Dobutamine-mediated heme oxygenase-1 induction via PI3K and p38 MAPK inhibits high mobility group box 1 protein release and attenuates rat myocardial ischemia/reperfusion injury in vivo

Jichun Wang, PhD, Hongxin Yang, PhD, Xiaorong Hu, PhD, Wenwen Fu, PhD, Jing Xie, PhD, Xiaoya Zhou, PhD, Weipan Xu, PhD, and Hong Jiang, MD, PhD*

Department of Cardiology, Renmin Hospital of Wuhan University, Cardiovascular Research Institute of Wuhan University, Wuchang, Wuhan, PR China

Abstract

Background: It has been reported that the induction of heme oxygenase-1 (HO-1) mediated by β1-adrenergic receptor inhibits high mobility group box 1 protein (HMGB1) release and increases the survival rate in cecal ligation and puncture-induced septic mice. The present study aimed to investigate whether dobutamine, a selective β1-adrenergic receptor agonist, could inhibit HMGB1 release via β1-adrenergic receptor-mediated HO-1 induction and attenuate myocardial ischemia/reperfusion (I/R) injury in rats.

Materials and Methods: Anesthetized male rats were pretreated with dobutamine (5 or 10 μg.Kg-1. min-1, intravenous) before ischemia in the absence and/or presence of LY294002 (0.3 mg/Kg), a phosphatidylinositol 3-kinase (PI3K) inhibitor; SB203580 (1 mg/Kg), a p38 mitogen-activated-protein kinase (p38 MAPK) inhibitor, and zinc protoporphyrin IX (ZnPPIX), 10 mg/Kg, a HO-1 inhibitor, respectively, and then subjected to ischemia for 30 min followed by reperfusion for 4 h. The myocardial I/R injury and oxidative stress were assessed. Likewise, the expressions of HO-1 protein, nuclear factor kappa B (NF-κB) p65, and HMGB1 were measured by Western blot analysis.

Results: Dobutamine significantly and dose-dependently attenuated myocardial I/R injury, reduced oxidative stress, and caused the induction of HO-1, the reduction of NF-κB activation and HMGB1 over expression. However, all the effects caused by dobutamine were significantly reversed by the presence of LY294002, SB203580, and ZnPPIX, respectively.

Conclusions: The present study demonstrated that dobutamine mediated the induction of HO-1 by selectively stimulating β1-adrenergic receptor via PI3K and p38 MAPK, which inhibited HMGB1 release and attenuated rat myocardial I/R injury in vivo.

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1. Introduction

Myocardial ischemia/reperfusion (I/R) injury is a complex pathophysiological process in which inflammatory response plays an important role [1–3]. High mobility group box 1 protein (HMGB1), as a ubiquitous nuclear protein, has been reported to act as a “late” inflammatory cytokine that contributes to the pathophysiological progress of myocardial I/R injury [4,5]. Hence, anti-HMGB1 release may become a novel therapeutic target for myocardial I/R injury [6].
Importantly, heme oxygenase-1 (HO-1), an inducible isoform of HO enzymes, has been reported to be anti-inflammatory, anti-apoptotic, and anti-fibrotic in several cell types, including cardiac myocytes [7,8]. Otherwise, it has been reported that β1-adrenergic receptor mediated HO-1 induction via phosphatidylinositol 3-kinase (PI3K) and p38 mitogen-activated-protein kinase (p38 MAPK) in RAW 264.7 cells, inhibited HMGB1 release in lipopolysaccharide-activated RAW 264.7 cells and increased the survival rate of cecal ligation and puncture-induced septic mice [9]. Furthermore, Salie et al. [10] showed that PI3K activation may be associated with the cardioprotective effects of pre-ischemic β1-adrenergic receptor stimulation during myocardial I/R process.

Taken together, we hypothesized that dobutamine, a selective β1-adrenergic receptor agonist, may protect against myocardial I/R injury. In the present study, we investigated the protective effect of dobutamine and its possible mechanisms during myocardial I/R in rats.

2. Materials and methods

2.1. Animal preparation and experimental designs

The experimental protocol conformed to the Guideline for the Care and Use of Laboratory Animals published by the US National Institutes of Health, revised 1996 and was approved by the Institutional Animal Care and Use Committee.

Male Sprague-Dawley rats (specific pathogen-free class, 250–300 g) were purchased from Hunan Slac Jingda Laboratory Animal Co., Ltd. (Hunan, China). All animals were kept in an environmentally controlled breeding room (temperature: 23 °C ± 2 °C, humidity: 60% ± 5%, 12 h dark/light cycle). Rats had free access to water and were adapted to the commercial pelleted feed for a week, and then were randomly assigned into seven groups receiving the following treatments:

Group 1: sham operated control (SO, n = 12): The rats were injected with saline via the femoral vein and underwent surgical manipulation without the induction of myocardial ischemia.

Group 2: Ischemia/reperfusion (I/R, n = 12): The rats were injected with saline via the femoral vein and subjected to left anterior descending coronary artery (LAD) occlusion for 30 min followed by reperfusion for 4 h. The rats were anesthetized with sodium pentobarbital (45 mg/kg, intraperitoneal), the rats were ventilated artificially with a volume-controlled rodent respirator at 70 strokes per minute. The rats were placed on an electric heating pad to maintain their body temperature at 37 °C ± 0.8 °C. Heparin (200 IU/kg, intravenous) was given before ischemia. Lead-II of the surface electrocardiogram was continuously monitored using a computer-based electrophysiology system (LEAD2000B; Jinjiang Ltd, China).

A thoracotomy through a left parasternal incision was performed to expose the anterior wall of the left ventricle. A 4-0 silk suture on a small curved needle was passed through the myocardium beneath the middle segment of the LAD branch coursing down the middle of the anterior wall of the left ventricle. A small vinyl flake was passed into both ends of the suture, which was then fixed by clamping the tube with a mosquito hemostat. A successful myocardial I/R model was confirmed by changes of ST segment elevation in Leads-II and regional cyanosis of the myocardial surface. The rats underwent a 30 min LAD occlusion, followed by a 4 h reperfusion.

2.2. Hemodynamic measurements

After being anesthetized with sodium pentobarbital (45 mg/kg, intraperitoneal), the rats were ventilated artificially with a volume-controlled rodent respirator at 70 strokes per minute. The rats were placed on an electric heating pad to maintain their body temperature at 37 °C ± 0.8 °C. Heparin (200 IU/kg, intravenous) was given before ischemia. Lead-II of the surface electrocardiogram was continuously monitored using a computer-based electrophysiology system (LEAD2000B; Jinjiang Ltd, China).

2.3. Assessment of myocardial injury

To assess the lactate dehydrogenase (LDH) and creatine kinase (CK), blood samples were collected, centrifuged and kept at −20 °C until analyses. Standard techniques were performed by using commercial kits according to the manufacturer’s instructions. Values were expressed in international units (IU) per L.
2.4. **Assessment of infarct size**

After 4 h reperfusion, the LAD was again occluded and 2 ml of 1.5% Evans blue dye was injected via the femoral vein. The risk area was analyzed by negative staining with Evans blue. The rats were then killed and the hearts were excised and frozen overnight. The atria and right ventricle were removed and the left ventricle was cut into transverse slices of 2 mm thickness from the apex to base. The risk area was separated from the colored non-ischemic area (blue) and then incubated with a 1% solution of 2,3,5-triphenyltetrazoliumchloride in 0.2M Tris buffer, pH7.4) stained for 20 min at 37°C. Viable myocardium was stained red by triphenyltetrazoliumchloride, whereas necrotic myocardium appears as negative staining with red.

In each slice, the infarct size and the risk area (left ventricular areas) were determined by a computer-assisted image analysis system (Image-Pro Plus 3.0, Media Cybernetics, Silver Spring, MD) and multiplied by the thickness of the slice to calculate volumes of risk area. Infarct size was expressed as a percentage of the risk area volume (% infarct size/risk area).

2.5. **Measurement of myocardium malondialdehyde (MDA) and superoxide dismutase (SOD) activity assay**

Myocardial tissue MDA concentration and SOD activity, indexes of oxygen free radical and lipid superoxide level in the myocardium, respectively, were measured by using commercial kits according to the manufacturer’s instructions.

2.6. **Measurement of inflammatory cytokine expression (tumor necrosis factor alpha [TNF-α], interleukin-6 [IL-6]) in myocardial tissues**

The titres of TNF-α and IL-6 in cardiac muscle samples were measured by using enzyme-linked immunosorbent assay technique according to the manufacturer’s recommendations.

2.7. **Western blot analysis for the expressions of the HO-1, nuclear factor kappa B (NF-κB), and HMGB1**

The cytoplasmic and nuclear protein extracts were prepared from pulverized frozen ischemia area of left ventricle samples as reported previously [16,17]. Western blot was performed according to the manufacturer’s procedures. Briefly, 50 μg of cytoplasmic or nuclear proteins were separated on 10% sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose membrane. Nonspecific binding sites were blocked with 5% non-fat dry milk in Tris-buffer saline-0.05% Tween. The membrane was subsequently probed with primary antibody (anti-HO-1 antibody, diluted 1:400; anti-HMGB1 antibody, and anti-NF-κB antibody, diluted 1:500, respectively) and incubated with horseradish peroxidase-conjugated secondary antibody (diluted 1:50000). The protein bands were visualized by an enhanced chemiluminescence system, and glyceraldehyde-3-phosphate dehydrogenase was used as an internal control to correct the variations of different samples. The expressions of the HO-1, activated NF-κB p65 and HMGB1 were normalized to glyceraldehyde-3-phosphate dehydrogenase expression.

2.8. **Statistical analysis**

All continuous values were expressed as mean ± SD. Student t-test was used for between-group comparisons. One-way ANOVA or Welch was used for comparisons among groups and the Student–Neuman–Keuls or Dunnett T3 was used for post-hoc multiple comparisons. P value < 0.05 was considered statistically significant.

2.9. **Materials**

Dobutamine hydrochloride, SB203580, LY294002, and ZnPPIX were purchased from Sigma-Aldrich, St. Louis., MO. Commercial kits for the LDH, CK, MDA, and SOD were purchased from Nanjing Jiancheng Bioengineering Institute, China. The rat TNF-α and IL-6 enzyme-linked immunosorbent assay kits were purchased from Wuhan Elabscience Biotechnology Co., Ltd., China. The antibodies used to recognize HO-1, NF-κB p65, and HMGB1 were purchased from Sigma-Aldrich.

3. **Results**

3.1. **Effects of dobutamine on cardiac function**

Cardiac function was assessed before LAD occlusion and after drugs administration (dobutamine, LY294002, SB203580, or ZnPPIX). As shown in Table 1, no significant difference in LVEF, HR, and MAP was found in groups (all P > 0.05).

After 4 h reperfusion, compared with I/R group, the LVEF was significantly improved in dobutamine-treated groups (both P < 0.05 versus I/R group), but no significant difference in HR and MAP was found between I/R and dobutamine-treated groups (both P > 0.05 versus I/R group). Meanwhile, there was no significant difference between the Dobutamine1-I/R group and Dobutamine2-I/R group in LVEF (P = 0.276, Table 2).

However, compared to Dobutamine1-I/R group or Dobutamine-2-I/R group, the improved effect of dobutamine on the LVEF was obviously reversed by the presence of LY294002, SB203580, and ZnPPIX, respectively (all P < 0.05 versus groups). No significant difference of other indices (HR and MAP) was found among groups (all P > 0.05).

3.2. **Effects of dobutamine on inflammatory cytokine expressions**

The results indicate that there was no significant difference in the expressions of inflammatory cytokine expression (TNF-α and IL-6) between I/R and dobutamine-treated groups (all P > 0.05).

3.3. **Effects of dobutamine on apoptosis-related protein expressions**

The results indicate that there was no significant difference in the expressions of apoptosis-related protein expression (Bax and Bcl-2) between I/R and dobutamine-treated groups (all P > 0.05).

3.4. **Effects of dobutamine on hemodynamic measurements**

Cardiac function was assessed before LAD occlusion and after drugs administration (dobutamine, LY294002, SB203580, or ZnPPIX). As shown in Table 1, no significant difference in LVEF, HR, and MAP was found in groups (all P > 0.05).

After 4 h reperfusion, compared with I/R group, the LVEF was significantly improved in dobutamine-treated groups (both P < 0.05 versus I/R group), but no significant difference in HR and MAP was found between I/R and dobutamine-treated groups (both P > 0.05 versus I/R group). Meanwhile, there was no significant difference between the Dobutamine1-I/R group and Dobutamine2-I/R group in LVEF (P = 0.276, Table 2).

However, compared to Dobutamine1-I/R group or Dobutamine-2-I/R group, the improved effect of dobutamine on the LVEF was obviously reversed by the presence of LY294002, SB203580, and ZnPPIX, respectively (all P < 0.05 versus groups). No significant difference of other indices (HR and MAP) was found among groups (all P > 0.05).

Table 1 – Summary of hemodynamic measurements before ischemia.

<table>
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<th>LVEF (%)</th>
<th>HR (bpm)</th>
<th>MAP (mmHg)</th>
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<tr>
<td>SO</td>
<td>77.3 ± 2.2</td>
<td>296 ± 9</td>
<td>80 ± 6</td>
</tr>
<tr>
<td>I/R</td>
<td>78.8 ± 2.6</td>
<td>286 ± 5</td>
<td>79 ± 5</td>
</tr>
<tr>
<td>Dobutamine1-I/R</td>
<td>82.8 ± 2.9</td>
<td>292 ± 9</td>
<td>82 ± 3</td>
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<tr>
<td>Dobutamine2-I/R</td>
<td>84.5 ± 2.6</td>
<td>298 ± 7</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>LY294002-Dobutamine2-I/R</td>
<td>85.3 ± 4.0</td>
<td>294 ± 8</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>SB203580-Dobutamine2-I/R</td>
<td>82.3 ± 3.4</td>
<td>298 ± 9</td>
<td>83 ± 4</td>
</tr>
<tr>
<td>ZnPPIX-Dobutamine2-I/R</td>
<td>82.0 ± 3.7</td>
<td>294 ± 8</td>
<td>81 ± 3</td>
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</table>

Summary of hemodynamic measurements before myocardial I/R and after drugs treatment. All data are expressed as means ± SD. No significant difference in LVEF, HR, and MAP was found in groups (all P > 0.05). SO: sham operated; I/R: ischemia/reperfusion; ZnPPIX: zinc protoporphyrin IX; LVEF: left ventricular ejection fraction; MAP: mean artery pressure; HR: heart rate.
Table 2 — Summary of hemodynamic measurements after 4 h reperfusion.

<table>
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<th></th>
<th>LVEF (%)</th>
<th>HR (bpm)</th>
<th>MAP (mmHg)</th>
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<tbody>
<tr>
<td>SO</td>
<td>77.8 ± 3.9</td>
<td>273 ± 5</td>
<td>77 ± 2</td>
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<tr>
<td>I/R</td>
<td>58.0 ± 2.2 ▲</td>
<td>275 ± 2</td>
<td>71 ± 4</td>
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<tr>
<td>Dobutamine1-I/R</td>
<td>67.3 ± 2.2 ▼</td>
<td>276 ± 5</td>
<td>73 ± 5</td>
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<tr>
<td>Dobutamine2-I/R</td>
<td>68.8 ± 3.0 ★</td>
<td>279 ± 8</td>
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<tr>
<td>LY294002-Dobutamine2-I/R</td>
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<td>78 ± 4</td>
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<tr>
<td>SB203580-Dobutamine2-I/R</td>
<td>56.3 ± 6.5</td>
<td>279 ± 6</td>
<td>74 ± 6</td>
</tr>
<tr>
<td>ZnPPIX-Dobutamine2-I/R</td>
<td>57.8 ± 2.1 ★</td>
<td>284 ± 4</td>
<td>74 ± 6</td>
</tr>
</tbody>
</table>

Summary of hemodynamic measurements after myocardial I/R. All data are expressed as means ± SD. ▲ P < 0.05 versus SO group; ▼ P > 0.05 versus I/R group; ★ P < 0.05 versus Dobutamine1-I/R or Dobutamine2-I/R groups. SO: sham operated; I/R: ischemia/reperfusion; ZnPPIX: zinc protoporphyrin IX; LVEF: left ventricular ejection fraction; MAP: mean artery pressure; HR: heart rate.

3.2. Effect of dobutamine on infarct size

After 4 h reperfusion, compared with I/R group, the pretreatment of dobutamine significantly reduced the infarct size (both P < 0.05 versus I/R group) in a dose-dependent manner. However, the decrease in infarct size caused by dobutamine was significantly reversed by the presence of LY294002, SB203580, and ZnPPIX, respectively (all P < 0.05 versus Dobutamine1-I/R group or Dobutamine2-I/R group) (Fig. 1).

3.3. Effects of dobutamine on LDH and CK levels in I/R rats

After 4 h reperfusion, compared with SO group, the levels of LDH and CK in I/R group were significantly increased (both P < 0.05 versus SO group). However, compared with I/R group, dobutamine significantly inhibited the increase of LDH and CK levels (both P < 0.05 versus I/R group). But there was no different significance between the Dobutamine1-I/R group and Dobutamine2-I/R group (P > 0.05). Meanwhile, compared with Dobutamine1-I/R group or Dobutamine2-I/R group, the reductions of LDH and CK release induced by dobutamine were significantly abolished by the presence of LY294002, SB203580, and ZnPPIX, respectively (all P < 0.05 versus Dobutamine1-I/R group or Dobutamine2-I/R group) (Fig. 2).

3.4. Effects of dobutamine on MDA and SOD activity assay

After 4 h reperfusion, compared with SO group, the level of MDA was significantly increased and the level of SOD was significantly decreased, respectively, in I/R group (both P < 0.05 versus SO group) (Fig. 3A, B). However, compared with I/R group, dobutamine dose-dependently inhibited the increase of the MDA level and the decrease of the SOD level (both P < 0.05 versus I/R group) (Fig. 3A, B). Conversely, compared with Dobutamine1-I/R group or Dobutamine2-I/R group, the effects of dobutamine were significantly reversed by the presence of LY294002, SB203580, and ZnPPIX, respectively (all P < 0.05 versus Dobutamine1-I/R group or Dobutamine2-I/R group) (Fig. 3A, B).

3.5. Effects of dobutamine on production of TNF-α and IL-6

After 4 h reperfusion, compared with SO group, both TNF-α and IL-6 were significantly increased in I/R group (both P < 0.05 versus SO group). But both dosages of dobutamine produced a statistically significant reduction in the production of TNF-α and IL-6 compared with the I/R group (both P < 0.05 versus I/R group). However, compared with Dobutamine1-I/R group or Dobutamine2-I/R group, the effects of dobutamine on the TNF-α and IL-6 production were completely abolished by the presence of LY294002, SB203580, and ZnPPIX, respectively (all P < 0.05 versus Dobutamine1-I/R group or Dobutamine2-I/R group) (Fig. 4).

3.6. Effects of dobutamine on HO-1, NF-κB p65, and HMGB1 expressions

After 4 h reperfusion, compared with SO group, the NF-κB p65 and HMGB1 expression levels were markedly increased in I/R group (both P < 0.05 versus SO group). Meanwhile, the HO-1 expression was also significantly increased in I/R group compared with the SO group (P < 0.05 versus SO group) (Fig. 5A, B).

Fig. 1 — Effect of dobutamine on infarct size during I/R. (n = 9 or 12 for each group). All data are expressed as means ± SD. ▲ P < 0.05 versus I/R group. ★ P < 0.05 versus Dobutamine1-I/R group. ▼ P < 0.05 versus Dobutamine1-I/R or Dobutamine2-I/R groups. I/R: ischemia/reperfusion; ZnPPIX: zinc protoporphyrin IX.
However, compared with I/R group, dobutamine significantly and dose-dependently mediated HO-1 induction and NF-κBp65 and HMGB1 inhibition (all \( P < 0.05 \) versus I/R group) (Fig. 5A, B). However, all the effects of dobutamine were significantly reversed by the presence of LY294002, SB203580, and ZnPPIX, respectively (all \( P < 0.05 \) versus Dobutamine1-I/R group or Dobutamine2-I/R group) (Fig. 5A, B).

4. Discussion

The present study demonstrated that dobutamine dose-dependently attenuated myocardial I/R injury and oxidative stress. Furthermore, the induction of HO-1 and inhibition of HMGB1 were shown to be involved in the effects of dobutamine on attenuating myocardial I/R injury in rats.

HMGB1, a novel pro-inflammatory cytokine, has been proven to play an important role in myocardial I/R injury [4]. In addition, a series of studies have demonstrated that HMGB1 may contribute to myocardial I/R injury by promoting the apoptosis of myocardium and inflammatory response in rats [5,18]. Moreover, Takamiya et al. [19] have further demonstrated that the expression levels of HMGB1 were higher in HO-1\(^{-/-}\) mice than in HO-1\(^{+/+}\) mice. Meanwhile, the HO-1 induction has been further proven to prevent the release of HMGB1 in endotoxin-activated macrophages in vitro and septic animals in vivo [20], which is further supported in myocardial I/R injury model in rats in the present study.

Importantly, since the HO-1 induction has been reported to depend on PI3K/p38MAPK signaling pathway in many cells [21–23], we focused on whether the signaling pathway involved \( β_1 \)-adrenergic receptor-mediated HO-1 induction during rat myocardial I/R injury in vivo. In the present study,
we found that dobutamine mediated HO-1 induction via selective β1-adrenergic receptor stimulation, and this effect was significantly reversed by LY294002, a specific PI3K inhibitor, and SB203580, a p38 MAPK inhibitor, respectively.

Of note, PI3K has been confirmed to be involved in growth factor signal transduction via receptor and non-receptor tyrosine kinases. Hence, inhibitors of PI3K may potentially give a better understanding of the function and regulatory mechanisms of PI3K. The compound, LY294002, has been proven to specifically abolish PI3K activity with a selective structure/activity relationship and with slight changes in structure [24]. Meanwhile, p38 MAPK is a member of enzymes
which are activated by dual phosphorylation upon threonyl and tyrosyl residues separated by a single amino acid. The compound, SB203580, has been proven to completely suppress the activation of mitogen-activated protein kinase-activated protein (MAPKAP) kinase-2 and not inhibit c-Jun N-terminal kinase or p42 MAP kinase. Therefore, SB203580 is useful for studying the physiological roles and targets of p38 MAPK and MAPKAP kinase-2 [25].

Importantly, the activation of NF-κB in the nucleus is critical for induction of inflammatory cytokines including TNF-α, IL-6, NO, and release of HMGB1 [26]. Hence, in the present study, we observed that dobutamine mediated HO-1 induction paralleled the inhibition of NF-κB p65 and HMGB1 release in the heart tissues. However, ZnPPIX, an inhibitor of HO-1, significantly reversed the effects of dobutamine, further confirming the fact that HO-1 activity plays an important role in inhibiting HMGB1 release during myocardial I/R injury in rats.

Taken together, as shown in Figure 6, dobutamine causes the induction of HO-1 via β1-adrenergic receptor stimulation in cardiac myocytes, which may depend on PI3K/p38MAPK signaling pathway. Additionally, HO-1 induction inhibits HMGB1 release and attenuates myocardial I/R injury in rats.

4.1. Study limitations

First, the present study only demonstrated that dobutamine mediated the induction of HO-1 via PI3K and p38 MAPK and inhibited HMGB1 release and attenuated rat myocardial I/R injury in vivo. However, other factors such as nuclear factor erythroid 2-related factor 2 translocation may also contribute to these cardioprotective mechanisms and should be considered in a future study [27–30] (Fig. 6). Second, we only observed that dobutamine reduced oxidative stress during myocardial I/R in rats; the precise mechanisms require future elucidation.

5. Conclusions

The present study demonstrated that dobutamine mediated the induction of HO-1 by selectively stimulating β1-adrenergic receptor via PI3K and p38 MAPK, inhibiting HMGB1 release and attenuating rat myocardial I/R injury in vivo.

Acknowledgments

This study was partially supported by a grant from National Natural Science foundation of China (no. 81100146), grant 111023 from the Fundamental Research Funds for the Central Universities and the Specialized Research Fund for the Doctoral Program of Higher Education of China (no. 20110141120060) and the Fundamental Research Funds of Wuhan City (No.2013070104010044).

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