Effects of Atrial Natriuretic Peptide After Prolonged Hypothermic Storage of the Isolated Rat Heart

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Abstract

Objective

 Primary graft failure (PGF) caused by ischemia-reperfusion injury (IRI), is the strongest determinant of perioperative mortality after heart transplantation. Atrial natriuretic peptide (ANP) has been found to reduce the IRI of cardiomyocytes and may be beneficial in alleviating PGF after heart transplantation, although there is a lack of evidence to support this issue. The purpose of this study was to investigate the cardioprotective effects of ANP after prolonged hypothermic storage.

Methods

 An isolated working-heart rat model was used. After the preparation, the hearts were arrested with and stored in an extracellular-based cardioplegic solution at 3−4°C for 6 h and followed by 25min of reperfusion. The hearts were divided into 4 groups (n=7 each) according to the timing of ANP administration: group 1 (in perfusate before storage), group 2 (in cardioplegia), group 3 (in reperfusate), and control (no administration of ANP). Left ventricular functional recovery and the incidence of ventricular fibrillation (VF) was compared.

Results

ANP administration at the time of reperfusion improved the percent recovery of left ventricular developed pressure (control, 45.5 ± 10.2; group 1, 47.4 ± 8.8; group 2, 45.3 ± 12 vs. group 3, 76.3 ± 7; $p < 0.05$) and maximum first derivative of the left ventricular pressure (control, 47.9 ± 8.7; group 1, 46.7 ± 8.8; group 2, 49.6 ± 10.8 vs. group 3, 76.6 ± 7.5; $p < 0.05$). The incidence of VF after reperfusion did not differ significantly among these 4 groups (71.4%, 85.7%, 57.1%, and 85.7% in groups 1, 2, 3, and control, respectively).

Conclusions

Administration of ANP at the time of reperfusion significantly improves the recovery of left ventricular function after prolonged hypothermic ischemia and may have the potential to decrease the incidence of PGF after heart transplantation.

Introduction

Primary graft failure (PGF) is the strongest determinant of perioperative mortality after heart transplantation, and is responsible for up to 42% of perioperative deaths (1). Acute ischemia-reperfusion injury (IRI) with myocardial stunning has been proposed to be a predominant factor in the development of PGF (2). Most donor hearts are stored in a cold preservation solution and transported on ice. Hypothermic storage slows but does not completely arrest cellular metabolism. Consequently, progressive ischemic injury is an inevitable consequence of prolonged storage.

IRI involves damage to cardiomyocytes, vascular smooth muscle, and endothelial cells. When cardiomyocytes are reoxygenated after a prolonged period of energy depletion, severe cytosolic calcium overload and reactivation of energy production results in deleterious hypercontracture, which leads to cell disruption in tissue (3). Recent experimental studies have demonstrated that reoxygenation-induced hypercontracture can be prevented if the contractile apparatus is temporarily blocked during the initial phase of reoxygenation to reestablish normal cytosolic calcium

control (4).

Myocardial guanosine 3', 5'-cyclic monophosphate (cGMP), which reduces the calcium sensitivity of myofilaments (5), is reduced in myocardial cells after prolonged ischemia (6). The stimulation of cGMP synthesis at the time of reoxygenation has an inhibitory contractile effect in reperfused myocardium, and is able to prevent reoxygenation-induced hypercontracture in isolated cardiomyocytes (7), isolated hearts (6), and in situ hearts (8).

Atrial natriuretic peptide (ANP) is known to stimulate the synthesis of the particulate guanylate cyclase, causing a consequent increase in cGMP synthesis. Thus, it is able to protect the myocardium against IRI and can preserve myocardial function.

Based on these findings, it seems likely that ANP could be beneficial in ameliorating PGF in a transplanted heart. Although there is evidence that ANP can have a cardioprotective effect in acute myocardial infarction (9, 10, 11) or cardiac surgery (12), its potential role in heart transplantation has not yet been investigated. Therefore, we investigated the ability of ANP to improve the functional recovery of isolated working rat hearts after

prolonged hypothermic storage in an extracellular-based cardioplegic solution.

Materials and Methods

Animals

Male Sprague-Dawley rats were used in the present study. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institute of Health (National Institutes of Health publication No. 85-23, revised 1996). The experimental protocol was approved by the Experimental Animals Committee of the Okayama University School of Medicine.

Isolated rat heart model

The composition of the modified Krebs-Henseleit bicarbonate buffer (KHB) used for organ perfusion was as follows (in mmol/L): NaCl, 118.0; $NaHCO_3$, 25.0; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KCl, 4.7; KH_2PO_4, 1.2; and glucose, 11. The pH was 7.4. The KHB was bubbled with 95% oxygen and

5% carbon dioxide gas at 38.0°C to maintain an aortic partial oxygen pressure of >400 mmHg. It was filtered through a cellulose acetate membrane (pore size 0.45 μm) to remove any particulate contaminants.

The composition of the extracellular-based cardioplegic solution was as follows (in mmol/L): Na^+, 142.0; K^+, 18.5; Mg^{2+}, 18.5; Ca^{2+}, 1.1; HCO_3^-, 1.9; and glucose, 11.1.

We modified and used an isolated, perfused, rat heart apparatus that was previously described by Fujii and associates (13). This circuit was designed to work in 2 interchangeable conditions, namely the unloaded and loaded modes. In the unloaded mode, the hearts were perfused through the aorta at a pressure of 80 cmH_2O and continued to beat without external work. In the loaded mode, the hearts were perfused in the same fashion, but beat against external force. These hearts were not paced and the coronary effluent was discarded.

The rats were anesthetized with an intraperitoneal injection of pentobarbital (50mg/kg), and heparin (100 IU/100g body weight) was injected into the exposed right femoral vein. The hearts were quickly excised and immersed in cold (4°C) modified KHB, and Langendorff

perfusion was establishied. The aorta was cannulated within 1 min after the excision. The pulmonary artery was incised to facilitate coronary drainage. The heart was then perfused in a retrograde manner under the perfusion conditions of the unloaded mode at 38.0°C for 10 min. Subsequently, the changes in heart rate (HR), left ventricular developed pressure (LVDP), and the first derivative of LV pressure (dp/dt max) were monitored under the loaded mode with commercially available software (PowerLab, ADInstruments, Sydney, Australia) using an intraventricular balloon inserted through the mitral annulus that was inflated with distilled water. During the pressure measurement, the left ventricular end-diastolic pressure was maintained at 4 mmHg using the intraventricular balloon. The coronary flow (CF) was measured by direct collection of coronary effluent dripping from the heart for 1 min.

Study protocol

  The study protocol is shown in Figure 1. Hearts were perfused in the unloaded mode for 10 min followed by the loaded mode for 15 min. During the later interval, we measured the baseline value of HR, LVDP, and dp/dt

max. The cardioplegic solution (at 3−4°C) was then infused into the coronary circulation for 1 min from a reservoir located 80 cm above the heart and the hearts were stored on ice (3−4°C) in 100 mL of the same cardioplegic solution for 6 h. At the end of this period, the hearts were remounted on the perfusion apparatus and reperfused in the unloaded mode for 10 min. The apparatus was then switched to the loaded mode and we recorded the aforementioned indices of cardiac function at 1, 5, 10, and 15min. Data beyond 15 min were not recorded because we observed no further recovery beyond 15 min. The recovery of each parameter was expressed as a percentage of its pre-storage value.

The CF and its cGMP concentration were also measured before storage, and at 1 and 15 min of the post-reperfusion loaded mode.

The hearts were removed from the apparatus at the end of the experiment. They were heated to 70°C for 14 days, and were then weighed to determine the dry weight of ventricular myocardium. The concentration of cGMP was determined by radioimmunoassay as previously described (14) and was expressed in pmol/g dry weight per min.

We also noted the incidence of ventricular fibrillation (VF) at the time of

reperfusion.

The rats were divided into 4 groups (n = 7 in each group). The control hearts were perfused with KHB. To determine the optimal timing for ANP administration, we added ANP (alpha-human atrionatriuretic peptide; Sankyo-Daiichi, Tokyo, Japan) to the perfusate before storage (Group 1), to the cardioplegic solution (Group 2), or to the perfusate during reperfusion (Group 3), using a dose of 0.1 μmol/L. It has been reported that administration of 0.1 μmol/L ANP induces a threefold increase in cGMP release into the coronary effluent without any effect on cardiac function in the isolated rat heart (15).

Exclusion

At the first hemodynamic evaluation, hearts beating at a rate less than 250beats/min were considered to have experienced myocardial damage during the preparation. These hearts were excluded from the study.

Statistical analysis

All data were expressed as mean ± standard deviation. The statistical

analysis was performed using commercially available software (SPSS for Windows; SPSS Japan, Tokyo, Japan). Differences among multiple groups were determined using one-way analysis of variance followed by Scheffe's test. The incidence of VF among groups was compared using the chi-square test. A p-value of <0.05 was regarded as statistically significant.

Results

Pre-ischemic data

Table 1 shows the body weight of the rats, HR, CF, dp/dt max, LVDP, and cGMP release in the coronary effluent during the pre-ischemic loaded perfusion. There were no statistically significant differences in HR, CF, dp/dt max, and LVDP. Only group 1 had a higher release of cGMP in the coronary drainage induced by the administration of ANP.

Post-ischemic cardiac functional recovery and changes in cGMP

The post-ischemic recovery of HR, dp/dt max, and LVDP was expressed as a percentage of the pre-ischemic value; the results are listed in Figure 2.

The recovery of LVDP and dp/dt max was better in group 3, than in the other groups (LVDP: control, 45.5 $\pm$ 10.2; group 1, 47.4 $\pm$ 8.8; group 2, 45.3 $\pm$ 12; vs. group 3, 76.3 $\pm$ 7; p < 0.05. dp/dt max: control, 47.9 $\pm$ 8.7; group 1, 46.7 $\pm$ 8.8; group 2, 49.6 $\pm$ 10.8; vs. group 3, 76.6 $\pm$ 7.5; p < 0.05). There was no difference in the recovery of HR (control, 82 $\pm$ 4.7; group 1, 90.3 $\pm$ 7.4, group 2, 87.8 $\pm$ 7.6; group 3, 89 $\pm$ 11.2).

The cGMP release into the CF increased after ischemia only in group 3 (control, 1.31 $\pm$ 0.23; group 1, 1.35 $\pm$ 0.15; group 2, 1.26 $\pm$ 0.28; vs. group 3, 5.32 $\pm$ 1.63; p < 0.05). There was no difference in the post ischemic recovery of CF (control, 79.7 $\pm$ 20.1; group 1, 85.6 $\pm$ 9.5; group 2, 78 $\pm$ 10.6; group 3, 83.6 $\pm$ 11).

The post-ischemic cardiac functional recovery was closely related to the significantly increased cGMP release due to the infusion of ANP at the time of reperfusion.

Incidence of ventricular fibrillation after reperfusion

The occurrence of VF after reperfusion was noted in 6 (85.7%), 5 (71.4%),

6 (85.7%), and 4 (57.1%) rats in the control group, group 1, group 2, and group 3, respectively. There was no significant difference among these groups (p = 0.552).

Discussion

The present study showed that the administration of ANP at the time of reperfusion elicited a significant improvement in the acute-phase post-ischemic recovery of left ventricular function after 6 h of hypothermic storage of the rat heart. This improvement was attributed to the increased cGMP release triggered by ANP, as demonstrated by previous investigators (11, 15). In contrast, the administration of ANP before ischemia or during cardioplegia was not associated with any significant improvement in the recovery of left ventricular function after reperfusion.

The timing of treatment aimed at increasing cGMP release for protection against hypoxia-reoxygenation or IRI has been controversial. Okawa and colleagues (16) reported that pre-ischemic infusion of ANP elicits myoprotective effects during ischemia reperfusion in isolated rat hearts. Agulló and colleagues (17) reported that L-arginine supplementation before

hypoxia increases cGMP release during reoxygenation and improves functional recovery in isolated rat hearts subjected to 40 min of hypoxia. Recent ischemia-reperfusion studies (6, 11) have demonstrated that urodilatin, a member of the natriuretic peptide family, improves functional recovery when administered at the time of reperfusion. Sangawa and colleagues (15) also reported that the administration of ANP at the time of reperfusion after 15 min of normothermic global ischemia, improved post-ischemic recovery; however, no improvement was observed when ANP was administered before ischemia. All of these studies were performed under conditions of acute ischemic heart disease or resuscitation from sudden cardiac arrest; therefore, the ischemic times ranged from only 15 to 60 min.

Besides promoting the synthesis of cGMP, ANP also appears to have an additional cardioprotective effect against IRI in the myocardium. An experimental study has presented evidence for the presence of an independently functioning and local renin-angiotensin system in the heart (18). In an isolated perfused rat heart, angiotensin II was found to exacerbate ischemia-induced ventricular fibrillation and impaired

cardiodynamics, whereas these effects were blocked by ANP (19). Morales et al. (20) reported that the direct blocking of the local renin-angiotensin system with angiotensin II receptor antagonists ameliorates myocardial stunning after global ischemia. Therefore, the functional antagonism of angiotensin II may underlie the protective effect of ANP against IRI.

Reperfusion-induced arrhythmia is one of the important factor for IRI. Several mechanisms are believed to be responsible for the development of reperfusion-induced arrhythmia. One of the important mechanisms is considered to be intercellular Ca^{2+} overload, caused by an involvement of H^+/Na^+ and Na^+/Ca^{2+} exchange (21, 22). The effect of ANP on reperfusion-induced arrhythmia remains unclear. Takata et al (23) reported that, in anesthetized dogs subjected to 30 min of left circumflex artery occlusion followed by 60 min of reperfusion, ANP infusion inhibited reperfusion-induced ventricular arrhythmias and preserved the high-energy phosphate content in the inner layer of the ischemic myocardium. The authors suggested that the beneficial effects of ANP were probably due to its direct effects via cGMP as a stimulator of Na^+/Ca^{2+} exchange, leading to a reduction of intracellular Ca^{2+} overload. However, 2 other reports failed

to detect any favorable effect on ventricular arrhythmias after coronary occlusion in dogs (9), or during reperfusion in the isolated rat heart after 30 min of regional ischemia (24). In the present study, the administration of ANP at the time of reperfusion did not prevent VF. However, as our findings indicated that ANP might reduce reperfusion-induced arrhythmia, we increased the number of rats to 13 in both the control group and group 3, as a complementary study to determine whether or not a significant difference in the incidence of VF would be observed. We noted that 12 of 13 rats (92%) in the control group and 8 of 13 rats (62%) in group 3 exhibited VF at the time of reperfusion. The incidence of VF tended to be lower in group 3 than in the control group, but the difference was not statistically significant ($p = 0.062$). The reason for the disparity between the results of different studies regarding the beneficial effect of ANP on reperfusion-induced arrhythmias is unclear. Differences in the experimental protocol (i.e., the duration of the ischemic periods, the dose of ANP, or the extent of the ischemia) might have affected the results. We believe that further investigation is required to confirm the effect of ANP on reperfusion-induced arrhythmias.

Despite the improvements in the treatment of PGF, it is still associated with high morbidity and mortality (2), and is the most common cause of death within the first month after heart transplantation. It has been reported that a prolonged total graft ischemic time, accelerating IRI is one of the risk factors for PGF (25, 26). But, because of the shortage of donor hearts, the use of marginal hearts that have a prolonged ischemic time may increase the likelihood of PGF. Thus, investigation of the safe storage of donor hearts and the preservation of their ventricular function is becoming increasingly important. The excellent recovery of left ventricular function after 6 h of cold ischemic arrest in the present study indicates that ANP may have a preventive effect against PGF after heart transplantation.

Limitations of the model

There are several limitations to our study. First, we did not use the heart transplant model to evaluate the cardiac function of the rat heart. Therefore, it might be difficult to extrapolate the results directly to heart plantation. Second, we did not confirm a cause and effect relationship between cGMP and postischemic recovery by using cGMP analogs and antagonists.

However, previous studies using isolated rat heart models demonstrated that improved functional recovery, induced by the administration of the natriuretic peptide urodilatin during initial reperfusion after 40 min of ischemia, was reproduced by the cGMP analog 8-bromo-cGMP (6), and that reduction in lactate dehydrogenase release by urodilatin after 60 min of ischemia was abolished by adding the ANP receptor antagonist isatin (11). Third, we used an isolated perfused preparation. Although the preparations were denervated, direct cardiac responses can be studied independently of the systemic effects of ANP. Finally, we used a crystalloid solution in the perfusion circuit. Blood perfusion may induce different results from those of crystalloid perfusion (27). Because each blood component serves different roles during ischemia and reperfusion and might affect the results, we used a simple crystalloid solution in this study.

Conclusion

The administration of ANP at the time of reperfusion after prolonged hypothermic storage significantly improved left ventricular function after reperfusion, although it did not significantly reduce the incidence of VF.

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ANP may be a beneficial adjunct to improve the ventricular function of a donor heart as a means of decreasing the incidence of PGF during heart transplantation.

Author contributions

Hitoshi Kanamitsu; corresponding author, Data analysis, Drafting article

Yasuhiro Fujii; Concept/design

Hideya Mitsui; Approval of article

Shunji Sano; Supervisor, Funding secured by

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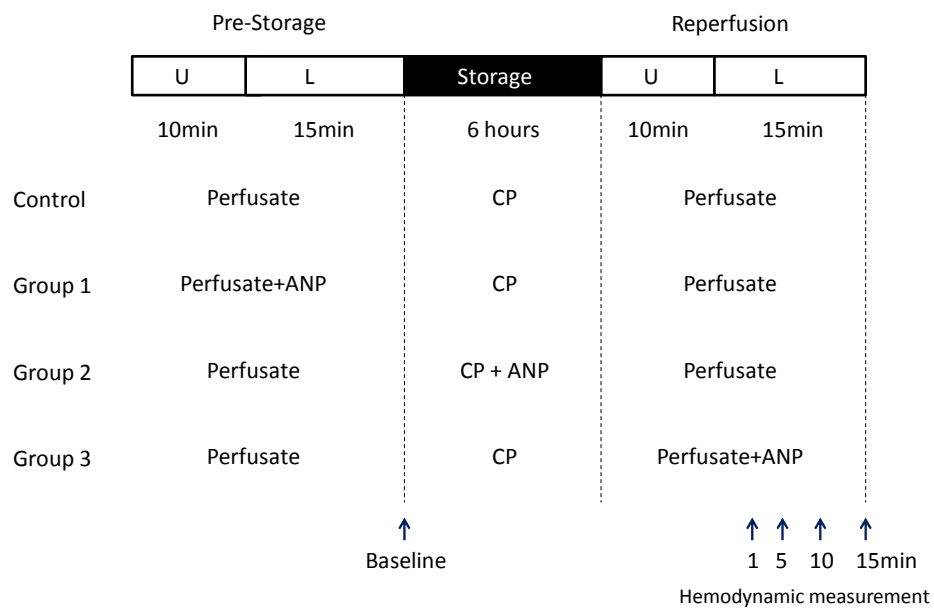
Table 1. Pre-ischemic data

	BW(g)	LVDP(mmHg)	d p/dt max (mmHg/sec)	HR(beat/min)	CF(mL/min)	cGMP (pmol/dry weight/min)
control	275±21.5	132.2±9.1	3811.4±378.6	279.3±19.7	9.5±2	1.54±0.25
group 1	272.7±18.9	127±9.2	3879±410	298±21	10.1±1.2	4.46±0.98 *
group 2	282.3±22.9	128.6±6.5	3965.1±530.9	290.7±23.8	10.4±1.4	1.45±0.32
group 3	272.9±4.6	135.6±9.3	3936.3±549.3	291.7±24.7	8.4±1.2	1.42±0.24

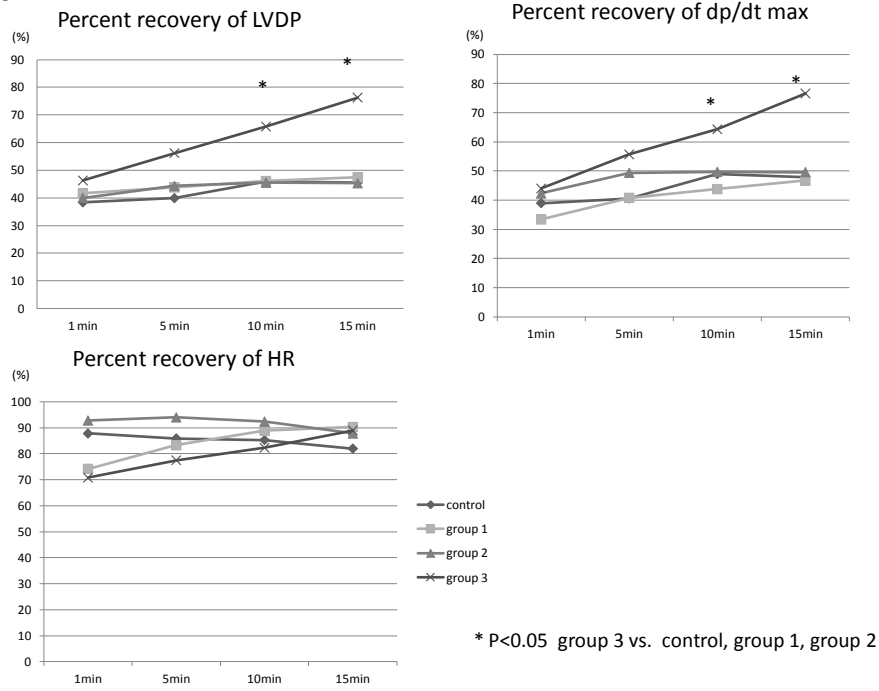* $P<0.05$ group1 vs control, group 2, group 3

Values are presented as mean ± standard deviation. BW, body weight; LVDP, left ventricular developed pressure; dp/dt max, first derivate of left ventricular pressure; HR, heart rate; CF coronary flow; cGMP, cyclic guanosine monophosphate

Figure 1. Experimental protocol

	Pre-Storage		Storage	Reperfusion	
	U	L		U	L
	10min	15min	6 hours	10min	15min
Control	Perfusate		CP	Perfusate	
Group 1	Perfusate+ANP		CP	Perfusate	
Group 2	Perfusate		CP + ANP	Perfusate	
Group 3	Perfusate		CP	Perfusate+ANP	

Baseline

1 5 10 15min

Hemodynamic measurement

U, Unloaded perfusion; L, Loaded perfusion; ANP, atrial natriuretic peptide; CP, Cardioplegia

Figure 2.

Percent recovery of LVDP

Percent recovery of dp/dt max

Percent recovery of HR

* P<0.05 group 3 vs. control, group 1, group 2

The postischemic recovery of the first derivative of left ventricular pressure (dp/dt max), heart rate (HR), and the left ventricular developed pressure (LVDP). The recovery of dp/dt max and LVDP was better in Group 3.