DESENSITIZATION OF MYOCARDIAL β -ADRENERGIC RECEPTORS AND DETERIORATION OF LEFT VENTRICULAR FUNCTION AFTER BRAIN DEATH

Brain death often results in a series of hemodynamic alterations that complicate the treatment of potential organ donors before transplantation. The deterioration of myocardial performance after brain death has been described; however, the pathophysiologic process of the myocardial dysfunction that occurs after brain death has not been elucidated. This study was designed to analyze the function of the myocardial β -adrenergic receptor and the development of left ventricular dysfunction in a porcine model of experimental brain death. Analysis of the β -receptor included determination of receptor density and adenylate cyclase activity after stimulation independently at the receptor protein, the G protein, and the adenylate cyclase moiety. Myocardial β -receptor density did not change after the induction of brain death. A decrease in stimulated adenylate cyclase activity was observed within the first hour after brain death at the level of the β -receptor, the G protein, and the adenylate cyclase moiety, which suggests the occurrence of rapid desensitization of β -receptor function. Significant deterioration of myocardial performance also occurred within the first hour after brain death, represented by a decrease in preload-recruitable stroke work compared with the baseline value. The deterioration of myocardial performance after brain death correlates temporally with desensitization of the myocardial β -receptor signal transduction system. The mechanism of impairment appears to be localized to the adenylate cyclase moiety itself. (*J THORAC CARDIOVASC SURG* 1995;110:746-51)

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The success of transplantation is contingent on adequate organ availability and the treatment of brain-dead organ donors. Brain death is often accompanied by hemodynamic instability and impaired cardiac function, as found both in experimental animals and in potential organ donors. Hemodynamic compromise after brain death threatens the viability of all of the organs in a potential donor, and optimal preservation of cardiac function would protect these organs for retrieval.

Alterations in preload and afterload may contribute to the hemodynamic instability that occurs after irreversible cerebral ischemia; however, recent studies demonstrate that brain death produces decreased myocardial contractility independently.¹⁻³ Numerous experimental models have been used to investigate the pathophysiologic process of brain death and myocardial dysfunction. The induction of brain death in experimental animals has been shown to induce autonomic storm, with significant elevation of serum concentrations of epinephrine and norepinephrine.⁴ This massive release of endogenous catecholamines produces calcium overflow injury to the myocardium, which results in contraction band necrosis, myocytolysis, and impaired contractile mechanisms in the myocyte.^{5, 6} Furthermore, the deterioration of myocardial performance after brain death has been quantified by the use of preload-recruitable stroke work, a load-independent index of left ventricular performance.¹ Several investigators have demonstrated that the hemodynamic alter-

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ations that follow brain death may be prevented by the administration of propranolol before the induction of cerebral ischemia,^{7,8} which implicates the β -adrenergic signal transduction pathway in the pathophysiologic process of impaired myocardial performance after brain death. However, direct function of the β -adrenergic receptor during cerebral ischemia and its direct role in the development of left ventricular dysfunction after the induction of brain death have not been investigated.

The diversity of the components involved in the membrane β -receptor complex, that is, the β -receptor protein, the guanine nucleotide binding regulatory protein (G protein), and the effector (the adenylate cyclase moiety), is depicted in Fig. 1. When a β -agonist, such as isoproterenol, binds to the receptor, the intermediary G protein (Gs) transduces the signal to the effector system (the adenylate cyclase moiety), which is coupled to production of a second messenger, cyclic adenosine monophosphate (cAMP). G protein function requires the hydrolysis of guanosine 5'-triphosphate (GTP) and may also be stimulated independently by sodium fluoride at another site. The activated form of the G protein is coupled to the adenylate cyclase moiety, which itself may be activated independently by forskolin.

This study attempted to describe the mechanism of the deterioration of myocardial performance in a porcine model of experimental brain death with use of a functional analysis of the β -receptor complex, including density and adenylate cyclase stimulation at the level of the β -receptor protein, the G protein, and the adenylate cyclase moiety.

Methods

All animals 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 National Academy of Sciences (NIH Publication No. 86-23, revised 1985). This study used a validated porcine model of brain death.^{1,9} After institutional animal care committee approval, 12 pigs (30 to 35 kg) were sedated with intramuscular ketamine (20 mg/kg) and anesthetically treated with sodium thiopental (15 mg/kg). Pressure-transducer catheters were placed in the inferior vena cava via the femoral vein and the thoracic aorta via the femoral artery to monitor central venous pressure and systemic arterial pressures, respectively. Crystalloid solutions were administered to maintain central venous pressure at 3 to 8 cm H₂O in all animals. Throughout the study, arterial blood gas values were monitored and appropriate interventions made to keep the pH value 7.35 to 7.45, carbon dioxide tension 35 to 45 torr, and oxygen tension greater than 100 torr.

Fig. 1. β -Adrenergic receptor complex (see text for details).

After median sternotomy, hearts were instrumented to measure left ventricular major and minor axis diameters with ultrasonic dimension transducers and left ventricular pressure with micromanometers. After baseline left ventricular pressure-volume data were obtained during transient vena caval occlusions and left ventricular transmyocardial biopsy samples obtained, brain death was induced ($n = 6$) by ligation of the innominate trunk and the left subclavian artery, which effectively interrupted perfusion from the carotid and vertebral systems bilaterally.^{1,10} This method of producing ischemic brain death has been demonstrated in our laboratory to reduce cerebral blood flow to less than 5% of control values. A second group of pigs ($n = 6$) was not subjected to brain death and served as a control group. Left ventricular pressure-volume data and transmyocardial biopsy samples were obtained at hourly intervals in all animals. Biopsy specimens were obtained in the anatomically identical place in the apex of the left ventricle with a 7F biopsy needle, immediately frozen in liquid nitrogen, and stored at -70°C . Studies in our laboratory have confirmed that taking these biopsy specimens does not alter ventricular function. Two-dimensional echocardiograms were recorded at each stage of the experiment for the determination of left ventricular wall volume. At the conclusion of each study the myocardium was dissected and left ventricular wall volume confirmed by saline solution displacement.

Left ventricular function. Left ventricular shell volume was calculated from the ultrasonographically determined epicardial base-apex (major) and anteroposterior (minor) axes with use of a modified ellipsoid shell model.⁹ Left ventricular cavity volume was determined by subtracting echocardiographically derived left ventricular wall volume, by the method described by Feneley and associates,¹¹ from the shell volume. Stroke work was calculated as the area of each left ventricular pressure-volume loop and related to end-diastolic volume. Preload-recrutable stroke work (PRSW), defined as the slope of the stroke work-end-diastolic volume relationship, was used as a load-insensitive index of myocardial performance¹² and expressed as a fraction of the baseline value (PRSW index).

β -Receptor analysis. Myocardial biopsy samples were thawed in ice-cold lysis buffer (Tris 5 mmol/L; ethylenediaminetetraacetic acid [EDTA] 2 mmol/L; pH 7.4 at 4°C) and homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, N.Y.) at 75% maximal speed for 5 seconds. The lysis buffer, and all subsequent buffers described for ligand binding or adenylate cyclase assays, contained the protease inhibitor benzamidine (10

Table I. Results of analysis of cardiac function before and after brain death

	Baseline	1 hr	2 hr	3 hr
PRSW index in experimental animals	1.0	0.83 ± 0.14 ($p < 0.05$)	0.90 ± 0.08 ($p < 0.05$)	0.62 ± 0.16 ($p < 0.01$)
PRSW index in control animals (not subjected to brain death)	1.0	0.98 ± 0.08 (NS)	0.96 ± 0.12 (NS)	0.97 ± 0.10 (NS)

Values given as mean plus or minus the standard deviation. NS, Not significant.

μg/ml), soybean trypsin inhibitor (10 μg/ml), and leupeptin (5 μg/ml). After homogenization, the particulate suspension was diluted 15-fold in lysis buffer and centrifuged at 20,000 rpm (Sorvall RC-5C centrifuge, Du Pont Company, Wilmington, Del.) for 15 minutes at 4° C. The pellet was resuspended in ice-cold resuspension buffer (Tris 75 mmol/L, MgCl₂ 5 mmol/L, EDTA 2 mmol/L, pH 7.4) and filtered through one layer of 100 μl nylon mesh to remove tissue clumps. Resuspension buffer was added to the membrane suspension to adjust the final concentration to 10 μl buffer per milligram tissue wet weight for assay.

β-Receptor radioligand binding. Myocardial β-receptor density was determined with the use of saturation [¹²⁵I]-iodocyanopindolol (ICYP) binding as previously described.¹³ For saturation binding studies, membranes (approximately 10 μg total protein) were incubated with saturating concentrations of ICYP (250 pmol/L) in the absence (total binding) and presence (nonspecific binding) of 1 mmol/L propranolol for 2 hours at 25° C. GTP (100 μmol/L) was included in the incubations to eliminate any retained agonist binding. The reactions were terminated by vacuum filtration over glass fiber (GF/C) filters, which were subsequently washed three times with ice-cold Tris buffer (10 mmol/L). Filters were counted in a gamma counter at 70% efficiency. Both receptor densities and adenylate cyclase activities were normalized to protein, which was measured by the copper–bicinchoninic acid method.¹⁴ Receptor-specific binding was calculated as the difference between total and nonspecific binding.

Adenylate cyclase activities. Myocardial adenylate cyclase activity was assessed by the method of Salomon, Londos, and Rodbell,¹⁵ as modified and described previously.¹⁶ Samples (approximately 20 μg total protein) were incubated in glass tubes in triplicate with various reagents in a reaction mixture that consisted of Tris 30 mmol/L (pH 7.4), MgCl₂ 2 mmol/L, EDTA 0.8 mmol/L, ascorbic acid 0.8 mmol/L to prevent catecholamine oxidation, adenosine triphosphate (ATP) 0.12 mmol/L, GTP 53.0 μmol/L, phosphoenolpyruvate 2.7 mmol/L, myokinase 1.0 IU, pyruvate kinase 0.2 IU, cAMP 0.1 mmol/L, and 50 μCi/ml of [^{α-32}P] ATP in a final volume of 50 μl for 30 minutes at 37° C. Reactions were stopped by adding 1.0 ml ice-cold “stop solution” (200 mg/ml ATP, 100 mg/L cAMP, 25 μl/L ³H-cAMP) in an ice bath. The final free Mg²⁺ concentration in these reactions is approximately 1 mmol/L.

Coupling to adenylate cyclase stimulation was tested at the level of the β-receptor (isoproterenol 100 μmol/L). In addition, the function of other components of the β-receptor/adenylate cyclase pathway was tested with the use of 10 μmol/L sodium fluoride (direct stimulation of the

guanine nucleotide, or G protein) and 100 μmol/L forskolin (direct stimulation of the adenylate cyclase moiety). The samples were then spun in a table-top centrifuge for 2 minutes at 4° C at maximum speed to pellet the membrane. The supernatant was processed by sequential chromatography to isolate [^{α-32}P] cAMP produced by the reaction. Sequential chromatography was done with dowex and alumina columns. Dowex columns were washed with deionized water and alumina columns were washed with 0.1 mol/L imidazole into scintillation vials. Final results were expressed as picomoles cAMP per milligram protein per 30 minutes.

Complete analysis of the β-receptor complex included determination of β-receptor density and quantification of β-receptor functional capacity by stimulation at three distinct points along the β-receptor signal transduction pathway before and hourly after brain death. Comparisons were made with paired two-tailed Student's *t* tests. A *p* value <0.05 was considered significant. Data are presented as mean plus or minus the standard deviation.

Results

Left ventricular function. The effect of brain death on systolic myocardial performance is demonstrated in Table I. Deterioration of myocardial performance occurred within the first hour after brain death, as illustrated by the significant ($p < 0.05$) decrease in the PRSW index. There was no further significant change in myocardial performance throughout the duration of the study. In control animals, not subjected to brain death, there was no significant change in cardiac function throughout the time course of the experiment.

β-Receptor function. The effect of brain death on the function of the β-receptor is summarized in Table II. β-Receptor density was stable after brain death. Isoproterenol-stimulated, sodium fluoride-stimulated, and forskolin-stimulated adenylate cyclase activity decreased significantly within the first hour after brain death. There was no further significant change in adenylate cyclase activity throughout the course of the study. When adenylate cyclase activity was normalized, the magnitude of the effect of brain death on adenylate cyclase stimulation by isoproterenol, sodium fluoride, and forskolin was

Fig. 2. Adenylate cyclase activity after brain death expressed as percent of baseline.

Table II. Results of analysis of β -adrenergic receptor density and activity before and after brain death (as compared with baseline values)

	Baseline	1 hr	2 hr	3 hr
β -Adrenergic receptor density (fmol/mg protein)	50 $\pm$ 14	37 $\pm$ 14 (NS)	45 $\pm$ 8 (NS)	46 $\pm$ 12 (NS)
Isoproterenol-stimulated adenylate cyclase activity (pmol cAMP/mg protein/30 min)	975 $\pm$ 168	475 $\pm$ 249 ($p < 0.01$)	417 $\pm$ 320 ($p < 0.001$)	425 $\pm$ 235 ($p < 0.01$)
Sodium fluoride-stimulated adenylate cyclase activity (pmol cAMP/mg protein/30 min)	7404 $\pm$ 2240	4393 $\pm$ 2026 ($p < 0.05$)	3346 $\pm$ 2860 ($p < 0.05$)	4214 $\pm$ 1680 ($p < 0.05$)
Forskolin-stimulated adenylate cyclase activity (pmol cAMP/mg protein/30 min)	10,626 $\pm$ 2508	6537 $\pm$ 2608 ($p < 0.01$)	4469 $\pm$ 4140 ($p < 0.05$)	5680 $\pm$ 2144 ($p < 0.01$)

Values given as mean plus or minus the standard deviation. NS, Not significant.

demonstrated to be similar (Fig. 2). In contrast, control pigs (not subjected to brain death) demonstrated no change in β -adrenergic receptor density or adenylate cyclase activity during the same period (Table III).

Discussion

Successful transplantation depends on appropriate treatment of potential organ donors. Brain death is followed by profound metabolic and cardiovascular alterations,⁴ increased production of catecholamines,¹⁷ calcium overflow injury in the myocardium,⁵ and cardiac dysfunction.^{1,2} The cardiovascular pathophysiologic process of brain death may inhibit ventricular function of a potential cardiac allograft and jeopardize the support of other organs before retrieval.

Cellular mechanisms responsible for development of myocardial dysfunction after brain death have not been investigated. Catecholamine levels have been shown to be uniformly elevated after the induction of experimental brain death.⁴ In addition, the ad-

ministration of β -adrenergic receptor antagonists before the induction of cerebral ischemia has been shown to prevent the development of myocardial injury.^{7,8} Thus the myocardial β -adrenergic receptor may be involved in the pathophysiologic process of brain death. Therefore the present study was designed to investigate the effect of brain death on the myocardial β -receptor with the use of parameters of receptor density and functional coupling of the receptor to the stimulation of adenylate cyclase.

Deterioration of myocardial performance is observed within the first hour after brain death. The development of cardiac dysfunction is not related to β -receptor density, which remained stable over the course of the study. However, analysis of the functional stimulation of the β -receptor demonstrated an inhibitory effect of brain death on adenylate cyclase stimulation (cAMP production) at all three locations along the signal transduction pathway (β -receptor, G protein, and adenylate cyclase moiety). Because no further decrease in adenylate cyclase stimulation is seen at the receptor or G protein

Table III. Results of analysis of β -adrenergic receptor density and activity in control animals (not subjected to brain death)

	Baseline	1 hr	3 hr
β -Adrenergic receptor density (fmol/mg protein)	34 $\pm$ 14	48 $\pm$ 16 ($p = 0.22$)	47 $\pm$ 21 ($p = 0.34$)
Isoproterenol-stimulated adenylate cyclase activity (pmol cAMP/mg protein/30 min)	310 $\pm$ 1121	393 $\pm$ 167 ($p = 0.20$)	351 $\pm$ 136 ($p = 0.56$)
Sodium fluoride-stimulated adenylate cyclase activity (pmol cAMP/mg protein/30 min)	1267 $\pm$ 627	1425 $\pm$ 557 ($p = 0.54$)	1496 $\pm$ 828 ($p = 0.54$)
Forskolin-stimulated adenylate cyclase activity (pmol cAMP/mg protein/30 min)	4060 $\pm$ 1527	4373 $\pm$ 1679 ($p = 0.52$)	3657 $\pm$ 1133 ($p = 0.48$)

Values given as mean plus or minus the standard deviation.

level, the effect of ischemic brain death on the β -adrenergic signal transduction system appears to be localized to the adenylate cyclase moiety (the final catalytic unit) over the time course studied. The lack of changes in β -receptor density and functional coupling in control pigs suggests that these results are not simply a result of time, operation, or anesthesia, but are specific for brain death.

Desensitization is a functional definition and refers to impaired responsiveness to a drug over time. Three mechanisms of desensitization are generally attributed to G protein-coupled receptors: (1) decreased receptor density secondary to transient receptor internalization (sequestration), (2) decreased receptor density secondary to internalization followed by destruction of receptors (down-regulation), and (3) impairment in the coupling of one of the functional components of the receptor system. In this model of brain death, because receptor number remained constant, impairment of coupling of β -receptors to their second messenger system appears to be the mechanism of myocardial dysfunction. Furthermore, this defect is localized to the adenylate cyclase moiety.

Depending on the model of brain death used, myocardial ischemia may or may not be present. Hence it is important to differentiate the effects of brain death per se from associated myocardial ischemia and its inherent effects on β -receptor function.¹⁸ Although myocardial blood flow has been demonstrated to decrease in this porcine model of brain death, the ratio of endocardial to epicardial flow is not affected.¹⁹ This suggests that myocardial ischemia is not a major component in the pathophysiologic process of myocardial dysfunction in our model of brain death. Thus this study provides a

relatively pure environment in which to investigate mechanisms of myocardial dysfunction after brain death.

In summary, this study confirms that significant dysfunction occurs within 1 hour after induction of brain death and is not related to β -receptor number in this porcine model. The pathophysiologic process of cardiac dysfunction after brain death may involve desensitization of the β -receptor. Deterioration of myocardial performance is temporally related to a defect in β -receptor/G protein/adenylate cyclase coupling. This defect appears to be localized to the adenylate cyclase moiety itself. Further studies will be required to elucidate the exact defect in the adenylate cyclase moiety responsible for deterioration in myocardial performance after brain death and to evaluate therapeutic approaches to the treatment of potential organ donors.

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