Endothelin-A and -B antagonists protect myocardial and endothelial function after ischemia/reperfusion in a rat heart transplantation model

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Received 12 January 1998; accepted 25 May 1998

Abstract

Objective: Previous studies suggested that endothelin-1 (ET-1) may play a pathophysiological role in myocardial ischemia/reperfusion injury. This study was designed to investigate the effects of the selective ET-A receptor antagonist BQ123 and the selective ET-B receptor antagonist BQ788 on myocardial and endothelial function after reversible deep hypothermic ischemia in a heterotopic rat heart transplantation model. Methods: Isogenic intraabdominal heterotopic transplantation was performed in Lewis rats. After 1 h of cold ischemic preservation reperfusion was started either after application of placebo (control), BQ123 (3 μmol/kg/min), BQ788 (3 μmol/kg/min), ET-1 (8 pmol/kg/min) or simultaneous infusion of BQ123 or BQ788 and ET-1, respectively (n=12 each). An implanted balloon was used to obtain pressure-volume relations of the transplanted heart. Myocardial blood flow (MBF) was assessed by the hydrogen-clearance method. Measurements were taken after 1 and 24 h of reperfusion. Endothelium-dependent vasodilation to acetylcholine (ACH) and endothelium-independent vasodilation to sodium nitroprusside were also determined. Results: Both BQ123 and BQ788 significantly improved myocardial and endothelial functional recovery during early reperfusion, whereas ET-1 significantly impaired myocardial and endothelial function. Simultaneous infusion of ET-1 diminished the effects of BQ123 and BQ788. Although myocardial function and baseline MBF were similar in all groups after 24 h of reperfusion, endothelium dependent vasodilation to ACH was still significantly higher in the BQ123 and BQ788 groups and lower in the ET-1 groups (p<0.05). Conclusions: These results suggest that endogenous ET release is involved in the pathogenesis of reperfusion injury after heart transplantation. ET-A and ET-B receptor antagonists may be useful to reduce ischemia/reperfusion injury. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Endothelin-antagonist; Reperfusion; Transplantation; Rat

1. Introduction

Cardiac transplantation is a successful treatment option for irreversible, final stage cardiac failure. Myocardial performance within the first hour after the surgical procedure determines the patient’s fate not only during the postoperative period, but also for the long time outcome. Most studies about the effects of myocardial ischemia and reperfusion focused on myocardial injury and the recovery of contractile function. It is now appreciated that the survival of the heart as a whole is in part dependent on the ability of the microcirculation to adequately deliver and distribute blood flow during reperfusion. Recent studies show the significance of protecting the microvasculature to attenuate reperfusion injury [1,2], which may be especially important in those hearts undergoing cold ischemic storage for transplantation [3,4].

As a paracrine tissue, the endothelium synthesizes nitric oxide (endothelium dependent relaxing factor) with vasodilative properties and endothelin (ET), the most potent vasoconstrictor known so far [5]. In previous studies [3,4], we showed that the preservation of the endogenous nitric oxide synthesis significantly improves myocardial and
endothelial function during reperfusion after heart transplantation. However, no data are available on the effects of ET in transplant models. To date, two different ET receptor subtypes are described: the ET-A receptor, which mainly mediates vasoconstriction, is predominantly present in smooth muscle layer, whereas the ET-B receptor, whose stimulation is assumed to primarily result in vasodilatation by enhancing nitric oxide synthesis, is predominantly located in vascular endothelium. However, recent experiments showed that ET-B receptors are in fact also present on smooth muscle cells and contribute to the vasoconstrictive effect of ET [6,7]. Furthermore, ET-A/B receptors could also be demonstrated in cardiac myocytes [8].

Ischemia and reperfusion result in enhanced production and release of ET [9,10], which may contribute to the ischemia/reperfusion injury. As previous studies on potential cardioprotective effects of ET-1 antagonist used only selective ET-A or mixed ET-A/ET-B blockers in models of regional or global ischemia, the purpose of the present study was to evaluate the effects of the selective ET-A receptor antagonist BQ123 and the selective ET-B receptor antagonist BQ788 as well as the application of exogenous ET-1 on myocardial and coronary endothelial function in a heterotopic rat heart transplantation model. This model was used to simulate the clinical conditions in terms of hypothermic cardiac preservation and whole blood reperfusion. It allows an observation time of 24 h, which is impossible in isolated organ models, and the assessment of myocardial function independently from the actual loading conditions.

2. Methods

2.1. Animals

Male Lewis rats (\( n = 72 \)) weighing 250 to 300 g were used in this experiment. All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

2.2. Preparation of the donor heart

The donor rat was anaesthetized with intraperitoneal xylazine (7 mg/kg). After the abdominal cavity was opened 500 units of sodium heparin were injected into the inferior vena cava. The diaphragm was then opened via the laparotomy incision and the heart was exposed by dividing each side of the thorax. The anterior chest wall flap was pulled cephalad and the pericardium was incised. After the inferior vena cava was ligated, a small forceps was passed through the transverse sinus. The superior vena cava and the pulmonary veins were tied en masse with a 4-0 single silk suture. The aorta and the pulmonary artery were divided and the heart was immediately placed into cold saline (4°C). The donor organ was prepared within 5 min.

2.3. Preparation of the recipient

The recipient rats were anaesthetized with intraperitoneal xylazine (7 mg/kg). Then the animals were intubated for mechanical ventilation to measure myocardial blood flow (MBF) by the hydrogen clearance technique after transplantation. The internal carotid artery and the external jugular vein were cannulated for simultaneous monitoring of blood pressure and heart rate and for drug administration and volume substitution. The abdomen was opened by a midline incision and the aorta and the vena cava were exposed by reflecting the intestines to the left side.

2.4. Heterotopic transplantation

Two centimeter segments of the infrarenal aorta and the vena cava were isolated and occluded by small vessel forceps. The aorta and the pulmonary artery of the donor heart were end to side anastomosed to the abdominal aorta and the vena cava of the recipient rat, respectively. This was achieved using 9-0 monofilament polyamide sutures operating under a 16-power magnification microscope. The heart was maintained at 4–6°C during the implantation period by wrapping it in cold gauze which was regularly irrigated with cold saline (4°C). The temperature was monitored by an intraventricularly placed thermometer. To minimize variability between experiments, the duration of the implantation was standardized to 60 min for all studies. After completion of the anastomoses the vessels were released slowly and the heart was then reperfused with blood in situ. Crystalloid volume substitution (Ringer’s solution) was adjusted via the external jugular vein to maintain mean arterial pressure between 75–85 mmHg for a 1 h reperfusion period.

2.5. Measurements

2.5.1. Left ventricular (LV) pressure–volume relations

After 1 h of reperfusion a latex balloon was introduced into the left ventricle via the apex and was connected to a precision calibrated syringe for administration and withdrawal of fluid to determine LV systolic pressure (LVSP), its first derivative (dP/dt) and enddiastolic pressure (LVEDP) by a Millar micromanometer (Millar Instruments, Houston, TX, USA) at different LV volumes. These parameters together with the arterial pressure were recorded on line with a Gould pressure transducer and an analog differentiator connected to an analog strip chart recorder (Astromed). The data were also collected onto a PC computer for later offline analysis.
Ventricular volumes were calculated as the volume of saline injected into the balloon plus the displaced volume by the empty balloon (0.02 ml). Data for a complete pressure-volume curve were obtained by incremental increases in ventricular volume by 0.03 ml until a ventricular volume of 0.14 ml was reached. From these data indices of LV myocardial contractility (systolic pressure-volume relationships) and compliance (diastolic pressure-volume relationships) were constructed.

2.5.2. Myocardial relaxation

Myocardial relaxation was characterized by the relaxation time constant ($T_E$) of the LV pressure fall. $T_E$ was calculated from time-expanded recordings of LV pressure; pressure records were digitized at 1 ms intervals beginning at peak negative $dP/dt$ began returning monophasically toward zero and terminating at an isovolumetric pressure ($P$) of 10% of peak systolic pressure. The coordinates were fit by a monoexponential equation: $P = P_0 e^{-t/T} + P_b$ where $P_0$ is the LV pressure at peak negative $dP/dt$ and $P_b$ is the baseline pressure towards which the monoexponential decays. Since the influence of intrathoracic or intrapericardial pressure of an in situ preparation could be neglected after heterotopic transplantation, we assumed that $P_b$ was equal to zero.

2.5.3. MBF

LV MBF was measured by the hydrogen gas clearance method [11–13] using self-constructed platine electrodes, nano-amperemeters (Knick) and a computer program developed by our laboratory. During the MBF measurements, the rats were mechanically ventilated with a mixture of room air and hydrogen (80:20%) for 15 to 20 s until the equilibration of myocardial tissue $H_2$ levels. The hydrogen gas clearance curve was plotted, from which myocardial blood flow was determined [11,12]. Coronary resistance was estimated by dividing the perfusion pressure by MBF. After baseline measurements a subsequent infusion of the endothelium-dependent vasodilator acetylcholine (ACH, 1 $\mu$mol/l) and the endothelium-independent vasodilator sodium nitroprusside (SNP, 10 $\mu$mol/l) were started in all groups for a period of 5 min and the measurements of MBF were repeated. Between the infusions, MBF was allowed to return to baseline levels. Vasodilator response was expressed as the percent change of MBF from baseline.

2.6. Study groups

Heart rate and MBF were evaluated in all animals before explantation. After completion of the anastomoses, the hearts were divided in six groups ($n=12$/group). Five minutes before reperfusion of the graft, an intravenous infusion was started of either (1) saline vehicle (control group), (2) ET-A receptor antagonist BQ123 (3 $\mu$mol/min/kg), (3) ET-B receptor antagonist BQ788 (3 $\mu$mol/kg/min), (4) ET-1 (8 pmol/kg/min), (5) BQ123 and ET-1 or (6) BQ788 and ET-1 and continued during the first 15 min of reperfusion. The above mentioned doses were selected based on the current literature [14–17]. After 1 h of reperfusion the measurements of systolic and diastolic function and MBF were completed. Then the abdominal cavity was closed and the animals were allowed to recover from the anesthesia. The animals of all groups received the same standard diet and normal drinking water. After 24 h the animals were reanesthetized and the abdominal cavity was reopened. The grafts were instrumented and the measurements were performed similarly as after 60 min of reperfusion.

2.7. Statistical analysis

Statistical analysis was performed by the SPSS software package. All the values were expressed as mean±standard error of the mean (SEM). Student’s paired $t$-test was used to compare individual means within the groups. Students unpaired $t$-test and repeated measures of analysis of variance followed by an unpaired $t$-test with a Bonferroni correction for multiple comparisons and the post-hoc Scheffe’s test were used to compare individual means between the groups. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Early reperfusion, 60 min

The recipient’s heart rate and aortic pressure were the same in all groups (Table 1). The hemodynamic parameters are shown in Table 2. Systolic functional recovery was significantly better in the BQ123 and BQ788 groups in comparison to control: LVSP and peak positive $dP/dt$ were significantly higher in the BQ123 and BQ788 groups ($p<0.05$). Systolic cardiac function curves showed a significant leftward shift in these groups in comparison to control (Fig. 1). The slope of the systolic pressure–volume relationship $E_{max}$ was significantly higher in both the BQ123 and BQ788 groups in comparison to control ($p<0.05$). Myocardial relaxation was better in the BQ123 and BQ788 groups as indicated by the significantly lower isovolumic relaxation constant $T_E$ ($p<0.05$). LVEDP did not differ in comparison to control. The diastolic compliance curves (enddiastolic pressure–volume relationships) were similar in all groups (Fig. 1). MBF was significantly higher and coronary vascular resistance was significantly lower ($p<0.05$) in the BQ123 and BQ788 groups in comparison to control (Table 3). Endothelium-independent vasodilation after SNP was similar to the control group. In contrast, endothelium-dependent vasodilation after ACH was significantly ($p<0.05$) better in the BQ123 and BQ788 groups than in the control group (Fig.
2). There was no significant difference in any of the parameters between the BQ123 and BQ788 groups. ET-1 led to a significantly impaired systolic function characterized by decreased LVSP, peak positive dP/dt and $E_{\text{max}}$ ($p<0.05$). Myocardial relaxation was also impaired as indicated by the significantly increased $T_{\text{E}}$ ($p<0.05$). MBF was significantly decreased and endothelium-dependent vasodilation after ACH was significantly impaired ($p<0.05$). Endothelium-independent vasodilation after SNP was similar to in the control group (Fig. 2). Simultaneous infusion of ET-1 to BQ123 or BQ788, respectively, abolished their myocardial and endothelial effects. All registered values were similar to control (Tables 2 and 3).

### 3.2. Late reperfusion, 24 h

The recipient animals had the same heart rate and aortic pressure (Table 1). In all groups of transplanted hearts, no hemodynamic differences were observed after 24 h of reperfusion. LVSP, peak positive dP/dt, $E_{\text{max}}$, $T_{\text{E}}$, LVEDP and baseline MBF were similar in the control and all treatment groups (Tables 2 and 3). In the control, ET-1, BQ123+ET-1 and BQ788+ET-1 groups, all these parameters showed a significant improvement in comparison to the values after 60 min of reperfusion ($p<0.05$). In the BQ123 and BQ788 groups, there were no significant differences in comparison to the values of 60 min of reperfusion. Systolic cardiac function curves and diastolic compliance curves of all groups were nearly identical (Fig. 1). As depicted in Fig. 2, after 24 h, endothelium-dependent vasodilation was significantly increased in all groups in comparison to the values after 60 min of reperfusion ($p<0.05$). Although baseline MBF was similar in all groups (Table 3), endothelium-dependent vasodilation after ACH was still significantly ($p<0.05$) better in the BQ123 and BQ788 group in comparison to control (Fig.

### Table 1

Hemodynamic parameters of the recipient

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BQ123</th>
<th>BQ788</th>
<th>ET-1</th>
<th>BQ123+ET-1</th>
<th>BQ788+ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (min)</td>
<td>1 h Reperfusion</td>
<td>342±14</td>
<td>310±36</td>
<td>302±21</td>
<td>332±19</td>
<td>322±24</td>
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<tr>
<td></td>
<td>24 h Reperfusion</td>
<td>336±28</td>
<td>330±27</td>
<td>330±22</td>
<td>318±22</td>
<td>315±38</td>
</tr>
<tr>
<td>AoP (mmHg)</td>
<td>1 h Reperfusion</td>
<td>78±8</td>
<td>81±8</td>
<td>75±7</td>
<td>84±6</td>
<td>77±8</td>
</tr>
<tr>
<td></td>
<td>24 h Reperfusion</td>
<td>83±5</td>
<td>85±9</td>
<td>85±6</td>
<td>83±5</td>
<td>77±9</td>
</tr>
</tbody>
</table>

Heart rate (HR) and aortic pressure (AoP) after 1 h and 24 h of reperfusion. All values are given as mean±SEM.

### Table 2

Hemodynamic parameters of the transplanted heart

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BQ123</th>
<th>BQ788</th>
<th>ET-1</th>
<th>BQ123+ET-1</th>
<th>BQ788+ET-1</th>
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<tbody>
<tr>
<td>HR (min)</td>
<td>1 h Reperfusion</td>
<td>167±17</td>
<td>168±14</td>
<td>188±12</td>
<td>181±10</td>
<td>178±19</td>
</tr>
<tr>
<td></td>
<td>24 h Reperfusion</td>
<td>223±9</td>
<td>207±7</td>
<td>230±10</td>
<td>208±16</td>
<td>199±21</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>1 h Reperfusion</td>
<td>95±7</td>
<td>143±15$^a$</td>
<td>171±17$^b$</td>
<td>61±6$^c$</td>
<td>110±8$^d$</td>
</tr>
<tr>
<td></td>
<td>24 h Reperfusion</td>
<td>160±6$^f$</td>
<td>152±12</td>
<td>165±10</td>
<td>144±12$^g$</td>
<td>158±8$^h$</td>
</tr>
<tr>
<td>dP/dt$_{\text{max}}$ (mmHg/s)</td>
<td>1 h Reperfusion</td>
<td>3312±247</td>
<td>6766±649$^a$</td>
<td>7789±763$^b$</td>
<td>1562±189$^c$</td>
<td>3413±336</td>
</tr>
<tr>
<td></td>
<td>24 h Reperfusion</td>
<td>6454±722$^b$</td>
<td>6329±715</td>
<td>6875±867</td>
<td>5805±616$^a$</td>
<td>5997±678$^b$</td>
</tr>
<tr>
<td>E$_{\text{max}}$ (mmHg/ml)</td>
<td>1 h Reperfusion</td>
<td>578±59</td>
<td>1154±94$^a$</td>
<td>1280±174$^b$</td>
<td>397±25</td>
<td>531±73</td>
</tr>
<tr>
<td></td>
<td>24 h Reperfusion</td>
<td>1250±87$^b$</td>
<td>1194±130</td>
<td>1033±129</td>
<td>970±116$^a$</td>
<td>1089±90$^b$</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>1 h Reperfusion</td>
<td>8.7±1.5</td>
<td>9.7±3.4</td>
<td>9.5±3.3</td>
<td>9.5±2.4</td>
<td>9.4±3.7</td>
</tr>
<tr>
<td></td>
<td>24 h Reperfusion</td>
<td>9.1±1.6</td>
<td>10.2±2.4</td>
<td>9.2±2.4</td>
<td>9.7±2.4</td>
<td>9.7±1.9</td>
</tr>
<tr>
<td>$T_{\text{E}}$ (ms)</td>
<td>1 h Reperfusion</td>
<td>12.2±1.5</td>
<td>6.9±1.9$^a$</td>
<td>6.1±0.5$^b$</td>
<td>19.5±0.9$^c$</td>
<td>13.4±2.1</td>
</tr>
<tr>
<td></td>
<td>24 h Reperfusion</td>
<td>7.5±2.0$^b$</td>
<td>7.4±0.6</td>
<td>7.7±0.8</td>
<td>8.3±1.1$^b$</td>
<td>7.9±0.3$^b$</td>
</tr>
</tbody>
</table>

All parameters were assessed after 1 h and 24 h of reperfusion. HR, heart rate; LVSP, left ventricular peak systolic pressure; dP/dt$_{\text{max}}$, peak positive left ventricular dP/dt; LVEDP, left ventricular enddiastolic pressure; $T_{\text{E}}$, isovolumic relaxation constant. All values are given as mean±SEM at an intraventricular volume 80 μl.

$^p <0.05 \text{ vs. control.}$

$^p <0.05 \text{ 1 h vs. 24 h of reperfusion.}$
Fig. 1. Left ventricular systolic and diastolic function in the heterotopically transplanted rat heart after 1 h of reperfusion (left panel) and 24 h of reperfusion (right panel). The peak systolic pressure–volume relationships are shown on the top and the enddiastolic pressure–volume relationships are shown on the bottom of the graphs. LVP, left ventricular pressure, LVV left ventricular volume. All values are given as mean ± SEM.

2). On the other hand, endothelium-dependent vasodilation after ACH was still significantly depressed in the ET-1 group in comparison to control (p<0.05). Endothelium-dependent vasodilation after ACH was nearly identical to control in the BQ123+ET-1 and BQ788+ET-1 groups (data not shown on Fig. 2). Endothelium-independent vasodilation after SNP was similar in all groups and showed no changes over the time.

4. Discussion

The present study demonstrates that infusion of BQ123, a selective antagonist of the ET-A receptor, and infusion of BQ788, a selective antagonist of the ET-B receptor, improve the recovery of myocardial and endothelial function after 1 h of cold ischemia and reperfusion in the heterotopically transplanted rat heart. ET-1 infusion impaired functional recovery. Simultaneous administration of ET-1 and BQ123 or BQ788 mutually antagonized their effects suggesting that the observed functional changes can be attributed to the inhibition of ET-1. These findings are in agreement with recent studies about the pathophysiological role of endogenous ET in the development of ischemia/reperfusion injury. In models of regional ischemia/reperfusion, BQ123 [18] and the mixed ET-A/ET-B receptor antagonist bosentan [19] reduced infarct size and increased coronary flow. In isolated rat hearts with global warm ischemia/reperfusion, bosentan caused an improvement of LV function and coronary flow [20] and BQ123 and BQ610, another selective antagonist of the ET-A receptor, delayed the time to ischemic contracture

<table>
<thead>
<tr>
<th>Parameters of the coronary circulation</th>
<th>Control</th>
<th>BQ123</th>
<th>BQ788</th>
<th>ET-1</th>
<th>BQ123+ET-1</th>
<th>BQ788+ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBF (ml/min/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h Reperfusion</td>
<td>1.9±0.2</td>
<td>2.8±0.3*</td>
<td>3.2±0.6*</td>
<td>1.0±0.2*</td>
<td>1.5±0.2</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>24 h Reperfusion</td>
<td>2.7±0.5*</td>
<td>3.3±0.4</td>
<td>4.3±0.7</td>
<td>2.8±0.3*</td>
<td>3.4±0.4b</td>
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<tr>
<td>CVR (U/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h Reperfusion</td>
<td>41±6</td>
<td>29±9*</td>
<td>23±4*</td>
<td>84±8*</td>
<td>51±6</td>
<td>46±5</td>
</tr>
<tr>
<td>24 h Reperfusion</td>
<td>30±7*</td>
<td>25±3</td>
<td>19±6</td>
<td>29±6*</td>
<td>22±6*</td>
<td>24±6*</td>
</tr>
</tbody>
</table>

Myocardial blood flow (MBF) and coronary vascular resistance (CVR) after 1 h and 24 h of reperfusion. All values are given as mean±SEM at an intraventricular volume 80 μl.

* p<0.05 vs. control.

b p<0.05 1 h vs. 24 h of reperfusion.
Fig. 2. Endothelium-independent vasodilation to sodium nitroprusside (top) and endothelium-dependent vasodilation to acetylcholine (bottom) in the heterotopically transplanted rat heart after 1 and 24 h of reperfusion. All values are given as mean±SEM. * p<0.05 vs. control. † p<0.05 1 h vs. 24 h of reperfusion.

and enhanced LV developed pressure and coronary flow [21,22]. Blocking of the ET-A receptor with BE-18257B significantly improved the recovery of myocardial function and coronary flow after global hypothermic ischemia, while application of exogenous ET decreased functional recovery [23]. Not only myocardial function, but also endothelial function may be preserved by selective ET-A receptor antagonists and mixed ET-A/ET-B receptor antagonists after global ischemia and reperfusion [20,24].

As no data are available on the role of selective ET-B receptor antagonism in ischemia/reperfusion injury, we examined the effects of the selective ET-B receptor antagonist BQ788. Similarly to the ET-A antagonist BQ123, BQ788 also improved the recovery of myocardial and endothelial function. These data indicate that both ET-A and ET-B receptor subtypes contribute to the beneficial role of ET antagonism. These data also suggest, that even if ET-B mediated pathways are supposed to induce vasodilation by enhancement of the endothelial nitric oxide synthesis, the net effect of ET-B receptors at least during ischemia/reperfusion is probably dominated by constrictor properties. Furthermore, Balwierczak [25] and Filep and coworkers [26,27] showed in Langendorff perfused rat hearts and in conscious rats, respectively, that both subtypes of the ET receptor (ET-A and ET-B) mediate vasoconstriction under nonischemic conditions. Moreover, ischemia and reperfusion lead to endothelial injury [1–4,28] which may reduce the number of endothelial (vasodilator) ET-B receptors resulting in an enhanced net vasoconstrictor response by ET-B receptors.

Wang et al. [10] showed in a pig model of regional ischemia and reperfusion that both ET-1 which has a higher affinity to ET-A receptors and Ala–ET-1, a selective ET-B agonist, cause coronary vasoconstriction in the posts ischemic myocardium. In a recent study [29], different activity of ET-A and B receptors was described under normal and pathophysiological conditions. In experimental heart failure ET-B receptor activation caused enhanced coronary vasoconstriction while it did not in normal hearts. One may speculate that other pathophysiological situations such as ischemia/reperfusion also lead to similar alterations of coronary vascular responsiveness to ET-A and ET-B receptor stimulation. On the other hand, local clearance of ET-1 by ET-B receptors was suggested [30] in a low flow ischemia model and thereby coronary vasoconstriction if ET-B receptor antagonist was applied. The relevance of the above findings for the clinical situation remains unclear. However, in humans, both ET-A and ET-B receptors have been shown to mediate contraction to ET-1 [7,31]. According to Rubanyi and Polokoff [31] ET-B receptor mediated vasodilation probably has a significance only in large vessels, but not in the coronary vasculature.

This is the first study, in which long-term effects of ET-1 receptor antagonists on global hypothermic ischemia/reperfusion injury were investigated. Although no more differences were found in systolic and diastolic function and baseline myocardial blood flow after 24 h of reperfusion, endothelial function was still depressed in the control group in comparison to the BQ123 and BQ788 treatment.
groups as indicated by the lower MBF response to ACH. This very interesting finding would suggest that there is a discrepancy between the recovery of myocardial and endothelial function after ischemia/reperfusion or, otherwise, the endothelium is more vulnerable to reperfusion injury than the myocardium. Schnabel et al. [28] showed in a recent ultrastructural study in human transplant biopsy specimens that while myocyte ultrastructural integrity recovers within 60 min of reperfusion, ultrastructural regeneration of the endothelium lasts from days up to 1 week. There are only a few reports [4,24,32] where endothelial and myocardial function were studied in the same model during ischemia/reperfusion. Godwin et al. [32] and Hagar [24] found that the blockade of the ET-1 pathway improves postischemic recovery of myocardial blood flow and endothelial function, but has no effect on the recovery of mechanical function. Hagar speculated about the involvement of endogenous nitric oxide synthesis in endothelial protection by inhibiting ET-A receptors: endogenous ET may selectively impair the production or increase the degradation of nitric oxide in the postischemic state.

The possible beneficial effects of ET receptor antagonists has not been fully understood yet. By inducing vasodilation ET receptor antagonists probably prevent the no-reflow phenomenon leading to an increased myocardial functional recovery or to a reduction of infarct size [18–24] which might be one of the most important modes of action of ET receptor antagonism during ischemia/reperfusion. However, flow-independent mechanisms may also contribute to the beneficial influence of ET receptor antagonists. ET-1 is shown to be involved in the activation and accumulation of neutrophils [33,34] which are also pathogenic factors in ischemia/reperfusion injury. ET may also enhance the production of superoxide anion [35]. Furthermore, ET receptor antagonists may have a direct effect on the myocardial ET receptors attenuating the increase of intracellular concentrations of calcium [36].

In contrast to the above mentioned studies, some authors did not find any influence of ET receptor antagonists during ischemia/reperfusion [37–40]. However, these conflicting results occurred mainly in models of regional ischemia/reperfusion which cannot directly be compared with the present study. The negative results in regional ischemia/reperfusion studies may be attributed at least partly to the fact that even in the control group the infarct size was too low or had a too high variance to perform an effective statistical comparison [37]. Dagassan et al. [39] did not observe any beneficial effect of bosentan after a relatively short time of global ischemia and reperfusion in isolated rat hearts. One may speculate that the degree of ischemic stimulus may explain the contradictory findings. In support of this, Illing et al. [22] found a more pronounced mechanical and metabolic effect of the treatment with the ET-A receptor antagonist BQ610 following 30 min of ischemia than following 15 min of ischemia.

In summary, we showed that ET antagonism reduces reperfusion injury after reversible deep hypothermic ischemia in a heterotopic rat heart transplantation model. Both subtypes of ET receptors, ET-A and ET-B, contribute to this effect. Furthermore, an initial treatment with ET-A or ET-B receptor antagonists had a persisting beneficial effect on endothelial function during late reperfusion. Further studies are necessary to clarify the exact pathomechanism of ET and ET receptors during ischemia/reperfusion and the interaction with the nitric oxide pathway.

Acknowledgements

This study was supported by the grant ‘Forschungsschwerpunkt Transplantation’ of the Land Baden-Württemberg.

References


