Beneficial effects of long-term selective endothelin type A receptor blockade in canine experimental heart failure

Gordon W. Moe\textsuperscript{a,\textcopyright}, Ana Albernaz\textsuperscript{a}, George O. Naik\textsuperscript{a}, Michael Kirchengast\textsuperscript{b}, Duncan J. Stewart\textsuperscript{a}

\textsuperscript{a}Terrence Donnelly Heart Centre, St Michael's Hospital, University of Toronto, Toronto, Ontario M5B1W8, Canada

\textsuperscript{b}Knoll AG, Ludwigshafen, Germany

Received 16 January 1998; accepted 26 May 1998

Abstract

Objectives: We examined the effects of chronic type A endothelin receptor (ET\textsubscript{A}) blockade in a dog model of pacing-induced cardiomyopathy. Methods: Eight dogs received an ET\textsubscript{A} antagonist, LU 135252 (50 mg/kg orally daily) and nine dogs received a matching placebo starting at day three of pacing and continued for the remainder of the three weeks of pacing. Results: In the placebo group, the mean pulmonary artery pressure and left ventricular end diastolic pressure increased from 16±3 and 8±2 mmHg, respectively, at baseline to 40±11 and 34±7 mmHg, respectively, at two weeks (both $p<0.001$ versus baseline). Cardiac output declined from 3.5±0.7 to 1.9±0.6 l/min ($p<0.001$). In the treatment group, LU 135252 attenuated the increase in mean pulmonary artery and left ventricular end diastolic pressure (16±3 and 9±1 mmHg at baseline to 29±3 and 27±3 mmHg, respectively, at two weeks ($p<0.001$), and the decline in cardiac output (3.2±0.3 to 2.6±0.8 l/min, $p<0.01$; $p<0.05$ versus placebo for the three parameters). Systemic and pulmonary vascular resistance increased only in the placebo group. Left ventricular end-diastolic volume increased to a similar degree. However, LU 135252 attenuated the increase in plasma norepinephrine level (placebo, 1.2±0.5 to 3.7±1.9 pmol/l; treatment, 0.8±0.3 to 2.4±0.6 pmol/l; both $p<0.001$ versus baseline; $p<0.05$ versus placebo). Conclusion: Our results suggest that endothelin-1 plays a role in the hemodynamic perturbations in canine pacing-induced cardiomyopathy. The favourable hemodynamic effects without concomitant aggravation of neurohormonal activation suggests that ET\textsubscript{A} receptor blockade may be beneficial in the treatment of heart failure. \copyright 1998 Elsevier Science B.V. All rights reserved.

Keywords: Endothelin; Heart failure; Hemodynamics; Remodeling; Natriuretic peptide

1. Introduction

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide with diverse biologic actions [1–3]. The endothelin peptides interact with two receptor subtypes: the type A (ET\textsubscript{A}) and type B (ET\textsubscript{B}) receptors [4–6]. The vasoconstrictive effect of ET-1 is mediated mostly by the ET\textsubscript{A} receptors [7]. Stimulation of the ET\textsubscript{B} receptors by specific agonists elicits contraction as well as vasorelaxation, which is mediated by the release of nitric oxide and prostaglandins [8–11]. The plasma ET-1 level is elevated in animals models of [12–14] and patients with heart failure [15,16].

The availability of specific antagonists of the endothelin receptors has allowed for investigations of the functional role of ET-1 in heart failure. Studies to date that have assessed the effect of these antagonists have involved mostly the use of small animal models of heart failure [17–21]. The chronic effects of endothelin receptor antagonists in large animal models of heart failure, which more closely simulate human heart failure, have not been assessed. Accordingly, the aim of the current study was to assess a potential pathogenetic role of ET-1 in chronic heart failure by examining the effects of chronic ET\textsubscript{A} receptor blockade on hemodynamics, left ventricular remodeling and neurohormonal parameters in a large animal model. To simulate clinical studies, we used a placebo-control design and employed a canine model of car-
diomyopathy that was induced by rapid pacing. This pacing model closely mimics human cardiomyopathy and has recently been recommended by a National Institute of Health executive report to be one of the heart failure models to be studied intensively [22].

2. Methods

The study group consisted of 17 male mongrel dogs with a mean weight of 22.1±1.4 kg (mean±SD). Nine dogs were randomized to receive placebo and eight dogs were randomized to receive an orally active ET<sub>A</sub> receptor antagonist. All paced dogs were preconditioned for at least one week prior to the start of the study. There was free access to water and dog chow. The investigation conformed with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication No. 85-23, revised 1985). In addition to the above dogs, three Beagle dogs were used to test the adequacy of ET<sub>A</sub> receptor blockade by undergoing an exogenous ET-1 challenge experiment as described below. For all studies, approval was obtained from the institutional Animal Care Committee before the studies commenced.

2.1. Endothelin challenge study

To test the adequacy of the dose of oral ET<sub>A</sub> receptor antagonist employed in the long-term study, synthetic ET-1 was infused into three Beagle dogs (weights 9 to 13.5 kg), with and without pre-treatment with an oral ET<sub>A</sub> receptor antagonist. The dogs were anaesthetized with intravenous pentobarbital sodium (30 mg/kg). Mean arterial pressure was measured via a catheter inserted into the femoral artery and connected to a Statham P 23 DB transducer. Endothelin-1 (0.75 nmol/kg) was dissolved in 0.5 ml/kg saline and injected into a leg vein over 2 min. Blood pressure was continuously recorded using a thermoprinter (Gould 2000), from 10 min before ET-1 until 30 min after injection. Two days later, the ET<sub>A</sub> receptor antagonist LU 135252 (50 mg/kg orally) was administered to the same dogs and the exogenous ET-1 challenge studies were repeated 2 h after the administration of LU 135252.

2.2. Induction of heart failure

The method of induction of heart failure has been described previously [23–25]. In brief, under general anesthesia, a unipolar pacing lead was advanced through the external jugular vein into the right ventricular apex. A programmable pulse generator (Medtronic SX-5985, Medtronic Canada, Missisauga, ON, Canada) was inserted into a subcuticular cervical pocket and a chronic cannula was placed through the carotid artery, advanced to the aortic arch, tunnelled underneath the skin and externalized through the dorsal midscapular surface. The animals were allowed to recover from the effects of general anesthesia for one week before the first baseline study measurements were taken. All hemodynamic studies were conducted using local anesthetics and light morphine sedation (5–10 mg). Right sided hemodynamics were obtained from a thermodilution catheter and left ventricular as well as aortic pressures were obtained from a micromanometer-tipped catheter (MIKRO-TIP<sup>®</sup>, Millar Instruments, Houston, Texas, USA), which was introduced via the femoral vein and artery, respectively. After baseline clinical, radiographic and echocardiographic assessments, as well as blood sampling for neurohormone measurements, the pacemaker was programmed to initiate continuous right ventricular pacing at 250 beats/min. All dogs were paced for three weeks. Clinical, radiographic and echocardiographic examinations, as well as arterial blood sampling from the chronic aortic cannula, were obtained in the morning at weekly intervals.

2.3. Chronic ET<sub>A</sub> receptor blockade

On the third day of pacing, the ET<sub>A</sub> receptor antagonist LU 135252 or matching placebo was administered orally at a dose of 50 mg/kg once daily and continued on the same daily dose until the end of the study at three weeks. The orally active compound LU 135252 is the active enantiomer of LU 127043 [26]. LU 135252 is highly selective for ET<sub>A</sub> receptors: it binds to human ET<sub>A</sub> receptors with high affinity (K<sub>i</sub>=2 nM) and the affinity to human ET<sub>B</sub> receptors is 184 nM [27]. In healthy volunteers, the agent has been shown to have an oral bioavailability of 90%, with rapid absorption (t<sub>max</sub> of 1 to 2 h) and a half-life of 10 to 15 h [28]. In the course of preclinical development of the compound, the pharmacokinetic data were also measured in dogs and the values were very similar to those reported in human volunteers (M Kirchengast, personal communication). The oral regimen employed in this study has been shown to significantly attenuate the pressor response to exogenous ET-1 [27] and this effect was reproduced in our ET-1 challenge studies (see Section 3). The rationale for administering LU 135252 on day three of pacing was to initiate ET<sub>A</sub> receptor blockade at a time point equivalent to early heart failure in humans, since our previous studies on this model have demonstrated significant hemodynamic perturbation and cardiac chamber enlargement after one week of pacing [29,30]. For both treatment groups, hemodynamic studies were repeated at a common time point of two weeks of pacing, to assess the impact of LU 135252 versus placebo treatment. Week two was chosen for the terminal hemodynamic study for the following reasons. First, our experience had been that, by week two, heart failure had already reached a moderately severe stage and yet sudden death rarely occurred in these animals at this time. Second, we had demonstrated that by the time of advanced heart failure, i.e. after four weeks or
more of pacing, it was often difficult to detect a treatment effect on hemodynamic parameters with agents such as the angiotensin converting enzyme inhibitor, enalapril, even though severe heart failure did take a longer time period to develop and coronary endothelial function did improve in the enalapril-treated group [31,32]. LU 135252 was withheld on the morning of the week two hemodynamic study in order to avoid measuring the acute hemodynamic effects of the drug. The hemodynamic study was conducted 15 min after the resumption of sinus rhythm. Pacing and drug treatment were resumed after the hemodynamic study to provide one further week of parallel assessment of echocardiographic and neurohormonal parameters of the two study groups as severe heart failure evolved.

2.4. Echocardiographic assessment of ventricular remodeling

Methods for the acquisition and analysis of sequential echocardiographic data have been described in detail elsewhere [30,33]. Data were acquired at baseline and weekly throughout the pacing period. Left ventricular volume and mass were calculated at end diastole using the formula for a hemispheric cylinder [30,34,35]. Left ventricular mean wall thickness was derived by taking the difference of the epicardial and endocardial short axis diastolic cross-sectional areas [30,35]. Left ventricular gloobularity was defined as the ratio of the chamber diastolic cross-sectional diameter ($D$) from the short axis view to the diastolic chamber length ($L$) from the long axis view [30]. Left atrial volume was derived from the formula: $(3/4) \times (\text{Area}) \times (\text{Length})$, where Area was the planimetered cross-sectional left atrial area obtained from the short axis view at the level of the aortic valve and Length was the longest anterior–posterior dimension of the left atrium obtained from the apical four-chamber view [36,37].

2.5. Plasma neurohormonal parameters

The plasma atrial natriuretic peptide (ANP) level was determined on plasma extracted from C$_{18}$ Sep-Pak columns (Waters, Milford, MA, USA) using a radioimmunoassay method. The plasma norepinephrine level was determined by a high-performance liquid chromatography method. Both assay methods have been reported in detail previously [37].

2.6. Statistical analysis

Data are expressed as mean value±SD except in the figures where the data are expressed as mean value±SEM. The effects of LU 135252 or placebo on hemodynamic, echocardiographic and neurohormonal data within the study group were analysed by repeated measures analysis of variance and the effects of LU 135252 and placebo were compared by analysis of repeated measures variance with two factor design followed by post-hoc comparison using Tukey’s protected $T$-test (GB-STATTM, Dynamic Microsystems Inc, Silver Spring, MD, USA). A probability value of <0.05 was considered to be statistically significant.

3. Results

3.1. Endothelin-1 challenge study

As shown in Fig. 1, administration of ET-1 induced a sustained increase in mean arterial pressure in the Beagle dogs. When ET-1 was readministered two days later, pretreatment with oral LU 135252 induced an initial decline in mean arterial pressure as well as completely inhibiting the ET-1-induced increase in blood pressure that was observed two days earlier.

3.2. Development of heart failure

After three weeks of continuous right ventricular pacing, both the placebo- and LU 135252-treated mongrel dogs developed severe heart failure, which was characterized by the development of lethargy, anorexia and ascites and accompanied by radiographic evidence of cardiomegaly and pulmonary congestion.

3.3. Hemodynamic parameters

The hemodynamic parameters of the placebo- and LU 135252-treated dogs obtained at baseline and after two weeks of pacing, obtained during temporary cessation of pacing, are shown in Table 1 and the four panels of Fig. 2. There were no statistically significant differences between the two groups in any of the hemodynamic parameters at

baseline. In the placebo-treated dogs, the intrinsic heart rate increased markedly after two weeks of pacing. Systolic arterial pressure declined from 153 ± 26 mmHg at baseline to 126 ± 27 mmHg (p < 0.01) at two weeks. Mean arterial pressure, left ventricular dP/dt and −dP/dt declined significantly, whereas both systemic and pulmonary vascular resistance increased markedly. Right atrial pressure, pulmonary artery pressure and left ventricular end diastolic pressure all increased markedly, whereas cardiac output declined. In the LU 135252-treated dogs, after two weeks of pacing, the intrinsic heart rate increased to a slightly greater degree than in the placebo-treated dogs, but the difference was not significant. Systolic arterial pressure declined from 147 ± 19 mmHg at baseline to 120 ± 22 mmHg (p < 0.001) at two weeks. Mean arterial pressure, dP/dt and −dP/dt also declined to a similar extent as in the placebo dogs. In contrast to the placebo dogs, the LU 135252-treated dogs had no significant increase in either systemic or pulmonary vascular resistance. In the LU 135252-treated dogs, there were significantly smaller increases in right atrial pressure, mean pulmonary artery and left ventricular end diastolic pressure, and a smaller decline

### Table 1
Hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>MAP</th>
<th>RAP</th>
<th>CO</th>
<th>dP/dt</th>
<th>−dP/dt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>beats/min</td>
<td>mmHg</td>
<td>mmHg</td>
<td>l/min</td>
<td>mmHg/s</td>
<td>mmHg/s</td>
</tr>
<tr>
<td><strong>Placebo (n=9)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>79 ± 16</td>
<td>118 ± 21</td>
<td>6.3 ± 1.9</td>
<td>3.5 ± 0.7</td>
<td>2043 ± 346</td>
<td>2019 ± 176</td>
</tr>
<tr>
<td>Week 2</td>
<td>127 ± 20*</td>
<td>105 ± 20*</td>
<td>11.4 ± 2.4</td>
<td>1.9 ± 0.6*</td>
<td>1071 ± 247*</td>
<td>1006 ± 152*</td>
</tr>
<tr>
<td><strong>LU 135252 (n=8)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>68 ± 16</td>
<td>116 ± 13</td>
<td>6.9 ± 0.8</td>
<td>3.2 ± 0.3</td>
<td>1827 ± 267</td>
<td>1782 ± 247</td>
</tr>
<tr>
<td>Week 2</td>
<td>137 ± 27*</td>
<td>101 ± 18*</td>
<td>9.7 ± 2.8*</td>
<td>2.6 ± 0.8*</td>
<td>1148 ± 230*</td>
<td>1033 ± 224*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD. HR, heart rate; MAP, mean arterial pressure; RAP, right atrial pressure; CO, cardiac output.

* p < 0.05, ‡ p < 0.001 versus baseline; § p < 0.05 versus placebo.
in cardiac output compared to the placebo dogs, reflecting the treatment effects of LU 135252 on these parameters. Finally, although LU 135252 had an effect on both systemic and pulmonary vascular resistance, the impact on pulmonary vascular resistance was greater than that on systemic vascular resistance.

3.4. Echocardiographic measures of cardiac remodeling

The echocardiographic parameters of both groups are shown in Table 2. The data at baseline of the two groups are comparable. In both study groups, the left ventricular ejection fraction declined whereas the left ventricular end diastolic volume and left atrial volume both increased markedly. The diameter/length ratio (D/L) increased progressively, indicating increasing globularity, whereas left ventricular mass and derived mean wall thickness did not change significantly, suggesting the absence of significant hypertrophy at the whole-organ level. The changes in echocardiographic parameters over three weeks in both study groups were similar, indicating that LU 135252 had no significant impact on left ventricular diastolic chamber size, shape or mass.

3.5. Neurohormonal parameters

Changes in arterial plasma ANP and norepinephrine levels are shown in Fig. 3. Baseline data were comparable in both groups. In both the placebo- and LU 135252-treated dogs, the plasma ANP level increased significantly after three days of pacing, increased further and plateaued after one week of pacing. Plasma norepinephrine increased gradually as severe heart failure developed over the three

![PLASMA ATRIAL NATRIURETIC PEPTIDE LEVEL](image1)

![PLASMA NOREPINEPHRINE LEVEL](image2)

**Fig. 3. Neurohormonal parameters (upper and lower panels).** Data are expressed as means±SEM. *p<0.01, **p<0.001 versus baseline, §p<0.05 versus placebo.

<table>
<thead>
<tr>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echocardiographic parameters</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Placebo</td>
</tr>
<tr>
<td>(n=9)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Week 1</td>
</tr>
<tr>
<td>Week 2</td>
</tr>
<tr>
<td>Week 3</td>
</tr>
</tbody>
</table>

| LU 135252| %    | ml/kg |     | g/kg  | mm/kg | ml/kg |
| n=8      |      |       |     |       |      |      |
| Baseline | 49±4 | 3.0±0.6 | 0.71±0.09 | 2.8±0.3 | 0.36±0.05 | 1.1±0.2 |
| Week 1   | 34±5* | 4.3±0.7* | 0.78±0.06 | 3.1±0.4 | 0.33±0.04 | 2.0±0.4* |
| Week 2   | 26±4* | 4.4±0.5* | 0.83±0.07 | 3.0±0.4 | 0.31±0.04 | 2.3±0.2* |
| Week 3   | 24±2* | 4.5±0.5* | 0.84±0.10* | 3.2±0.4 | 0.32±0.05 | 2.5±0.4* |

Data are expressed as means±SD.

LVEF, left ventricular ejection fraction; LVV, LV end diastolic volume; D/L, LV cross-sectional diameter/length ratio, an index of globularity; MASS and H, LV mass and mean wall thickness, respectively; LAV, left atrial volume.

* p<0.05, ** p<0.01 versus baseline.
weeks. Compared to the placebo group, there was a trend towards a smaller increase in plasma ANP levels in the LU 135252-treated group, although the between-group differences were not significant. On the other hand, the active-treatment group had a significantly smaller increase in plasma norpinephrine level compared to the placebo group.

4. Discussion

This investigation examined for the first time the effects of long-term ET<sub>A</sub> receptor blockade using a canine model that closely mimics human cardiomyopathy and using an experimental design and study parameters that are commonly employed in clinical trials. The following are our key findings. First, ET<sub>A</sub> receptor blockade significantly attenuated the increase in pulmonary artery and left ventricular end diastolic pressure, the decline in cardiac output and the increase in both systemic and pulmonary vascular resistance. Second, chronic ET<sub>A</sub> receptor blockade does not appear to have significant effects on left ventricular chamber dimension, shape or mass, parameters that are commonly used in clinical trials to assess cardiac remodeling. Third, unlike certain afterload reducing agents used clinically, the vasodilator effects of ET<sub>A</sub> blockade are not accompanied by an increase in plasma norepinephrine levels.

4.1. Adequacy of ET<sub>A</sub> blockade

Administration of 50 mg/kg orally of LU 135252 completely abolished the pressor response to exogenous ET-1. In addition, exogenous ET-1 in the presence of LU 135252 produced a slight initial decline in arterial pressure, suggesting an unmasking of ET<sub>B</sub> receptor-mediated vasodilation.

4.2. Hemodynamic effects of chronic ET<sub>A</sub> receptor blockade

Consistent with our previous reports in this model [23,24,29], chronic pacing resulted in a marked elevation in pulmonary artery, right- and left-sided cardiac filling pressures, a significant decline in cardiac output and a marked increase in both systemic and pulmonary vascular resistance. LU 135252 ameliorated the elevation in pulmonary artery and left ventricular end diastolic pressures.

This was accompanied by an improvement in cardiac output. The marked increase in systemic and pulmonary vascular resistance observed in the placebo dogs was attenuated in the LU 135252-treated dogs. Indeed, the impact of LU 135252 on pulmonary vascular resistance appears to be more pronounced than that on systemic vascular resistance, an effect that is distinct from that of conventional afterload reducing agents. Given the almost comparable increase in intrinsic heart rate in the two groups, the improvement in cardiac output produced by ET<sub>A</sub> receptor blockade was mediated predominantly by improved stroke volume. ET<sub>A</sub> receptor blockade did not improve dP/dt. This hemodynamic profile of LU 135252 therefore suggests that the agent may act predominantly as a systemic as well as a pulmonary vasodilator by blocking the pressor effects of increased endogenous ET-1 level, which has been documented in several pacing models of cardiomyopathy [12,21]. The marked lowering of pulmonary artery pressure and the near normalization of systemic and pulmonary vascular resistance, without the lowering of systolic arterial pressure or dP/dt, are hemodynamic effects that are desirable when considered for use in patients with heart failure.

Most studies to date which reported on the hemodynamic effects of either the specific ET<sub>A</sub> or mixed ET<sub>A</sub> and ET<sub>B</sub> receptor antagonists in heart failure have mostly involved the acute administration of the antagonists [17,18,38,39]. Only a limited number of studies have assessed the hemodynamic effects of long-term endothelin receptor blockade in heart failure. All of these studies that involved chronic ET<sub>A</sub> receptor blockade have used small animal models [19–21]. In rats with coronary artery ligation [19], infusion of the selective ET<sub>A</sub> receptor antagonist, BQ 123, on the day after the infarction resulted in lower right ventricular systolic and diastolic pressures and central venous pressure, but had no effects on mean arterial pressure, left ventricular end diastolic pressure, or dP/dt. Cardiac output was not reported in this study. While their finding of reduced right-sided cardiac filling pressures compliments our observations of reduced pulmonary artery pressure, the lack of an effect on left ventricular end diastolic pressure in their study contrasts with our observations. It is possible that following myocardial infarction, complex processes, such as tissue inflammation, necrosis and collagen formation, are actively underway, and ET-1 may play a pathogenetic role in one or more of these processes [40], thereby confounding the interpretation of the effects of endothelin receptor blockade. In a second study by the same investigators using the same model but with ET<sub>A</sub> blockade initiated at ten days after the infarction, left ventricular end diastolic pressure was significantly reduced [20]. In a rabbit model of pacing-induced heart failure [21], treatment with an ET<sub>A</sub> receptor antagonist was initiated before the onset of pacing. The animals were studied after three weeks of pacing. The only in vivo hemodynamic parameters reported were mean arterial and pulse pressures, which were both higher those of vehicle-treated rabbits. Cardiac output and cardiac filling pressures were not measured. It is therefore difficult to compare this study with ours in terms of in vivo hemodynamic parameters.

4.3. Effects of ET<sub>A</sub> receptor blockade on left ventricular remodeling

Consistent with our previous reports [24,25,30], pacing in the dog resulted in a markedly increased left ventricular
volume and globularity, left atrial volume, and a marked decline in the left ventricular ejection fraction. Although there was a trend towards increased mass and reduced mean wall thickness, these differences were not significant. Although one cannot rule out a type-II error for a modest increase in mass, cardiac hypertrophy at a whole-organ level is likely to be modest, a finding also observed in other pacing models [21]. The similar evolution of changes in echocardiographic parameters in the placebo- and actively treated animals suggests that, in this model, chronic ET$_\alpha$ receptor blockade has no significant impact on left ventricular remodeling, at least using measures that are commonly employed in clinical trials. The lack of a significant improvement in ejection fraction and a reduction in left ventricular diastolic volume was unexpected, given the marked improvement in cardiac output and the reduction in filling pressures in the treated animals. The reasons for these discrepant findings are unclear. It is possible that the unloading effects of LU 135252, as administered in the current protocol, was not sufficient to produce changes in cardiac dimensions that are detectable by echocardiography. It is interesting to note that, in the rabbits, chronic ET$_\alpha$ receptor blockade also produced only a small decrease in echocardiographically derived left ventricular end diastolic dimensions [22]. Like our dog model, pacing also did not increase left ventricular mass in the control or treated rabbits. Left ventricular fractional shortening was slightly greater in the treated rabbits. However, the contractile performance of isolated myocytes was completely restored in the treated rabbits, whereas myocyte dimensions were not altered to any great extent. One may therefore postulate that while ET$_\alpha$ receptor blockade may improve isolated myocyte function, this benefit may not necessarily be manifested as a major improvement in the systolic performance at the whole-organ level.

4.4. Effects of chronic ET$_\alpha$ receptor blockade on neurohormonal activation

The time course of the changes in plasma ANP and norepinephrine levels are consistent with our previous reports [37,41]. The lack of a significant lowering effect of chronic ET$_\alpha$ blockade on plasma ANP levels was unexpected, given the substantial lowering of cardiac filling pressures associated with ET$_\alpha$ blockade, as well as the known stimulatory action of ET-1 on the secretion of ANP [42], an effect that is presumably mediated by an ET$_\alpha$ receptor [43]. It is possible that the ET-1-induced ANP release is predominantly an in vitro phenomenon. The lack of a significant decrease in left atrial volume by ET$_\alpha$ receptor blockade suggests that there had not been sufficient relief of atrial stretch, the major stimulus for the release of ANP [44], even though atrial pressures were reduced significantly. The lack of a further increase in plasma norepinephrine levels by ET$_\alpha$ receptor blockade despite vasodilation may be related to the improvement in hemodynamic status and, therefore, an improvement in baroreflex function [45]. Given the ongoing concern regarding the relation of neurohormonal activation and increased mortality, raised by the results of clinical trials of vasodilator therapy in heart failure [46], the lack of a further increase in plasma norepinephrine levels associated with LU 135252 suggests that ET$_\alpha$ receptor blockade may be better tolerated than other vasodilators in patients with heart failure.

4.5. Study limitations

Several aspects of our study warrant further discussion. First, the hemodynamic effects of ET$_\alpha$ blockade was not accompanied by any significant changes in cardiac chamber dimensions. The heart failure induced in this model may have been too severe for ET$_\alpha$ receptor blockade to be sufficient to reverse the cardiac remodeling process. Indeed, it is unclear whether or not we would have observed hemodynamic benefit if treatment with LU 135252 was initiated in advanced stages of heart failure. Finally, we have limited our assessment to the effects of selective ET$_\alpha$ blockade. Since ET$_\beta$ receptors can mediate significant vasoconstrictor, in addition to vasodilator, effects [8,11], the response to chronic ET$_\beta$ receptor blockade may be different. To date, no studies have compared the effects of long-term ET$_\alpha$ with ET$_\beta$ or with mixed ET$_\alpha$ and ET$_\beta$ receptor blockade using the same model.

5. Conclusions

Using a large animal model of heart failure, which closely resembles human dilated cardiomyopathy, we have demonstrated that the administration of an ET$_\alpha$ receptor antagonist is associated with favourable hemodynamic effects. Unlike some direct-acting vasodilators, these beneficial hemodynamics effects are not accompanied by aggravation of neurohormonal activation. The results of our study support an important pathophysiologic role of ET-1 in chronic heart failure. Further studies that compare chronic blockade of the receptor subtypes will provide more insights into the role of ET-1 as a potentially new therapeutic modality in heart failure.

Acknowledgements

The authors wish to thank Mary Joe McDonald and Shelley Belford for technical assistance. We also wish to thank Medtronic of Canada for providing the pacing device. This work was supported by an operating grant from the Medical Research Council of Canada.
References


