</td>
</tr>
</tbody>
</table>

*Values are presented as mean ± SEM.
†P < 0.05 vs baseline values.
‡P < 0.05 vs third-hour values.
‡‡P < 0.05 vs BD pig values.
Brain death leads to a series of pathophysiological changes with deleterious consequences on cardiac function that have been well documented. They are characterized by a massive but transient release of circulating catecholamines, associated with a sustained increase in myocardial norepinephrine (NE) and neuropeptide Y (NPY) interstitial levels, directly released from cardiac sympathetic nerve endings, which result in a striking increase in myocardial oxygen demand as estimated by the increase in the RPP (Table).

No significant correlations were observed between interstitial myocardial adenosine concentrations and the hemodynamic changes observed in this experimental model.

Discussion

Brain death leads to a series of pathophysiological changes with deleterious consequences on cardiac function that have been well documented. They are characterized by a massive but transient release of circulating catecholamines, associated with a sustained increase in myocardial norepinephrine (NE) and neuropeptide Y (NPY) interstitial levels, directly released from cardiac sympathetic nerve endings, which result in a striking increase in myocardial oxygen demand as estimated by the increase in the RPP (Table).

Given the coronary vasoconstricting properties of NE and NPY, we hypothesized that the autonomic storm might contribute to impairment of the myocardial oxygen supply-to-demand balance, thus switching myocardial metabolism from an aerobic to an anaerobic pattern. This hypothesis is consistent with previous results from our laboratory and more recently from other groups suggesting abnormal CBF regulation after brain death. The pathophysiological paradigm is that increased oxygen demand in parallel with impaired coronary reserve results in functional myocardial ischemia that contributes to the myocardial dysfunction observed after brain death.

In the present study, to confirm this hypothesis, hemodynamic parameters and CBF measurements were performed to estimate the changes induced by brain death on LV contractility and myocardial oxygen demand and supply in the presence or absence of α/β-adrenergic blockade. The choice of a mixed α/β-adrenergic antagonist was based on the widely documented observation that brain death resulted in NE release directly from cardiac sympathetic nerve endings, leading to the activation of both α- and β-adrenergic receptors. Intersitial myocardial adenosine and lactate concentrations were monitored as an estimate of the potential mismatch between energy supply and demand, and myocardial ischemia.

A sustained increase in interstitial adenosine concentrations was observed in BD pigs after brain death induction. It reached peak values as of the first microdialysis sampling period (first 20 minutes) after brain death. Similar increases in adenosine concentrations have been previously reported to elicit maximal coronary dilatation, leading to a 300% to 400% increase in CBF in pigs. However, in our experiments, a limited increase in CBF was observed during the autonomic storm. This should be opposite the maximal increases in HR, LV dP/dt, RPP, and thus myocardial oxygen demand observed during the same experimental period. Simultaneously, a sustained increase in interstitial lactate levels was observed. Taken together, increased adenosine and lactate interstitial concentrations are consistent with the onset of an imbalance between oxygen supply and demand.

On the other hand, BD+Lab pigs exhibited limited hemodynamic modifications after brain death. These results confirmed the effect of labetalol administration before brain death induction, suggesting that the consequences of the myocardial sympathetic storm could be attenuated by early α- and β-adrenergic blockade. Moderate increases in HR and CBF were recorded. These modifications might result from incomplete β-receptor blockade or from the intrinsic β-mimetic properties of this drug. Moreover, the slight increase in CBF we observed is consistent with previous results observed in patients with coronary artery disease who exhibited increased CBF after labetalol administration resulting from α-receptor blockade. This increased CBF might also be a consequence of the moderate increase in adenosine interstitial levels observed in BD+Lab pigs.

The adenosine molecules present within the myocardial extracellular space may be derived from either the degradation by ATP pyrophosphohydrolase and ecto-5'-nucleotidase of ATP coreleased with NE and NPY from cardiac sympathetic nerve endings and/or directly from cardiac myocytes. They are considered to exhibit a "retaliatory effect" when energy delivery does not match energy demand but seem to...
essentially play a role in the early phase of the vasodilatory response, although not in the continuation of hyperemia.21 However, adenosine production and sympathetic activation have been shown to have complex interactions. Blockade of adrenergic receptors has been shown to abolish the adenosine nucleotides and adenosine efflux induced by norepinephrine from rat caudal and aortic arteries.22 Antagonists of β-adrenergic receptors have been found to prevent adenosine formation associated with myocardial hypoperfusion not only through their β-blocking activities but also through membrane stabilizing and antioxidant activities.23,24 Therefore, the limited increase in adenosine interstitial levels observed in labetalol-treated animals could be the result of reduced oxygen demand, direct α1-β-adrenergic receptor blockade, or both. Nevertheless, the increased adenosine concentration, in parallel with increases in CBF after both brain death induction and volume expansion, suggested a preserved regulatory role of myocardial adenosine after labetalol administration. This preserved regulatory role of adenosine is further confirmed by the stability of lactate interstitial concentrations observed in the BD+Lab group after both brain death induction and volume expansion.

Three hours after brain death, a volume expansion protocol was performed. Although this procedure does not provide precise and direct information concerning systolic or diastolic LV function, it allows for adequate clinical evaluation of potential cardiovascular reserve. After rapid fluid infusion, both groups exhibited profoundly different hemodynamic and biochemical changes. As expected, brain-dead animals presented a decrease in dp/dt, associated with a limited increase in CO and increased QLAD. This apparent hemodynamic failure was associated with increased adenosine and lactate interstitial concentrations. These results are consistent with our previous observations and suggest that despite a preserved regulatory role of adenosine on CBF, the myocardium of brain-dead animals was unable to adequately use oxygen, as attested to by the increased lactate interstitial concentrations. On the contrary, labetalol-treated animals exhibited a significant increase in dp/dt, CO, and QLAD. These modifications were associated with increased adenosine interstitial concentrations in relation with increased myocardial work,25 when lactate interstitial concentrations were not significantly modified.

In conclusion, in the present experimental model, brain death led to a dramatic increase in myocardial oxygen demand associated with a limited increase in CBF. This resulted in a sustained increase in myocardial interstitial adenosine and lactate concentrations consistent with oxygen supply-demand imbalance. The causal relationship among the autonomic storm, inadequate CBF, and myocardial dysfunction is strongly supported by the protective effects of α-/β-adrenergic blockade. These results suggest that the strategies designed to expand the quality and number of grafts available for heart transplantation should take into account the consequences of myocardial oxygen supply-demand imbalance resulting from the initial sympathetic activation.

Acknowledgment

This work was supported by a grant from Etablissement Français des Greffes, Paris, France.

References

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_Circulation_. 2001;104:I-197-I-201
doi: 10.1161/hc37t1.094714
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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