Preservation of ischemia and isoflurane-induced preconditioning after brain death in rabbit hearts

PASCAL CHIARI,1 VINCENT PIRIOU,1 GUYLAINE HADOUR,2 CLAIRE RODRIGUEZ,3 JOSEPH LOUFOUAT,2 JEAN-JACQUES LEHOT,1 MICHEL OVIZE,2 AND RENÉ FERRERA2

1Service d’Anesthésie Réanimation, Hôpital Cardiologique Louis Pradel; 2Institut National de la Santé et de la Recherche Médicale E0226, Faculté de Médecine Lyon Nord, Université Claude Bernard Lyon I; and 3Laboratoire de Biochimie, Hôpital Cardiologique Louis Pradel, 69394 Lyon Cedex 03, France

Received 24 April 2002; accepted in final form 3 July 2002

Address for reprint requests and other correspondence: P. Chiari, Service d’Anesthésie Réanimation, Hôpital Cardio-Vasculaire Louis Pradel, BP Lyon-Monchat 69394 Lyon Cedex 03, France (E-mail: p.chiari@vnumail.com).

PHARMACOLOGICAL STIMULATION of α-adrenergic receptors, either with exogenous norepinephrine or via release of endogenous catecholamines, has been shown to trigger preconditioning in some preparations (3, 4, 16, 25). These experimental observations might be of major clinical importance in the settings of brain death (BD), which is accompanied by an acute and dramatic sympathethic stress (5, 22). Hearts of brain-dead patients may be further used as donor organs and thus submitted to prolonged ischemia followed by reperfusion before transplantation. Despite protection of the cardiac graft by using hypothermia and preservation solutions, the myocardium may be damaged, sometimes irreversibly.

It is therefore of major clinical importance to determine whether BD-induced catecholamine release may protect the heart, and if not, whether it retains the ability to be preconditioned to improve overall cardiac graft protection.

The general objective of the present study was to investigate whether BD may precondition the rabbit heart, and, if not, whether preconditioning can still be induced by using the clinically available mitochondrial ATP-sensitive K⁺ channel (K⁺ATP) activator isoflurane.

MATERIALS AND METHODS

All animals were treated in accordance 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 National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

Surgical preparation. New Zealand White rabbits of either sex, weighing 2.5 ± 0.5 kg, were premedicated with an intramuscular injection of ketamine (50 mg/kg) and anesthetized with xylazine (50 mg/kg im), as previously described (10). Anesthesia was maintained by a continuous infusion of thiopental (30 mg·kg⁻¹·h⁻¹ iv). After tracheotomy, animals were mechanically ventilated (Servo ventilator 900B, Siemens-Elema; Solna, Sweden) by using a tidal volume of 15 ml/kg, a frequency of 35 breaths/min, and an oxygen fraction of 50%. When needed, adjustments were made to keep the end-tidal carbon dioxide within the physiological range. End-tidal gas concentrations were measured continuously by using a gas analyzer (Capnomac Ultima, Datex; Helsinki, Finland). Limb lead II of the ECG was recorded throughout the experiment. Core temperature was maintained between 38 and 39°C by means of a heating system incorporated into the operating table. Systemic blood pressure was monitored by use of a Gould pressure transducer connected to a fluid-filled catheter inserted in the left femoral artery. Infusion of fluids (hetastarch, 5 ml·kg⁻¹·h⁻¹) and drugs was performed via a catheter positioned into an ear vein.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
After an intravenous bolus administration of fentanyl (25 μg), the heart was exposed via a left thoracotomy and suspended in a pericardial cradle. A 4-0 suture was passed around the first large marginal branch of the circumflex artery to further permit coronary occlusion. In all animals, a craniotomy was performed ∼5 mm from the sagittal suture, at the fusion of the parietooccipital plates. An electroencephalogram (Reega Minihuit-TR, Alver Electronic, Montreuil-Paris, France) was recorded using electrode needles positioned on the two parietooccipital skull areas. After the surgical preparation, 30 min of stabilization were allowed.

Induction of BD. In BD groups, a 10-Ch Foley catheter was introduced into the subdural space. BD was induced by injection of 5 ml of a normal saline solution into the catheter balloon over 10 s. Disappearance of electroencephalogram waves, occurrence of a bilateral fixed mydriasis, and disappearance of spontaneous respiration ascertained BD. After injection, the catheter was kept inflated until the end of the experience.

Experimental design. The present study was aimed at determining: 1) whether BD may protect the heart against infarction, and 2) whether preconditioning may still be induced in BD rabbits by using either ischemia or halogenated anesthetics as a trigger.

In all groups, the coronary artery was occluded for 30 min. Myocardial ischemia was confirmed by the appearance of a regional cyanosis, akinesia, or dyskinesia and a marked S-T segment elevation in the ECG. After 30 min, the snare was released and reperfusion was allowed for a period of 4 h. Reperfusion was visually confirmed by the disappearance of epicardial cyanosis.

Before the sustained 30-min coronary artery occlusion, three groups of rabbits underwent BD, either alone (BD group) or associated with two episodes of 5 min of ischemia and 10 min of reperfusion (BD + IPC group), or with one episode of 15 min isoflurane inhalation (1 minimum alveolar concentration = 2% end tidal concentration), followed by a 15-min washout period (BD + Iso group) (8) (Fig. 1). In the three remaining groups, BD was not performed before the sustained occlusion; rabbits underwent either no intervention (C group), two episodes of 5 min ischemia and 10 min of reperfusion (IPC group), or one episode of 15 min of isoflurane inhalation (1 MAC = 2% end-tidal concentration), followed by a 15-min washout period (Iso group), as previously described (9). For each animal receiving isoflurane, end-tidal concentration was <0.1% at the end of the washout period.

Area at risk and infarct size measurement. At the end of the 4-h reperfusion period, the coronary artery was briefly reoccluded. Uniperser blue (Ciba-Geigy; Hawthorne, NY) was injected via the ear vein catheter to delineate the area at risk. Euthanasia was then induced, under deep anesthesia, by an intravenous injection of 4 meq of KCl. The heart was excised and, after removal of the right ventricle, cut into five or six 2-mm thick transverse slices. Each slice was weighed. Its basal surface was photographed. Each slice was then incubated for 20 min in triphenyl tetrazolium chloride (TTC) (at 37°C) and rephotographed for measurement of infarct size. Extent of left ventricular (LV) area, area at risk, and area of necrosis were quantified by computerized planimetry and corrected for the weight of tissue slices. Total weights of area at risk and area of necrosis were then calculated and expressed as weight (in grams).

Plasma catecholamines. Arterial plasma samples were immediately centrifuged (3,000 rotations/min, at +4°C, for 10 min) and stored at −80°C until measurement. Plasma levels of epinephrine and norepinephrine were assessed using the Chromsystems kit for high-performance liquid chromatography analysis with electrochemical detection. Normal reference values were <0.27 nmol/l for E and <1.77 nmol/l for NE. Plasma catecholamines were measured as baseline and at 1 and 5 min after BD (baseline, T1, and T5, respectively) as well as just before the prolonged coronary occlusion (preocclusion).

Hemodynamics. Heart rate, systolic, and diastolic blood pressure (HR, SBP, and DBP) were assessed at baseline, 1 and 5 min after BD (baseline, T1, and T5, respectively) just before coronary occlusion (preocclusion), at the end of coro-

**Fig. 1.** Experimental protocol. BD + IPC, brain death + ischemic preconditioning group; BD + Iso, brain death + isoflurane group.
Hemodynamics. In the control group, heart rate and blood pressure remained stable throughout the experiment (Table 1). As expected, BD resulted in a dramatic increase in systolic arterial pressure and heart rate (Table 1). This hemodynamic response was however short lived because both systolic blood pressure and heart rate returned to near-control values at the onset of the prolonged coronary artery occlusion. In the IPC and Iso groups, blood pressure and heart rate did not significantly differ from control throughout the experiment. Both BD + IPC and BD + Iso groups displayed a hyperdynamic response comparable to that of the BD group following BD induction. During the final reperfusion, systolic blood pressure was consistently lower in the three BD groups compared with controls (Table 1).

Catecholamines. The hyperdynamic response was simultaneous to a major rise of plasma catecholamine levels in the BD groups (Table 2). One minute after BD, norepinephrine averaged 8.64 ± 5.79, 4.48 ± 3.26, and 13.86 ± 17.22 nmol/l (P < 0.05 vs. controls), in the BD, BD + IPC, and BD + Iso groups, respectively, significantly different from 0.41 ± 0.15 in the C group.
Epinephrine plasma levels averaged 1.82 ± 1.85, 1.20 ± 1.15, and 2.82 ± 3.27 nmol/l in the BD, BD + IPC, and BD + Iso groups, respectively, versus 0.34 ± 0.17 in the control group (P < 0.01 for all groups) (Table 2). At T1 or T5, plasma levels of epinephrine and norepinephrine were not significantly different among BD, BD + IPC, and BD + Iso groups. Plasma catecholamine levels failed to significantly vary in IPC and Iso groups.

**Infarct size.** LV weight and area at risk were comparable among the different groups (Table 3 and Fig. 2). BD failed to significantly alter infarct size that averaged 0.45 ± 0.27 g in the BD group versus 0.49 ± 0.34 g in controls (P = not significant). This was confirmed when the weight of the infarct size was plotted versus the weight of the area at risk (Fig. 3). Clearly, all points in the BD group lie close to the control regression line, indicating that for any value of area at risk, BD hearts developed infarct size comparable to controls. As expected, IPC and Iso-treated animals developed significantly smaller infarcts than controls: 0.11 ± 0.04 g and 0.21 ± 0.15 g in IPC and Iso groups, respectively (P < 0.05 versus controls). This infarct size limitation persisted in the BD + IPC and BD + Iso groups, with a mean area of necrosis averaging 0.10 ± 0.09 and 0.22 ± 0.10 g, respectively (P < 0.05 vs. controls) (Table 3 and Fig. 2). These results were confirmed when the weight of the infarct size was plotted versus the weight of the area at risk (Fig. 4). As depicted in Fig. 4A, data points for the IPC and the BD + IPC groups lie below the control line, indicating that for any size of the risk region, IPC alone as well as BD + IPC in animals resulted in significantly smaller infarcts than controls. Figure 4B shows a similar result within the Iso-treated groups: when performed with or without BD, Iso inhalation significantly decreased infarct size irrespective of the size of the risk region. There was no correlation between infarct size and plasma levels of catecholamines in the BD groups (BD, BD + IPC, BD + Iso).

**DISCUSSION**

In the present study, we demonstrated that BD alone fails to protect the rabbit heart, yet does not prevent induction of preconditioning using a brief episode of ischemia or pharmacological activation of mitochondrial K<sub>ATP</sub> channels.

Several studies suggested that endogenous release of catecholamines before a prolonged ischemic insult can protect the heart. Transient induction of norepinephrine release by tyramine before a prolonged coronary artery occlusion limits infarct size in the rabbit heart (23). Depletion of presynaptic nerve terminals of norepinephrine stores using reserpine prevents IPC (3, 24). In addition, exogenous norepinephrine can trigger a protection that is abolished by the α<sub>1</sub>-adrenoceptor blocker prazosin in both in vivo rabbit or isolated rat hearts (4, 13). Although important, these experimental designs do not truly refer to clinical situations.
In contrast, catecholamine release in BD experimental preparations clearly depicts the clinical scenario preceding cardiac transplantation. BD induces a transient and massive catecholamine release, and the donor heart further undergoes prolonged global ischemia before reperfusion at the time of transplantation. Although the above-cited studies would suggest that the soon-to-be transplanted heart may be protected following BD, the question remains unresolved. In the present study, we were unable to demonstrate any beneficial effect of BD-induced catecholamine release on the heart. Plasma catecholamine release was transient but of major amplitude, like in the clinical situation, and involved both norepinephrine and epinephrine. Absence of a protective effect cannot be due to a detrimental effect of the hemodynamic response, because both heart rate and blood pressure returned to near baseline levels at the onset of the sustained coronary artery occlusion, and heart rate and systolic blood pressure are not major determinants of infarct size in this preparation. One cannot rule out that the ischemia-reperfusion challenge designed in our protocol might have been too severe for a norepinephrine-induced protective effect to be effective, whereas such a putative protection may have been unmasked following prolonged global hypothermic ischemia and reperfusion as it occurs in the clinical settings. The apparent discrepancy of our results with studies demonstrating a role for catecholamines in preconditioning is unclear. One must yet mention that, in reserpinized rabbits, Ardell et al. (1) were able to induce IPC by using four, but not one, cycles of brief ischemia-reperfusion. Haessler et al. (11) failed to prevent IPC with α1-adrenoreceptor blockers. Sebbag et al. (21) could not protect the dog heart by intracoronary administration of the α1-adrenoreceptor agonist methoxamine. In contrast, our results are in close agreement with those of de Zeeuw et al. (8) who reported that transient intracerebral hypertension cannot precondition the pig heart despite a major myocardial norepinephrine release, as demonstrated by microdialysis. Also, Kirsch et al. (15) recently showed that BD does not trigger preconditioning in the rabbit. Conversely, some reports established a detrimental influence of catecholamines on the myocardium (17, 20). After an acute increase in intracranial pressure, BD results in a major neuronal depolarization and catecholamine release that may induce myocardial contractile dysfunction and, in some case, minimal focal necrosis (12, 22). Recently, Communal et al. (6, 7) demonstrated that norepinephrine induces apoptosis of cultured adult rat cardiomyocytes via β1-adrenoreceptors activation. One may hypothesize that BD might induce some irreversible damage, e.g., through apoptosis, that may have blunted the putative preconditioning effect of catecholamine release on infarct size limitation. This is, however, unlikely because myocardial damage possibly induced by catecholamine rarely exceed small foci of necrosis. Finally, it is possible that, although plasma levels of norepinephrine and epinephrine were dramatically increased in our preparation, their concentration within the myocardium remained beyond a given threshold necessary to trigger preconditioning, as suggested by de Zeeuw et al. (8).

The lack of donor organs currently limits the availability of heart transplantation, and as much as 20% of potential cardiac grafts display myocardial dysfunction. Interestingly, in the present study, hearts from BD animals could still be protected against further ischemia-reperfusion. These hearts preserved the ability to be protected by IPC with an infarct size reduction similar to that observed in non-BD preconditioned rabbits. This indirectly suggests that the absence of infarct size limitation following BD alone is likely not due, like hypothesized above, to the fact that the putative norepinephrine-induced preconditioning was masked by a concurrent catecholamine cardiotoxicity, but rather simply reflects the lack of efficiency of the catecholamine stimulus to precondition the heart in our experimental conditions. Our results are in contradiction with those of Kirsch et al. (15), who reported that IPC cannot be triggered in brain-dead rabbits. These discrepancies between the two studies may be because: 1) TTC determination of infarct size was performed after only 90 min of reperfusion in their study (vs. 4 h in the present work), and 2) mostly, Kirsch et al.
REFERENCES


18. Miura T, Kawamura S, Tatsumo H, Ikeda Y, Miikami S, Iwamoto H, Okamura T, Iwateate M, Kimura M, Dairaku Y, Maekawa T, and Matsuoka M. Ischemic preconditioning attenuates cardiac sympathetic nerve injury via ATP-sensitive potassium channel opener, isoflurane. The present observation is of potential major clinical importance because isoflurane inhalation is feasible in the situation of human BD to further protect the donor organ before cardiac transplantation. This however needs further investigations to be fully determined.

We express our gratitude to Colette Budat, Jean Paul Sastre, Colette Berthet, Florence Arnal, and Sylviane Conti for technical assistance.

This work was supported in part by a grant from Aventis.