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Glycine application and right heart function in a porcine heart transplantation model

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Summary

Glycine reduces ischemia-reperfusion injury after experimental liver transplantation. We hypothesized that glycine might also protect right heart function in an isovolumic cardiac transplantation model. In six domestic donor pigs 150 ml of a 300 mmol L-glycine solution were administered intravenously. The hearts were then arrested with histidine-tryptophan-ketoglutarate solution. Animals without prior glycine infusion served as controls ($n = 6$). After 4 h of ischemia, hearts were transplanted into recipients. An isovolumic model was used in which the right ventricular (RV) volume was controlled *in vivo* using an intracavitary high-compliance balloon. After 1 and 2 h of reperfusion the RV balloon volume was gradually increased and the developed pressures were recorded ($P_{developed} = P_{systolic} - P_{diastolic}$). Right ventricular failure was defined as a decrease in developed intracavitary pressure. Glycine hearts could be loaded with a significantly increased volume after 1 h (glycine: 53 ± 13.7 ml vs. control: 32 ± 11.7 ml; $P = 0.015$) and after 2 h (67 ± 18.6 ml vs. 43 ± 8.2 ml; $P = 0.018$). Maximal RV developed pressures were not significantly different between groups. Postischemic RV end-diastolic compliance was significantly higher in glycine-treated animals ($P = 0.04$). Glycine protects early postischemic RV compliance, but has no important influence on maximal developed pressures.

Introduction

Ischemia-reperfusion injury, incurred during organ harvest, distant procurement and subsequent reperfusion causes right ventricular (RV) dysfunction in the early course after heart transplantation, leading to substantial morbidity and mortality [1,2]. This results in an ongoing quest for improved donor heart preservation.

L-Glycine is a nonessential amino acid and has been shown to have a protective effect against hypoxia and ischemia-reperfusion injury in renal tubules [3], kidney [4], hepatocytes [5,6], and liver [7,8]. In liver tissue, the proposed mechanism of cytoprotection in ischemia-reperfusion injury by glycine would include prevention of intracellular calcium accumulation by hyperpolarizing the cell membrane, thereby impeding the calcium influx by voltage-dependent calcium-channels through a glycine-gated chloride-channel in Kupffer cells [9]. This would lead to decreased release of inflammatory cytokines, proteases, and free radicals [8], thus ameliorating the severity of ischemia-reperfusion injury. A study from Omasa *et al.* showed in an isolated rat lung preparation that preservation using a glycine-enhanced pulmonary flush solution reduces oxidative damage and suppresses apoptosis in that organ [10], indicating the effect being not restricted to the liver and/or the kidney. The protective effect of glycine on the RV myocardium has not been investigated yet. Theoretically, glycine-mediated prevention of intracellular calcium accumulation should be beneficial in cardiac preservation, where calcium-dependent contraction of cardiomyocytes has deleterious effects [11].

We previously developed a porcine isovolumic right heart transplantation model, which allows total control of RV function after transplantation under conditions of constant, controlled left ventricular hemodynamics [12]. In that model we obtained beneficial results from the

intracoronary injection of C1-esterase inhibitor during reperfusion [12], from the addition of 2,3-butanedione-2-monoxime (BDM) to histidine-tryptophan-ketoglutarate (HTK) solution [11] and from the application of Celsior solution for cardiac preservation [13].

The purpose of this study was to evaluate the potential protective effect of glycine infusion before donor heart harvest by means of measuring RV function after heart transplantation in a porcine model.

Materials and methods

Donor animal preparation

In 12 landrace pigs (24.9 ± 3.3 kg; no statistically significant difference between groups, Table 1) anesthesia was induced with azaperon (5 mg/kg, i.m.), atropine (0.5 mg total dose, i.m.), and thiopental sodium (15 mg/kg) intravenously. Animals were intubated and underwent mechanical ventilation with $FiO_2 = 0.5$. In six pigs 150 ml of a 300 mmol L-glycine (Sigma, St Louis, MO, USA) solution (in saline) were administered intravenously before surgery. The other six animals served as donor animals for control experiments. All animals received humane care in compliance with the German animal protection legislation, the 'Principles of Laboratory Animal Care', and the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press (revised 1996).

Donor heart preparation

After median sternotomy the pericardium was opened and the superior (SVC) and inferior vena cava (IVC) and the pulmonary artery were encircled with tapes. Heparin (300 IU/kg) was administered intravenously, and a perfusion cannula inserted into the ascending aorta. Then, the SVC was ligated, the IVC was transected, the left atrial appendage cut and the ascending aorta cross-clamped. Administration of HTK solution (1000 ml at a perfusion pressure of 40 mmHg and 4 °C; Custodiol, Dr F. Köhler Chemie, Alsbach-Hähnlein, Germany) was initiated. HTK solution was composed of: (mM) 15.0 NaCl, 9.0 KCl, 1.0 potassium hydrogen-2-ketoglutarate, 4.0 $MgCl_2$, 180.0 histidine, 2.0 tryptophane, 30.0 molmannit, 0.015 $CaCl_2$. Additionally, the hearts were topically cooled with slushed ice. After completion of perfusion, hearts were excised in standard technique, and placed on slushed ice consisting of isotonic saline solution. To precisely control RV volume *in vivo*, an isovolumic model was used in which the RV volume was regulated using an intracavitary balloon. A high-compliance latex balloon, connected to a 2 cm diameter polyurethane tube was inserted into the RV through the transected pulmonary artery. The tricuspid valve was closed by a doubled running suture to prevent balloon herniation and thus provide absolute volume control in this model. To ensure that the balloon could fill the entire RV cavity and conform maximally to its internal contour, all tricuspid valve chordae tendinae were cut. A 14-gauge cannula was inserted into the RV free wall for drainage of Thebesian venous blood accumulating within the cavity of the RV. Measured amounts of saline solution were added or withdrawn through a port at the end of the tubing. The heart preparation is schematically illustrated in Fig. 1.

Table 1. Mean values (±SD) are given for glycine ($n = 6$) and control ($n = 6$) groups (differences between groups were not statistically significant).

Morphometric analysis	Glycine	Control
Donor weight (kg)	25.6 ± 3.6	24.1 ± 1.9
Recipient weight (kg)	32.5 ± 2.2	34.9 ± 2.7
Total heart weight (g)	107.8 ± 15.7	104.7 ± 11.4
Right ventricular free wall (g)	30.3 ± 5.8	27.9 ± 4.3
Left ventricular free wall (g)	50.5 ± 8.4	49.6 ± 4.7
Interventricular septum (g)	27.0 ± 4.7	27.2 ± 3.9

Recipient preparation

In 12 landrace pigs (34 ± 2.6 kg) anesthesia and ventilation were induced as described above. Mean animal weights did not differ significantly between groups (Table 1). The mean body weight of recipient animals was chosen 10 kg heavier than donor animals for technical ease of subsequent transplantation. Furthermore, earlier studies revealed superior hemodynamic stability in heavier recipients. Maintenance anesthesia was administered intravenously as a continuous thiopental sodium (5 mg/kg/h) and fentanyl (1 μg/kg/h) infusion. A carotid artery catheter was used for monitoring systemic arterial pressure. After median sternotomy and intravenous administration of 300 IU/kg heparin, total cardiopulmonary bypass was implemented via bicaval venous and bifemoral arterial cannulation. Animals were kept normothermic.

Transplantation

Total ischemic time of the hearts was 4 h (including transplantation). After excising recipient hearts, donor hearts were transplanted with left atrial and aortic 4-0 polypropylene running suture anastomoses. After deairing, the aortic cross-clamp was removed and the hearts were defibrillated and paced at a constant rate of 120 beats per minute.

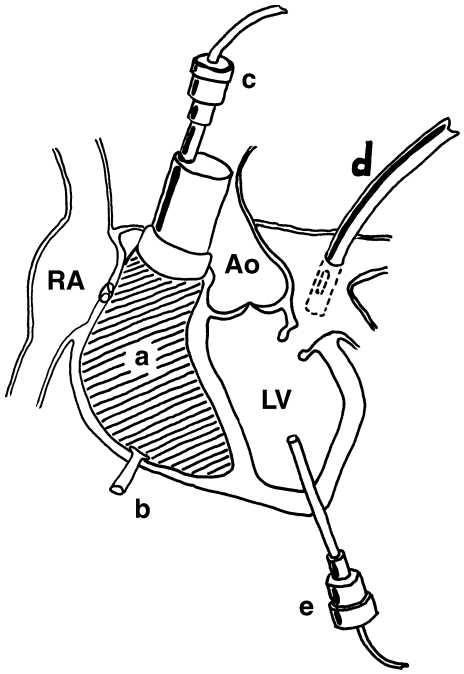

Figure 1 Schematic experimental set up. The tricuspid valve is closed by suture and placed in the right ventricle is a high-compliance latex balloon (a), i.e. secured in the main pulmonary artery. Thebesian vein blood is drained from the right ventricle by a small cannula (b). The right ventricular balloon is connected to a micromanometer-tipped catheter (c). Selective left ventricular (LV) blood flow is provided by an arterial line from the heart-lung-machine (d) and left ventricular pressure is recorded by a second micromanometer-tipped catheter (e).

In this model, the RV was isolated from the circulation by complete drainage of systemic venous return and coronary sinus effluent to a pump oxygenator. Oxygenated blood was returned to the systemic arterial circulation via the femoral arteries. The coronary arteries remained directly perfused from the ascending aorta.

Thirty minutes after opening the cross-clamp, an additional arterial line from the extracorporeal circulation circuit was inserted into the left atrium. Utilizing this technique, left heart cardiac output could be controlled by pumping blood into the left atrium at a constant flow rate of 200 ml/min. Hence, a possible volume-related influence of left ventricular function on the interventricular septum and RV performance could be avoided. Mean systemic arterial pressure was kept constant at 60 mmHg via heart lung machine flow adjustment. No drug therapy was necessary. Pump flow rates did not differ significantly between groups.

Experimental protocol

After transplantation hearts were reperfused for 1 h, and thereafter the RV balloon volume was increased by 10 ml increments until RV failure occurred. The point of RV failure was defined as a decrease in RV developed pressure (RVDP) after administration of a further RV balloon 10 ml volume increment. RVDP was defined as RVDP = $P_{systol} - P_{diastol} - P_{compliance}$. Hence, the compliance of the RV balloon and tubing system was evaluated before each experiment. The results were used to correct measured values for developed RV pressure [14]. RVDP were measured via a pressure transducer (Micron miniature transducer MP 15, volume displacement: 9×10^{-5} mm^3/mmHg; Micron Instruments, Simi Valley, CA, USA) attached to a separate port at the polyurethane tubing on the RV balloon-tubing system. RV diastolic compliance was calculated from the RV balloon volume in millilitre and the respective end-diastolic RV pressure in mmHg at the last measurement before onset of RV failure. Experiments were terminated following the second hemodynamic measurement after 2 h by an overdose of thiopental. The transplanted hearts were removed and the RV and LV free wall and the interventricular septum were separated and weighed.

Blood samples for measurement of creative kinase (CK) activity and creative kinase isoenzyme B (CKMB) and troponin-T concentrations were drawn at the following intervals: after insertion of a carotid artery catheter (baseline) and after 10, 60 and 120 min of reperfusion of the transplanted heart. Samples after 10, 60 and 120 min were collected from the coronary sinus to obtain exact measurements of myocardial marker release. CK activity and CKMB and troponin-T serum concentrations were determined using standard laboratory tests.

Statistical analysis

Data are expressed as mean ± SD. The Student's *t*-test was used to compare mean values of groups. When repeated measurements were recorded, two-way ANOVA was used to compare mean values of groups and changes over time. For all tests, values were considered to be statistically significant at $P < 0.05$.

Results

RV balloon volume and developed pressure

Results of heart morphometric analyses are summarized in Table 1. As illustrated in Fig. 2, the RV could be loaded with up to an average of 53 ml in the glycine group when compared with only 32 ml in the control group before the onset of RV failure after 1 h of reperfusion. After 2 h of reperfusion, the volume load capacity climbed to an average of 67 ml in the glycine group, whereas in the control group the RV failed above 43 ml on average. These differences were statistically significant,

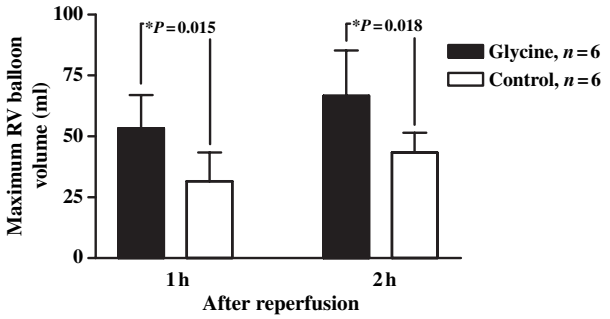

Figure 2 Maximum right ventricular balloon volume before onset of right ventricular failure in millilitre after 1 h (left) and after 2 h of reperfusion (right). Mean values (±SD) are given for glycine ($n = 6$) and control ($n = 6$) groups. *Statistically significant differences between groups after 1 and 2 h of reperfusion (repeated-measures ANOVA).

as indicated in Fig. 2. During reperfusion, RV volume capacity, measured at maximum RVDP, increased in both groups over time ($P = 0.04$, 1 h vs. 2 h after reperfusion; repeated-measures ANOVA). There was no significant difference in maximum RVDP at RV volumes just short of the onset of RV failure between groups after 1 h ($P = 0.22$) or 2 h of reperfusion ($P = 0.19$) because of substantially lower diastolic and systolic RV pressures in the glycine group at higher volumes (Fig. 3). Consequently, there was a trend toward higher developed pressures in the control group.

RV diastolic pressure–volume relationship

The RV diastolic compliance at maximum balloon volume load before onset of RV failure after 1 h of reperfusion was 3.98 ± 2.48 vs. 1.99 ± 0.92 ml/mmHg in glycine and control animals, respectively. After 2 h of reperfusion this difference decreased to 2.55 ± 0.79 vs. 1.99 ± 0.72 ml/mmHg ($P = 0.04$; repeated-measures ANOVA). However, these calculations are influenced by the high diastolic pressures of above 30 mmHg for some animals and time points, especially after 2 h of reperfusion, at these measurements due to our definition of 'onset of RV failure' upon a decrease of RVDP. A better illustration of RV compliance are the RV end-diastolic pressure–volume curves for RV balloon volumes from 0 to 80 ml, which show a significant shift to the right in the glycine-treated group after 1 h ($P < 0.0001$; Fig. 4a) and after 2 h of reperfusion ($P < 0.0001$; Fig. 4b).

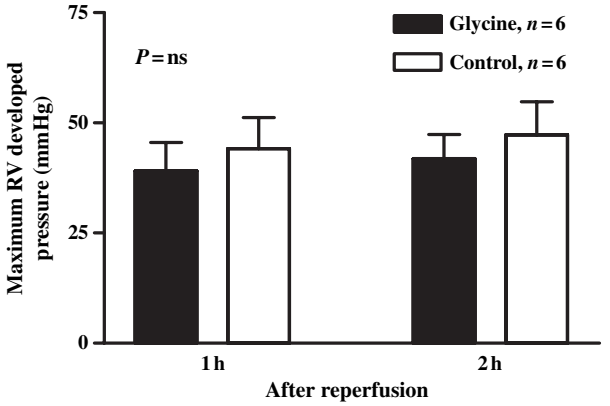

Figure 3 Right ventricular developed pressures (RVDP) at maximum volume load before onset of right ventricular failure in mmHg after 1 h (left) and after 2 h of reperfusion (right). Mean values (±SD) are given for glycine ($n = 6$) and control ($n = 6$) groups. No statistically significant differences were observed (repeated-measures ANOVA).

Markers of myocardial injury

The increase in serum CK activity in the glycine group from 502 ± 159.1 at baseline to 2488 ± 1418.5 U/l at the end of the experiment was reduced when compared to the control group with 575 ± 393.5 at baseline to 4284 ± 1457.2 U/l at the end of the experiment ($P =$

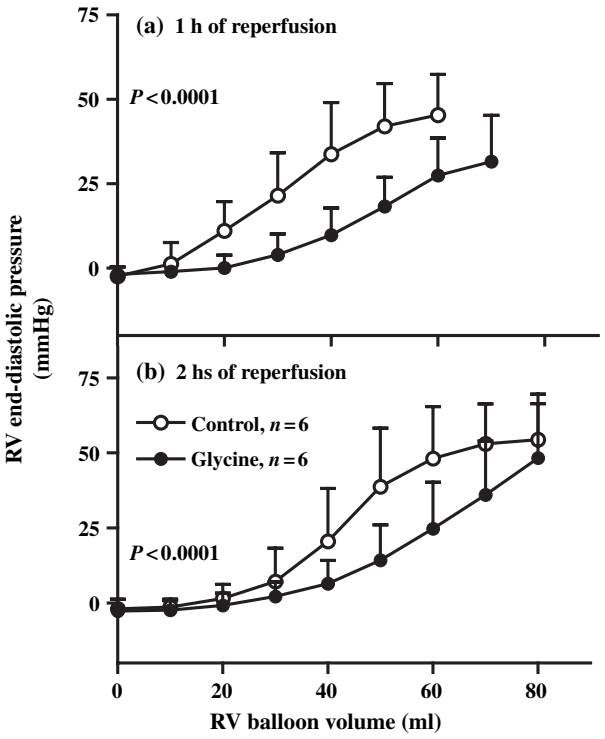

Figure 4 Right ventricular (RV) end-diastolic pressure–volume relationship for RV balloon volume increments from 0 to 80 ml (including measurements after the respective onset of RV failure) after 1 h (a) and after 2 h of reperfusion (b). Mean values (±SD) are given for glycine ($n = 6$) and control ($n = 6$) groups. Statistically significant differences were observed between groups after 1 and 2 h of reperfusion (repeated-measures ANOVA).

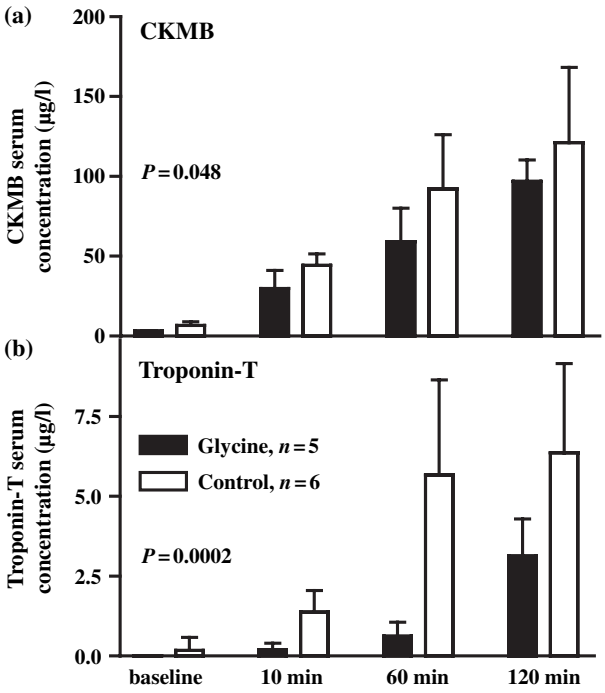

Figure 5 Serum samples for CKMB and troponin-T concentrations in µg/l were collected before surgery from an arterial line (baseline) (a) and after 10, 60 and 120 min of reperfusion from the coronary sinus (b). Mean values (±SD) are given for glycine ($n = 5$) and control ($n = 6$) groups. Statistically significant differences were observed between groups (repeated-measures ANOVA).

0.09, ANOVA). CKMB concentration increased over 10-fold and troponin-T serum levels increased over 100-fold from before surgery to 2 h after reperfusion (Fig. 5).

Discussion

The intravenous infusion of L-glycine to donor animals before organ harvest resulted in a significant improvement of postischemic RV compliance in recipients of cardiac grafts in this study, while the RVDP had a nonsignificant tendency to be higher in control animals, although at much lower RV filling volumes. Our protocol was designed to especially address the question of RV function, which remains a critical issue in clinical heart and heart–lung transplantation.

Glycine has been shown to ameliorate ischemia-reperfusion injury in the liver [7,8] and that effect has been attributed to its ability to inhibit Kupffer cell function [9]. Early studies have been based on dietary glycine [15]. More recent studies administered glycine intravenously before donor organ harvest [8] or added it to preservation solutions with encouraging success in counteracting ischemia-reperfusion injury [10]. We chose an intravenous route of delivery because the majority of the previously published work on liver preservation has utilized this technique.

For our experiments, a porcine model was chosen, because extrapolation of results from the more usual experiments in rat hearts that are related to calcium metabolism is probably not feasible as calcium metabolism might differ between rats and other mammals [16]. Therefore, results need to be corroborated in a species comparable to human calcium metabolism, with the pig as an acceptable model in this respect. Above that, the question of RV protection and recovery, which is clinically of overwhelming importance, has never been addressed satisfactory in rat heart preparations.

The isovolumic model selected for this study enables total control of confounding variables for right heart function and compensation mechanisms in right heart failure. While it does not exactly mimic the clinical situation, the complexity of RV hemodynamics provided the rationale for its use. As left heart hemodynamics influence maximum RVDP [17], observations in this study were made under conditions of controlled, constant left heart hemodynamics, with constant left heart output and systolic left ventricular (and hence, aortic) pressure. Thus, the present model allowed optimal control of potential variables that may influence data obtained on RV function. Pulmonary vascular resistance is a very inconsistent parameter in models of orthotopic heart transplantation. This problem was avoided in the isovolumic right heart preparation used in this study. During measurements the RV is subjected to increasing balloon volumes, and thereby increasing afterload in a controlled fashion, as the volume is not ejected. However, one limitation of the model is the condition of RV afterload during the remainder of the reperfusion period. While the balloon is empty, the effective RV afterload is very low. Additionally, left ventricular output was restricted to 200 ml/min and left-sided afterload was adjusted to 60 mmHg. Thus, the total load for the transplanted heart was relatively low. While this might be different from the clinical situation of early reperfusion after heart transplantation, it provided the advantage that no inotropic support was necessary. Weaning of experimental animals off the heart-lung-machine, albeit desirable in theory, is invariably associated with usage of substantial inotropic support, and might thus not be useful for studies of right heart function. Maintaining the experimental animals on the heart-lung-machine beyond 2 h leads to hemodynamic instability with rapidly progressive demand for inotropic support. We therefore had to accept the relatively short observation period of 2 h for this study.

It has been shown in previous studies [18] that improved RV function does not necessarily have to be expressed as increased RV pressure. The ventricle may

also react with increased volume load at constant pressures, i.e. increased ventricular compliance. In this study maximum developed RV pressure did not differ significantly between groups, but it is of importance to state that both groups revealed relatively high developed pressures and systolic pressures of 70–80 mmHg before the onset of RV failure, indicating that further improvement of systolic function might be difficult to proof. Strikingly, after glycine application maximum developed RV pressures were recorded at significantly higher RV balloon volumes differing up to 23 ml on average from control animals. A 10 ml increase in RV volume could mean that the RV, paced at a rate of 120/min, could pump 1.2 l of volume more per minute after protection using glycine. Thus, glycine-treated animals revealed better myocardial preservation than controls by the high RV compliance at maximum volume load before the onset of RV failure and in general upon analysis of end-diastolic RV pressure–volume relationships (Fig. 4). Diastolic properties of the heart are an important indicator of myocardial function and a high ventricular compliance is a prerequisite of effective ventricular work [19]. The higher volume handled by the RV at similar end-diastolic RV pressures in the glycine group would functionally translate into a significant shift of the RV Frank-Starling curve toward higher stroke volumes.

Better protection from ischemia-reperfusion injury in glycine animals might also be concluded from a significantly decreased release of CKMB and troponin-T after reperfusion. CK activity was lower in glycine-treated animals, but without significant differences between groups with a possible explanation being the ligation of the femoral arteries for cannulation, causing an increased CK release from the pig's lower extremities without respect to cardiac preservation.

The mechanism of putative myocardial protection by glycine remains speculative. While for example, inhibition of Kupffer cells plays a major role in liver ischemia-reperfusion injury, the effect of glycine in the lung has been attributed to the inhibition of alveolar macrophages, which exhibit glycine-gated chloride-channels, analogous to the Kupffer cells in the liver [20]. While it is not known, which cell population would be the main target of glycine in cardiac tissue, the effect of a reduced Ca^{2+}-influx into the cell should be a very attractive mechanism for the purpose of myocardial preservation that is aimed at reducing the adverse effects of Ca^{2+}-induced contraction [21]. However, prolonged inhibition of the Ca^{2+}-influx into cardiomyocytes after reperfusion of the transplanted heart could cause an early cardiodepressant effect. This effect could potentially be the explanation for the tendency of glycine-enhanced cardiac preservation to show slightly lower developed RV pressures than controls. It is not known how long this potential overhanging cardiodepressant effect after glycine treatment could last. Thus, the relatively short postoperative observation period of 2 h in our study might have fallen short of revealing an improvement of RV systolic function that could have developed after homeostasis of myocardial Ca^{2+}-metabolism would be re-established.

In conclusion, our study demonstrates that RV compliance after transplantation is significantly improved after glycine application. Using an isovolumic, porcine right heart transplant preparation with total control of left ventricular function, maximum volume load of the RV before the onset of RV failure was significantly higher and end-diastolic ventricular pressure was significantly lower in glycine animals when compared to controls without glycine application. This data suggest that administration of glycine may protect the myocardium from ischemic injury after prolonged ischemia during transplantation. However, future effort is mandatory to clarify the role of low RVDPs after glycine treatment and to elucidate the mechanism of myocardial protection provided by glycine.

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