Heat stress-induced resistance to myocardial infarction in the isolated heart from transgenic [(mREN-2)27] hypertensive rats

Marie Joyeux, Caroline Lagneux, Giampiero Bricca, Derek M. Yellon, Pierre Demenge, Christophe Ribuot

Abstract

Objective: Heat stress (HS) is known to confer protection against ischaemia–reperfusion injury, including mechanical dysfunction and myocardial necrosis. However, the effects of disease states on this HS-induced cytoprotective response are less known. Therefore, we investigated the effects of prior heat stress on the infarct size in the isolated rat heart and on the myocardial heat stress protein (HSP) 72 synthesis, in transgenic [(mREN-2)27] hypertensive (TGH) rats or normotensive (NT) controls.

Methods: TGH or NT rats were either heat stressed (42°C for 15 min) or sham anaesthetised. After 24 h, their hearts were isolated, perfused using the Langendorff technique, and subjected to a 35-min occlusion of the left coronary artery followed by 120 min of reperfusion. Myocardial HSP72 content was measured 24 h after HS or sham treatment using electrophoresis coupled with Western blot analysis.

Results: Infarct-to-risk (I/R) ratio was significantly reduced in HS (15.5±1.2%) compared to sham (42.2±2.1%) hearts of NT rats. This reduction in infarct size was maintained in TGH hearts (I/R: 20.0±1.0 vs. 48.0±3.8%). Risk zones were similar between all experimental groups. The incidence of ventricular arrhythmias during ischaemia and reperfusion periods was not different between the four experimental groups. Western blot analysis of the myocardial HSP72 content showed a heat stress-induced increase of this protein, in both TGH and NT animals.

Conclusion: These results demonstrate that the myocardial protective effect induced by heat stress could extend to a pathological animal model like the transgenic [(mREN-2)27] hypertensive rat and is correlated with a myocardial HSP72 induction.

Keywords: Heat stress; Infarct size; Transgenic [(mREN-2)27] hypertensive rats; Heat stress protein

1. Introduction

Environmental stresses, including heat stress (HS), are known to induce synthesis of heat stress proteins (HSP), which play an important role in the cell’s ability to survive noxious stresses (for a recent review, see reference [1]). In particular, the myocardial induction of HSP72, occurring 24 h following whole body hyperthermia, is associated with protection against ischaemia–reperfusion injury [2–5]. Moreover, this HSP72 induction has been directly correlated to the degree of HS-induced ischaemic tolerance [6,7].

Although the cytoprotective response induced by heat stress is reasonably well known in non-pathological animals, our knowledge of the effects of disease states on this HS-induced cardioprotection is limited. Since cardiac events are usually associated with underlying cardiovascular diseases or risk factors such as hypertension, it is important to study the development of the stress response in animal models of hypertension. As a matter of fact, ischaemic damage has been shown to be enhanced in various models of hypertension. In this respect, the incidence of ischaemia-induced arrhythmias is increased in hearts from spontaneously hypertensive rats [8].

On the other hand, the cardioprotection, induced 24 to 48 h following heat shock, may be analogous to that seen during delayed ischaemic preconditioning (second window

*Corresponding author. Tel.: 00 33 476 637 108; Fax: 00 33 476 637 152; E-mail: Christophe.Ribuot@ujf-grenoble.fr

Time for primary review 22 days
of protection) [9–11]. The effects of hypertension on ischaemic preconditioning have been investigated. Spechly-Dick et al. [12] have demonstrated that the hypertrophied myocardium of rats with mineralocorticoid-induced hypertension was protected by ischaemic preconditioning in vivo. It has also been observed that preconditioning significantly improves mechanical performance in hearts isolated from spontaneously hypertensive rats [13]. Moreover, Randall et al. [14] have shown that cardiac preconditioning is substantially enhanced in isolated hearts from transgenic [(mREN-2)27] hypertensive rats.

Therefore, in this study, we investigated the protective effects of heat stress on isolated hearts from male, heterozygous, transgenic [(mREN-2)27] hypertensive (TGH) rats. Littermates normotensive (NT) rats were used as the appropriate controls [15]. The heterozygous TGH animals have enhanced activity of local renin–angiotensin systems [16] and develop fulminant hypertension associated with cardiovascular changes, such as left ventricular hypertrophy [17]. The effects of heat stress on myocardial infarct size and the incidence of ventricular arrhythmias after an ischaemia–reperfusion sequence and on induction of cardiac HSP72 synthesis were assessed [18].

2. Methods

2.1. Materials

The male Sprague-Dawley (16 weeks old) TGH or NT rats used for these studies were obtained by crossing heterozygous TGH females with non-transgenic males. Transgenicity was controlled by polymerase chain reaction with primers that were specific for the transgene, according to Lee et al. [16]. The care and use of animals in this work were in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Unisperse blue dye was from Ciba-Geigy (France); 2,3,5-triphenyltetrazolium chloride was from Sigma (France). All other reagents were of analytical reagent quality.

2.2. Experimental groups

This study was conducted in two parts. In the first part, TGH or NT rats were submitted to either HS or anaesthesia without hyperthermia (sham). Subsequently, all animals were allowed to recover for 24 h. In the second part, ischaemia (35 min)–reperfusion (120 min) was performed in isolated hearts. Prior to this procedure, the arterial pressure of all animals was assessed in vivo.

Four experimental groups were studied: (1) Sham–NT (n=8), normotensive rats submitted to sham HS; (2) Sham–TGH (n=8), transgenic [(mREN-2)27] hypertensive rats submitted to sham HS; (3) HS–NT (n=8), normotensive rats submitted to HS and (4) HS–TGH (n=8), transgenic [(mREN-2)27] hypertensive rats submitted to HS. The experimental protocol is summarised in Fig. 1.

2.3. Heat stress protocol

Whole body hyperthermia was achieved as previously described [18] by placing anaesthetised (with 25 mg/kg i.p. sodium pentobarbitone) TGH or NT animals in an environmental chamber under an infrared light. Their body temperature, recorded with a rectal probe, was increased to 42±0.2°C for 15 min. Sham animals were anaesthetised only.

2.4. Ischaemia–reperfusion protocol

Twenty four hours after heat stress, the rats were heparinised (1000 U/kg, i.p.) and anaesthetised with sodium pentobarbitone (60 mg/kg i.p.). Arterial pressure was measured in vivo using a catheter inserted in the carotid artery and connected to a pressure transducer (Statham) and a polygraph (Windograph, Gould Instrument). The chest was then opened and the heart was rapidly excised and immediately immersed in 4°C Krebs-Henseleit buffer solution (NaCl 118.0, KCl 4.7, CaCl2 1.8, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25.2 and glucose 11.0 mM). The aortic stump was then cannulated and the heart perfused using the Langendorff technique at a constant pressure (75 mmHg) with oxygenated Krebs-Henseleit buffer.

![Fig. 1. Experimental protocol.](https://example.com/fig1.png)
buffer. A water-filled latex balloon, coupled to a pressure transducer (Statham) was inserted into the left ventricular cavity via the left atrium for pressure recordings. Left ventricular end-diastolic pressure (LVEDP) was adjusted to between 8 and 12 mmHg. Myocardial temperature was measured by a thermoprobe inserted into the left ventricle and was maintained constant at close to 37°C. For temporary occlusion of the left coronary artery (LCA), a 3/0 silk suture (Mersilk W546, Ethicon) was placed around the artery a few millimeters distal to the aortic root [19]. After 20 min of stabilisation, regional ischaemia was induced by tightening the snare around the LCA for 35 min [3]. Thereafter, the heart was reperfused for 120 min. Coronary flow (CF) was measured throughout the ischaemia–reperfusion procedure, by collecting the effluent. Heart rate (HR) and left ventricular developed pressure (LVPD = difference between left ventricular systolic pressure and LVEDP) were continuously recorded on a polygraph (Windograph, Gould Instrument). The rate pressure product (RPP) was calculated as the product of HR and LVPD. At the end of the reperfusion period, the coronary artery ligature was retied and unisepse blue dye was slowly infused through the aorta to delineate the myocardial risk zone. After removal of the right ventricle and connective tissues, the heart was frozen at −18°C for 1 h and then cut into 2 mm transverse sections from apex to base (six–seven slices/heart). Once defrosted, the slices were incubated at 37°C with 1% triphenyltetrazolium chloride in phosphate buffer (pH 7.4) for 10–20 min and fixed in 10% formaldehyde solution, to distinguish clearly stained viable tissue and unstained necrotic tissue [20]. The left ventricular infarct zone (I) was determined using a computerised planimetric technique (Minichromax; Biolab) and expressed as the percentage of the risk zone (R) and of the left ventricle (LV).

2.5. Quantification of arrhythmias

Arrhythmias were classified in accordance with the Lambeth Conventions guidelines [21]. Electrogram recordings were analysed for the incidence (%) of ventricular tachycardia and/or fibrillation (VT/VF) occurring during ischaemia and reperfusion.

2.6. Determination of myocardial HSP72 content

For myocardial HSP72 content determination, additional animals (n = 2 in each group) were submitted only to the first part of the experimental protocol. Twenty-four hours after HS or sham anaesthesia, TGH or NT rats were anaesthetised (60 mg/kg sodium pentobarbitone, i.p.), heparinised (1000 U/kg, i.p.) and the hearts were quickly excised, as described in Section 2.4. Left ventricular tissue samples (50 mg) were rapidly powdered in liquid nitrogen and suspended in 500 μl of sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer (20% glycerol, 6% sodium dodecyl sulphate, 1.4% Tris–HCl, pH 6.8). 2-Mercaptoethanol (10%) was added and the samples were heated at 100°C for 10 min. Samples were cooled and centrifuged at 11,000 g for 5 min. Bromophenol blue (8%) was added to the supernatant and the samples were stored at −20°C. Proteins were separated by electrophoresis on 12.5% polyacrylamide gels. Gels were stained in Coomassie blue R250 and subsequently destained, to confirm equivalence of protein loading. For Western blot analysis of HSP72, proteins were transferred electrophoretically onto nitrocellulose membrane (Hybond-C, Amersham, UK) overnight at 180 mA and 4°C. The membrane was placed in washing buffer for 30 min (phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20 and 0.1% dried milk powder) to block non-specific binding sites. The filter was first incubated (60 min) with a mouse monoclonal IgG that crossreacted with HSP72 (SPA-810, StressGen) at 1:1000 dilution and subsequently was incubated (60 min) with horseradish peroxidase-conjugated rabbit anti-mouse IgG (P260, Dako, Denmark) at 1:2500 dilution. The filter was developed using an enhanced chemiluminescence detection system (Amersham).

2.7. Statistical analysis

Arrhythmia incidences were compared using exact Fisher’s tests. The other data are presented as mean±SEM. Mean arterial pressure, body weight and heart weight data were analysed by Student’s t-tests. Comparisons of CF, HR, LVPD and RPP were determined by repeated measures ANOVA. Infarct size was analysed by one-way ANOVA with post-hoc multiple comparison Tukey tests. P values <0.05 were considered to be significant.

2.8. Exclusion criteria

Only hearts with CF within 13–20 ml/min and LVPD >70 mmHg at the end of the stabilisation period were included in this study. The efficiency of coronary occlusion was indicated by a CF diminution >30%. Hearts that developed ventricular fibrillation during ischaemia–reperfusion which did not reverse to normal sinus rhythm within 2 min were excluded. Moreover, the risk zone determined at the end of the ischaemia–reperfusion procedure had to represent 40–60% of the LV. Two hearts were excluded because of non-conformity with these predetermined criteria: One from the sham-NT group, because the coronary occlusion was not efficient, and one from the HS–NT group, because the risk zone was too large.

3. Results

3.1. Mean arterial pressure, body weight and heart weight in NT and TGH rats

Table 1 presents in vivo mean arterial pressure (MAP),
body weight and heart weight values assessed in NT and TGH animals. In anaesthetised TGH rats, the MAP was significantly higher than in age-matched NT rats ($p < 0.001$ by Student’s t-test). Moreover, heart weight was significantly higher while body weight was no different in TGH compared to NT rats.

3.2. Haemodynamic data

Table 2 summarises CF, HR, LVDP and RPP data recorded in the four experimental groups during the stabilisation and the ischaemia–reperfusion periods. Twenty-four hours after HS or sham anaesthesia, there were no statistically significant differences in haemodynamic performance between the four experimental groups.

3.3. Arrhythmias data

The incidence of ventricular arrhythmias during ischaemia and reperfusion periods is presented in Table 3. Twenty-four hours after HS or sham anaesthesia, there was no statistically significant difference in VT/VF incidence between the four experimental groups.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>NT</th>
<th>TGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>$133 \pm 5$</td>
<td>$174 \pm 4^*$</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>$428 \pm 14$</td>
<td>$427 \pm 11$</td>
</tr>
<tr>
<td>Heart weight (mg/g of body weight)</td>
<td>$3.8 \pm 0.1$</td>
<td>$4.4 \pm 0.1^*$</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±SEM. $^*$ $p \leq 0.001$ vs. NT group.

### Table 2

Haemodynamic data

<table>
<thead>
<tr>
<th>Group</th>
<th>Stabilisation</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF (ml/min)</td>
<td>Sham–NT</td>
<td>16.2 ± 0.4</td>
<td>10.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>HS–NT</td>
<td>14.8 ± 0.4</td>
<td>9.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Sham–TGH</td>
<td>18.6 ± 0.4</td>
<td>10.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>HS–TGH</td>
<td>14.7 ± 0.5</td>
<td>9.0 ± 0.5</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>Sham–NT</td>
<td>289 ± 8</td>
<td>275 ± 10</td>
</tr>
<tr>
<td></td>
<td>HS–NT</td>
<td>266 ± 7</td>
<td>266 ± 13</td>
</tr>
<tr>
<td></td>
<td>Sham–TGH</td>
<td>290 ± 5</td>
<td>275 ± 7</td>
</tr>
<tr>
<td></td>
<td>HS–TGH</td>
<td>282 ± 7</td>
<td>267 ± 10</td>
</tr>
<tr>
<td>LVDP (mmHg)</td>
<td>Sham–NT</td>
<td>84 ± 3</td>
<td>20 ± 2</td>
</tr>
<tr>
<td></td>
<td>HS–NT</td>
<td>95 ± 7</td>
<td>22 ± 2</td>
</tr>
<tr>
<td></td>
<td>Sham–TGH</td>
<td>99 ± 6</td>
<td>24 ± 2</td>
</tr>
<tr>
<td></td>
<td>HS–TGH</td>
<td>98 ± 6</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>RPP (mmHg/min×10³)</td>
<td>Sham–NT</td>
<td>24.3 ± 0.9</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>HS–NT</td>
<td>24.2 ± 1.6</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Sham–TGH</td>
<td>28.9 ± 2.0</td>
<td>6.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>HS–TGH</td>
<td>27.6 ± 1.3</td>
<td>6.3 ± 0.5</td>
</tr>
</tbody>
</table>

CF: coronary flow; HR: heart rate; bpm, beats per minute; LVDP: left ventricular developed pressure; RPP: rate pressure product ($=LVDP \times HR$).

Sham–NT (normotensive sham-anasthetised rats, $n = 8$), Sham–TGH (transgenic hypertensive sham-anasthetised rats, $n = 8$), HS–NT (normotensive heat-stressed rats, $n = 8$) and HS–TGH (transgenic hypertensive heat-stressed rats, $n = 8$).

Data are expressed as the mean±SEM.

### Table 3

Incidence (%) of ventricular tachycardia and or fibrillation (VT/VF) during the ischaemia and the 120 min reperfusion periods, in Sham–NT (normotensive sham-anasthetised rats), Sham–TGH (transgenic hypertensive sham-anasthetised rats), HS–NT (normotensive heat-stressed rats) and HS–TGH (transgenic hypertensive heat-stressed rats)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham–NT</th>
<th>HS–NT</th>
<th>Sham–TGH</th>
<th>HS–TGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>VT/VF ischaemia</td>
<td>75</td>
<td>38</td>
<td>42</td>
<td>88</td>
</tr>
<tr>
<td>VT/VF reperfusion</td>
<td>50</td>
<td>88</td>
<td>57</td>
<td>63</td>
</tr>
</tbody>
</table>

Data are expressed as mean values.

### Table 4

Table 4 summarises infarct size data, expressed as the percentage of the risk zone (I/R) or of the left ventricle (L/V) for the four experimental groups.

#### Table 4

Risk (R) and infarct (I) sizes, expressed as a percentage of the left ventricle (L/V)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham–NT</th>
<th>HS–NT</th>
<th>Sham–TGH</th>
<th>HS–TGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>R/LV (%)</td>
<td>51.6 ± 0.7</td>
<td>52.9 ± 1.3</td>
<td>52.1 ± 1.3</td>
<td>52.6 ± 0.6</td>
</tr>
<tr>
<td>I/LV (%)</td>
<td>22.5 ± 1.0</td>
<td>8.3 ± 0.9*</td>
<td>24.6 ± 1.6</td>
<td>10.9 ± 0.5*</td>
</tr>
<tr>
<td>I/R (%)</td>
<td>42.2 ± 2.1</td>
<td>15.5 ± 1.2*</td>
<td>48.0 ± 3.8</td>
<td>20.0 ± 1.0*</td>
</tr>
</tbody>
</table>

Sham–NT (normotensive sham-anasthetised rats), Sham–TGH (transgenic hypertensive sham-anasthetised rats), HS–NT (normotensive heat-stressed rats) and HS–TGH (transgenic hypertensive heat-stressed rats).

Data are expressed as mean±SEM.

$^*$ $p \leq 0.001$ vs. Sham–NT group, $^b$ $p \leq 0.001$ vs. Sham–TGH group.
subjected to an ischaemia–reperfusion sequence, in accordance with previous in vivo studies [3,5]. We provide the first demonstration that this delayed resistance to myocardial infarction can be reproduced in a pathological rat model of hypertension. Moreover, myocardial HSP72 synthesis was enhanced by heat stress in both normotensive and transgenic [(mREN-2)27] hypertensive animals.

The high rate of chronic hypertension among cardiac surgery patients implies that experimental therapies that protect normotensive myocardium will be more clinically relevant if they also protect chronically hypertensive as well as hypertrophic myocardium. For this reason, the effectiveness of experimental therapies known to protect normotensive myocardium from ischaemic injury have been investigated in different hypertensive rat models. For example, Boutros and Wang [13] have demonstrated that adenosine-pretreatment or ischaemic preconditioning are cardioprotective in both normotensive and spontaneously hypertensive rats. It has also been observed that myocardial protection conferred by ischaemic preconditioning was more pronounced in transgenic [(mREN-2)27] hypertensive rats than in normotensive controls [14]. Since left ventricular hypertrophy is a common consequence of hypertension [22], the protection induced by ischaemic preconditioning was investigated in this setting and was found to be effective [12,23]. However, few studies have explored the myocardial protection induced by heat stress in pathological models. It has been observed that ischaemic tolerance of rat hearts hypertrophic by aortic banding was improved by prior hyperthermia [24,25]. In the present study, we have demonstrated that heat stress was able to protect hypertrophied myocardium from transgenic [(mREN-2)27] hypertensive rats against infarction. Although the myocardial protection induced by ischaemic preconditioning was more pronounced in transgenic [(mREN-2)27] hypertensive than in normotensive animals [14], the heat stress-induced protection reported here was similar in both groups. However, since the end-point used in both studies was different, it is difficult to compare the protection induced by ischaemic preconditioning and heat stress. Moreover, it cannot be excluded that the protection conferred by hyperthermia, but not by ischaemic preconditioning, is an all or nothing process.

In contrast with previous studies performed on spontaneously hypertensive rats [8], Sham–TGH rats tended to have a lower incidence of ischaemia-induced ventricular tachycardia or fibrillation compared to Sham–NT rats. Also, heat stress tended to increase the incidence of ischaemia–induced arrhythmias in TGH rats while it tended to be protective in NT rats. However, these results are not statistically significant and need to be confirmed by adequately aimed studies.

Another purpose of this study was to evaluate myocardial HSP72 expression following hyperthermia, in the presence or absence of hypertension. In the absence of heat stress, our results show that there is only a weak synthesis

induced a 63% decrease in infarct size in hearts from NT animals and a 58% one in hearts from TGH rats \( p \leq 0.001 \) by one-way ANOVA, Fig. 2). Similar results were observed concerning the I/LV ratio (Table 4). Myocardial risk size, expressed as the percentage of the left ventricle \( (R/LV) \) was similar for all groups (Table 4). Differences in infarct size, therefore, did not result from variability in the risk zone.

3.5. HSP72 analysis

In NT animals, Western blot analysis of myocardial HSP72 content (Fig. 3) showed a marked increase of this protein following HS (HS–NT vs. Sham–NT groups). This confirms the adequacy of the HS protocol. The marked increase of HSP72 induced by heat stress was also seen in TGH rats (HS–TGH vs. Sham–TGH groups). Moreover, weak HSP72 expression was seen in the myocardium of Sham–TGH rats.

4. Discussion

In this study, we observed in normotensive rats that prior heat stress led to delayed cardioprotection, by significantly reducing infarct size in the isolated heart...
of this protein in hearts from transgenic [(mREN-2)27] hypertensive, but not from normotensive, rats. Indeed, hypertension is a chronic pathology that induces a small basal expression of myocardial HSP72 [26,27]. However, in accordance with the direct correlation seen between cardioprotection and the amount of HSP induced [6], the small amount of HSP72 synthesis observed in TGH rats appears to be insufficient to protect the myocardium. Twenty-four hours after heat stress, myocardial HSP72 expression was enhanced in both groups. These results are in accordance with observations on spontaneously hypertensive rats [26,27]. Since better functional recovery has been observed in isolated perfused transgenic mouse and rat hearts overexpressing HSP72 and subjected to an ischaemia–reperfusion sequence [28,29], we can presume that myocardial induction of HSP72 is responsible, at least in part, for the resistance to infarction induced by heat stress in hypertensive hearts.

In parallel, there is a growing amount of evidence that cytoprotective proteins, such as HSP72, manganese-superoxide dismutase and unidentified others, play a role in the delayed protection associated with ischemic preconditioning (for review, see reference [30]). Activation of protein kinase C appears to be a crucial intermediate step in the resistance to myocardial infarction induced by heat stress [18], as well as by ischemic preconditioning [30]. Since protein kinase C activity and concentration were described to be increased during the development of ventricular hypertrophy induced by pressure overload [31], it could be of interest to explore its implication in the actual setting.

In summary, this study provides the first observation that heat stress may protect the isolated heart of transgenic [(mREN-2)27] hypertensive rats against ischaemia–reperfusion injury. This effect appears to be as potent as the one observed in normotensive rats. Finally, this protection seems to be associated with the myocardial synthesis of HSP72.

References


