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BRAIN DEATH DOES NOT CHANGE EPICARDIAL ACTION POTENTIALS AND THEIR RESPONSE  
TO ISCHEMIA-REPERFUSION IN OPEN CHEST PIGS

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**KEYWORDS:** Ischemia, Brain death, Transplantation, Action potential

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## **Abstract**

**Background:** It is debated whether brain death (BD) causes transient functional ischemia. Here we used monophasic action potential (AP) recording, as a sensitive means to assess, during BD, (i) whether ischemia was present, (ii) the effect of BD on a subsequent ischemia-reperfusion challenge.

**Methods:** Period 1: BD was induced (BD group, six pigs) or not (sham maneuver, C group, six pigs) and effects were followed up for 3 hours. Period 2: LAD ligation ischemia was applied for 20 min to all hearts, and was followed by 60-min reperfusion.

**Results:** Period 1: plasma norepinephrine was 3.1, 6.3 and 5 fold larger in BD versus C at respectively 1, 120 and 180 minutes and systolic blood pressure was larger by 26% at 1 min and 35% at 120 minutes. The arterio-venous difference in lactate was similar or lower in BD versus C. In both groups, at all times, AP had rectangular plateau shape and the action potential duration ( $APD_{50}$ ) followed a linear relationship to the RR interval ( $R^2 = 0.89$  and  $0.73$ , slope =  $0.42 \pm 0.02$  and  $0.46 \pm 0.06$  in BD and C respectively). Period 2: ischemia caused a similar (50%) APD shortening in BD and C. Restoration of APD upon reperfusion was complete in both groups.

**Conclusions:** These data suggest that BD does not cause direct cardiac ischemia and does not change the response of the heart to subsequent ischemia-reperfusion challenge.

## Introduction

Besides its fundamental interest, the identification of the pathophysiology of BD appears of importance in the current context of shortage of organ supply,<sup>1</sup> as it may help to better define the management of the organ donor aiming at heart graft improvement.<sup>2</sup> Myocardial injury after BD is a primary cause of graft refusal.<sup>3</sup> It is multifactorial in origin and still incompletely understood.<sup>2</sup> There is controversy about whether BD directly produces cardiac ischemia or contributes less directly to myocardial dysfunction. Several works presented evidence in favor of myocardial ischemia during BD. BD was shown to induce major changes in circulating hormones, namely a large increase in plasma catecholamines at the onset of BD, especially dopamine, epinephrine and norepinephrine that are released from the autonomic nervous system (Cushing reflex), whereas decreased levels of cortisol, insulin and triiodothyroxine were evidenced.<sup>4-7</sup> In addition, endothelin 1 is released after BD,<sup>8</sup> which may cause coronary vasoconstriction. Furthermore, endothelium function is depressed which leads to the loss of its vasorelaxant potency and to increased sensitivity of coronary vessels to the constrictive effects of serotonin.<sup>9</sup> These hormonal changes converge in favoring the occurrence of a coronary artery vasospasm causing decreased organ perfusion.<sup>10</sup> These hormonal disorders may also switch the mode of cardiac metabolism from aerobic to anaerobic,<sup>11</sup> leading to depletion of high energy phosphates (HEP) and to depressed myocardial metabolism.<sup>12-15</sup> Mertes' group further showed that the early phase of BD was associated with both myocardial adenosine and lactate increase,<sup>16</sup> corroborating the ischemia hypothesis. Moreover, Ryan et al. recently confirmed reversible functional impairment again suggesting that an ischemic phenomenon occurs during BD.<sup>17</sup> However, other experimental and clinical studies strongly suggest that BD does not affect cardiac energetic metabolism. Bruinsma et al. showed that BD-related myocardial injury in cats was not associated with HEP compounds depletion.<sup>18</sup> Furthermore, Stoica et al. demonstrated that myocardial HEP were preserved in human donor hearts during BD period, both in right and left ventricles.<sup>19</sup> In a subsequent study, Bruinsma et al. confirmed that experimental BD did not induce depletion in HEP compounds, whereas energetic failure became apparent only upon reperfusion after harvesting the heart in cold storage solution.<sup>20</sup> Similarly, Bittner et al. noted that adenosine triphosphate and creatine phosphate remained unchanged before and after BD,<sup>6</sup> while Szabo et al. did not evidence an ischemic phenomenon during experimental BD.<sup>21</sup>

There has been so far no attempt to directly assess a loss of myocardial perfusion as a consequence of BD. Myocardial ischemia as due to myocardial underperfusion, and in such a way as to subsequently induce a major loss of function, should be present over the whole organ. Such a phenomenon should be detected from any region of the left ventricle. In the present study, we recorded monophasic action potentials (AP) from the epicardial region of the left anterior descending coronary artery (LAD) territory, as the least invasive method to assess the presence of an episode of myocardial ischemia in response to BD. We further used an ischemia-reperfusion challenge to assess the responsiveness of local action potential shape to global ischemia of each heart in the control and BD groups.

## **Materials and methods**

All animals used in the present investigation received humane care in compliance with *The Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and *The Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH Publication No 86-23, revised 1985).

### *Animal preparation*

Twelve pigs weighing 35 to 40 kg were allocated into two groups of six pigs: group (C) as the control group and group (BD) as the brain-dead group. Both groups were submitted to the same protocol, with the exception of brain death in the BD group. The animals were premedicated with intramuscular injection of 5 mg/Kg ketamine followed by an intravenous injection of 3 mg/Kg xylazine (2%). Anesthesia was maintained with inhaled halothane 2% supplemented with oxygen 80%. The animals were intubated with a cuffed endotracheal tube and ventilated at 130 ml.Kg<sup>-1</sup>.min<sup>-1</sup>. A Foley catheter was introduced into the subdural space through a parietal burr hole in the skull. A carotid canula was inserted to monitor systemic arterial blood pressure and the right jugular vein was cannulated for venous access. After sternotomy and opening of the pericardium, the heart was exposed. A ligature was prepared around the LAD, just below the departure of the first coronary artery bifurcation.

### *Brain death model*

A rapid injection of 15 ml of saline inflated the balloon of the subdural catheter, which caused an acute increase in intra-cranial pressure. Brain death occurred within a few minutes in all pigs. Disappearance of electroencephalographic activity, irreversible and complete bilateral mydriasis, and cessation of spontaneous respiration confirmed brain death, as previously validated.<sup>22</sup>

### *Measurements*

Baseline hemodynamic data and AP's were recorded 15 minutes before brain death induction. Subsequent measurements were performed at various times after subdural balloon inflation: 1, 30, 60, 120 and 180 minutes after brain death. In the control group, data were obtained at identical times. Arterial and venous blood samples were taken at initial baseline and at each of the above times in control and BD pigs for off-line dosage of norepinephrine and lactate (see below). Continuous monitoring of the electrocardiogram (ECG) and arterial pressure was done (without recording) using a HP78534A (Hewlett Packard) that was adjusted to report an alarm upon fibrillation or cardiac pause.

### *Regional ischemic challenge*

After the initial period of 180 minutes of follow-up, with or without brain death, the anterior coronary artery was ligated for 20 minutes after which reperfusion was followed up for 60 minutes. In one of the control pigs, the AP recording electrode became unstable. Thus, for data under ischemia, n=5 in the control group. In two pigs subjected to BD, we experienced one coronary vessel rupture upon clamping; and in another, the heart went into fibrillating arrest. Thus, for data under ischemia, n=4 in the BD group.

### *Biochemical analysis*

Plasma norepinephrine and arterio-venous difference (A-V) in serum lactate were evaluated as described by our group.<sup>22</sup>

### *Monophasic action potential (AP) recording*

A suction electrode with concentric chlorided silver wire leads was prepared according to Reid and Duker.<sup>23</sup> The device was connected via a twisted pair to a differential amplifier. Signal output was sampled at 1000 Hz through a MacLab interface and the Chart software (AD Instruments Ltd., Hastings, East-Sussex, GB). The electrode was applied onto the epicardial surface of the left ventricle near the apex using as low suction as needed to maintain it in place. After stabilization of the amplitude of the signal (30 sec to 1 min), APs were recorded during 1 minute at stable heart rate. AP's were detected and analyzed, as previously described,<sup>24</sup> under MATLAB (The MathWorks Inc., Natick, MA, USA) for AP plateau amplitude and action potential duration at 50% repolarisation from the plateau level (termed action potential duration, APD). Time zero for computing APD was taken at the time of the maximum upstroke velocity of the AP.

### *Statistical analysis*

All values are mean  $\pm$  standard error of the mean (SEM). Unpaired Student's t test was used to compare the two groups. A two-way analysis of variance (ANOVA) with repeated measures was used to compare baseline values and post-brain-death data. A difference was considered statistically significant at  $p < 0.05$ . Parameters estimated by linear regression of APD versus the RR interval of the ECG are reported with their 95% confidence interval.

## Results

### *Hemodynamic and plasma changes during BD (Table 1)*

Baseline values of plasmatic norepinephrine were similar in control and BD groups. From one minute post-BD and during all the time course of the experiment, we observed a significant increase of plasma norepinephrine in the BD group (Table 1). Concomitantly, there was an expected slight increase in systolic blood pressure. The arterio-venous difference in lactate contents was similar in both groups throughout the time course of the experiment, with the exception of a slightly lower value in BD group versus control group at time 120 minutes of the initial period (Table 1). Thus at no time was there any increase in the arterio-venous lactate difference after induction of BD.

### *Changes in ventricular epicardial AP after BD*

Baseline monophasic action potentials were recorded after a stabilization period once the animal was operated and all measuring devices and intra-cranial balloon set in place. These action potentials (see traces labeled *b* in the two upper panels of Figure 1) present a shape quite comparable to action potentials recorded in pigs either by suction electrode,<sup>25</sup> contact electrode<sup>26,27</sup> or microelectrodes.<sup>26,28</sup> This suggests that our recording procedure was reliable. In control pigs (left column in Figure 1), the balloon was not inflated, so that no brain death was induced. The changes in action potential plateau shape visible in the left panel A of Figure 1 may principally be attributed to slight changes in the position of the recording electrode on the heart surface, as the electrode was not left in place between one acquisition and the next one, to avoid injury to the myocardial tissue by maintained suction. However, no large changes in action potential duration occurred.

During the three hours after BD induction, the shape of the ventricular action potential in the pigs subjected to BD (right column in Figure 1) remained comparable to that in control pigs (upper right panel in Figure 1). In the present model of experimental BD in pigs, we previously evidenced a biphasic increase in catecholamines and ventricular function with a transient maximum at 1 minute followed by a return to baseline and a secondary increase throughout 30, 60, 120 to 180 minutes.<sup>7,22</sup> Measurements at these key times in the present study do not provide evidence of action potential shortening that would reflect acute ischemia.

### *Changes in AP during ischemia and at reperfusion*

In both groups of pigs, ischemia as provoked by LAD coronary artery ligation rapidly induced a dramatic change in ventricular action potential shape characterized by a loss of the action potential plateau resulting in triangular-shaped action potentials with a drastic shortening of APD (Figure 1 middle panels) that was highly significant (see *Isch* in Figure 2). The magnitude of APD shortening was the same in both groups. These changes were identical to the action potential changes under ischemia reported for pigs.<sup>25,29</sup> Upon reperfusion, the action potential recovered a rectangular shape with prominent plateau as before ischemia (lower panels of Figure 1) and APD returned to near

baseline values after 60 minutes reperfusion (*Reperf* in Figure 2). Such large APD shortening with triangular action potentials as under ischemia was not seen during 3 hours after BD, which suggests an absence of ischemia during BD.

However, it may be noted that a sizeable dispersion of APD values was present in both groups, as attested in control recordings in upper left panel of Figure 1 and by the values of the SEM (Figure 2). Such dispersion either might be due to a change of action potential duration per se, or to changes in the heart rate, that are known to alter the APD. Therefore, we further analyzed the relation of APD to the last RR interval before each AP recorded.

#### *Relation of APD to the RR interval in BD and after provoked ischemia*

The AP-duration versus RR relation was analyzed and compared in sham-operated and in brain death groups (Figure 3). APD values for each pig at baseline and at each time of the 3 hours experimental period were plotted against the corresponding duration of the RR interval and the data set was evaluated by linear regression analysis. Values of ADP for sham operated controls followed the RR interval along a linear relation (slope  $0.46 \pm 0.06$ ; APD =  $17 \pm 39$  ms at RR=0) with a correlation coefficient ( $R^2$ ) of 0.73 (see lozenges and continuous line in Figure 3). For brain-dead pigs, the RR values were found to span over shorter durations, and the APD also varied with the RR interval along a linear relation (slope  $0.42 \pm 0.02$ ; APD =  $52 \pm 13$  ms at RR=0,  $R^2 = 0.89$ , circles and dashed line in Figure 3). As neither the slope nor the ordinate at origin were significantly different from one regression line to the other, we concluded that both series of APD values were related to the RR interval along the same linear relationship (not shown in Figure 3). During ischemia, if a genuine action potential shortening would occur, it is expected that the relation of APD to RR changes and becomes quite different from the one analyzed in the preceding 3 hours recording period (see above). The plot of APD durations during the ischemic period (Figure 3) shows that, indeed, most APD durations fall far from the linear relation of APD to RR in BD pigs (x symbols in Figure 3) as it does in control pigs (+ symbols). The coefficient  $R^2$  of the linear regression for APD versus RR under ischemia being 0.02 in control pigs and 0.12 in BD pigs. Thus, ischemia induced a large decrease of AP duration that was not related to changes in the RR interval. There was no sign of such shortening of the APD during 3 hours after induction of BD.



## Discussion

In this study, we clearly demonstrated that BD (Figure 1) induced no ischemia-related changes in epicardial action potential shape. In particular, there was no significant difference in AP duration between BD and control groups (Figure 2), as all changes in AP duration outside ischemia and reperfusion were accounted for by frequency changes (Figure 3). The sensitivity of the regional action potential duration to ischemia was established by Kingaby et al.<sup>30</sup> who demonstrated, in the pig heart in-situ, a linear relationship of action potential (AP) duration to local ischemic index with a correlation coefficient  $R^2$  of 0.76. A decrease in the regional myocardial blood flow by as little as 5% would have been detected by this method.<sup>30</sup> In case of a significant ischemia, a drastic shortening of AP duration should be present.<sup>25,27,29</sup> Damages induced by ischemia were reported to be more severe at the sub-endocardial layer of the left ventricle than at the sub-epicardial layer.<sup>31</sup> If so, epicardial action potentials, as recorded here, might therefore have failed to report any ischemia that would be confined to the sub-endocardial layer. However, in open chest pigs, ischemia induced by LAD coronary occlusion caused a similar response of the epicardial layer of the left ventricular free wall to that of the endocardial layer.<sup>32</sup>

The primary conclusion of our study is that our epicardial action potential data do not support the hypothesis of metabolic impairment accompanying a putative global myocardial ischemia that would be caused by BD. Thus, myocardial perfusion presumably remained adequate after BD. Under this condition, the absence of an increase in arterio-venous lactate difference during BD (Table 1) suggests that no major switch to anaerobic metabolism occurred at the times explored in this study.

In the second part of the study, we tested whether BD was responsible for a change in sensitivity of the heart to an ischemia-reperfusion challenge, as encountered in clinical settings. In response to LAD coronary artery ligation, there appeared a characteristic triangular shape of the repolarisation phase resulting in a large shortening of APD.<sup>30</sup> APD shortening under provoked ischemia was similar in BD and in controls (Figure 2), suggesting a similar sensitivity of the hearts to ischemia in both groups.

Another likely consequence of this result is that, if an undetected significant **ischemic** episode had taken place during BD, it might have resulted in preconditioning of the heart. Indeed, it was shown in pigs that the duration of epicardial monophasic APs of the left ventricle shortened twice more during a second period of 8 minutes of LAD coronary artery ligation than during the first one, manifesting the presence of a preconditioning effect.<sup>33</sup> In the present study, the similar shortening of APD observed in both groups during provoked ischemia suggests the absence of a preconditioning effect as would result from a previous ischemic episode related to BD.

The reversibility of the AP duration shortening upon reperfusion was complete in both groups (Figure 2), and action potential returned to baseline shape (Figure 1). Thus, restoration of electrical activity after ischemia was as complete in BD pigs as in control ones, which confirms the absence of changes in myocardial response to ischemia in BD versus control.

The present results do not support the idea of a significant hypoperfusion of the left ventricle within 3 hours after BD and suggest that more indirect mechanisms are likely to cause myocardial function impairment associated to BD.

## Conclusion

In the present model of BD in pigs, BD-induced ischemia was not supported. When ischemia was induced by LAD ligation, BD did not affect the response to ischemia and subsequent recovery.

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Figures

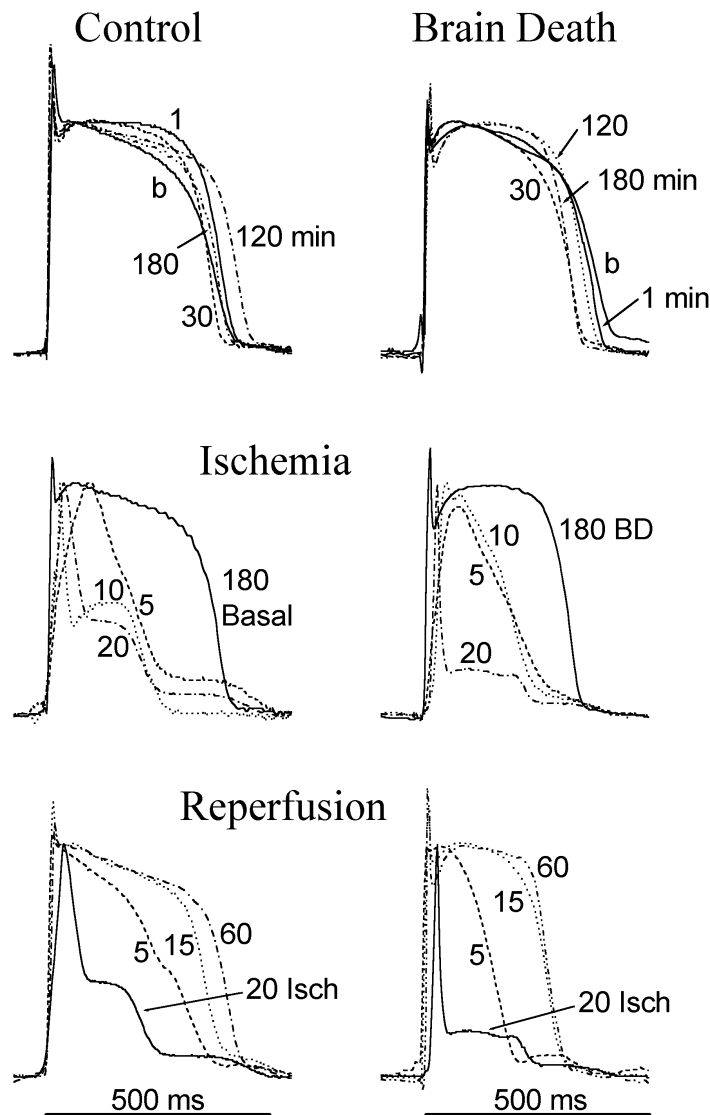


Figure 1: Representative monophasic action potentials (AP) recorded with an epicardial suction electrode near the apex of the heart in a *Control* pig (left column) and in a pig subjected to *Brain Death* (right column). One recording was taken before the start of the experiment, in open chest condition, (trace noted "b" for baseline) and at various times (indicated in minutes by numbers near the traces) up to 180 minutes after the onset of the experiment (upper panels, no intervention for the control pig, brain death induction at time 0 for the BD pig). Thereafter, a 20-minutes ischemic period was applied (*Ischemia*, middle panels, numbers are times in minutes from LAD occlusion, trace at 180 minutes ("180 Basal" or "180 BD") of the first period is superimposed for comparison). It was followed by a reperfusion period (*Reperfusion*, lower panels, numbers are times in minutes after releasing the LAD ligature). In *Ischemia* and *Reperfusion*, the last recording under ischemia ("20 Isch") is shown for comparison. All monophasic action potentials were normalized in amplitude and diastolic voltage level to enable detailed comparison of AP shapes.

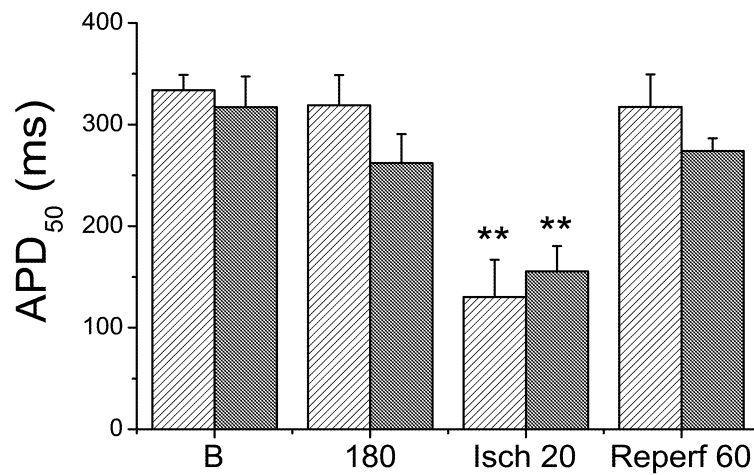


Figure 2: Mean action potential duration (APD, measured at 50% repolarisation) values at baseline (5 minutes before the beginning of the experiment), 180 minutes after inducing brain death (BD), after 20 minutes of subsequent ischemia and after 60 minutes of reperfusion. Means  $\pm$  SEM of APD are plotted for sham-operated pigs (striped bars) and for BD pigs (gray filled bars) (n=6 in each group). Significant decrease in APD versus baseline value is indicated by \*\* ( $p < 0.01$ ).

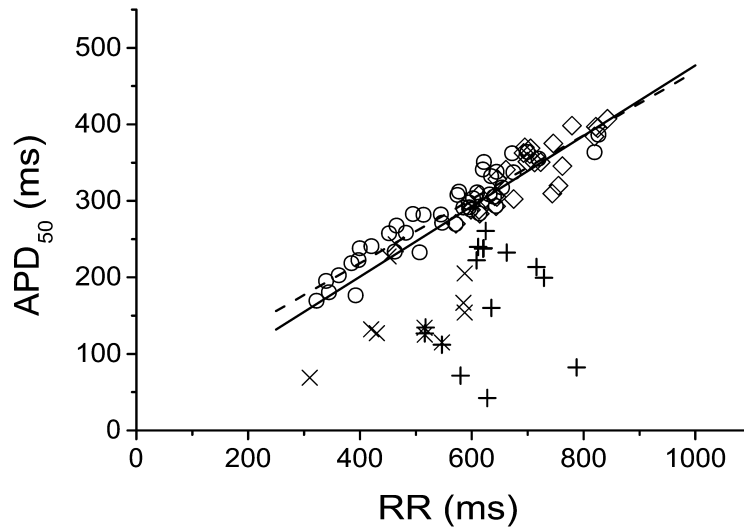


Figure 3: Relation of action potential duration (APD) to the RR interval. Values at baseline and for all times, after 1, 30, 60 and 180 min are plotted for sham-operated (Control) pigs (diamonds) and for brain death (BD) pigs (circles). The regression lines for data from control pigs and for data from BD pigs are represented by a continuous and a dotted line, respectively. The line equation for BD data was  $APD = RR * 0.42 (\pm 0.02) + 52 (\pm 13)$  ms with  $R^2 = 0.89$  and for Control data  $APD = RR * 0.46 (\pm 10.06) + 17 (\pm 39)$  ms with  $R^2 = 0.73$ . Values of APD during the ischemic period (at 5, 10 and 20 min ischemia) are shown for control (+ symbols) and BD animals (x symbols). Note that almost all APD values under ischemia fall well off the linear region. The correlation to RR was negligible: the  $R^2$  coefficient being respectively 0.02 and 0.12.



**Table 1**

		Basal	T1	T30	T60	T120	T180
Norepinephrine (ng/l)	C	64.7 ± 19.3	64.5 ± 19.1	50.3 ± 11.5	57.7 ± 14.4	54.5 ± 17.1	97.5 ± 14.5
	BD	70.8 ± 9.9	200 ± 42*	77.9 ± 17.7	162 ± 57	341 ± 105*	495 ± 118**
Lactate A-V (mmol/l)	C	0.1 ± 0.05	0.10 ± 0.05	0.08 ± 0.07	0.07 ± 0.09	0.17 ± 0.05	0.05 ± 0.02
	BD	-0.01 ± 0.03	-0.07 ± 0.09	0.05 ± 0.08	0.10 ± 0.08	-0.12 ± 0.07*	0.06 ± 0.10
Systolic blood Pressure (mm Hg)	C	67.8 ± 3.8	66.7 ± 3.4	64.5 ± 3.3	63.0 ± 3.3	60.2 ± 4.5	58.8 ± 5.6
	BD	76.3 ± 3.9	84.0 ± 5.7*	68.0 ± 2.9	75.6 ± 4.9	81.0 ± 6.1*	77.6 ± 6.9

Table 1: Six pigs were sham-operated (C) and maintained for 3 hours (see methods). Six other pigs were subjected to brain death (BD). Plasma norepinephrine, arterio-venous difference (A-V) in serum lactate and systolic blood pressure were evaluated during initial control period (Basal) and after start of experiment, at times 1 minute (T1), 30 minutes (T30), 1 hour (T60), 2 hours (T120) and 3 hours (T180). Values are means ± SEM. Significant differences of BD versus control are noted with \* for p<0.05, \*\* for p<0.01.