The role of endothelin-1 in mediating changes in cardiac sympathetic nerve activity in heart failure

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Brief title: Endothelin-1 and CSNA

Total word count: 2365 (excl. abstract, fig legends, references)

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Keywords: heart failure, endothelin-1 receptor blockade, tezosentan, cardiac sympathetic nerve activity
Abstract

Heart Failure (HF) is associated with increased sympathetic nerve activity to the heart (CSNA), which is directly linked to mortality in HF patients. Previous studies indicate that HF is associated with high levels of plasma endothelin-1 (ET-1), which correlates with the severity of the disease. We hypothesized that blockade of endothelin receptors would decrease CSNA. The effects of intravenous tezosentan (a non-selective $\text{ET}_A$ and $\text{ET}_B$ receptor antagonist) (8 mg/kg/hr) on resting levels of CSNA, arterial pressure and heart rate were determined in conscious normal sheep (n= 6) and sheep with pacing induced HF (n= 7). HF was associated with a significant decrease in ejection fraction (from 74 ± 2% to 38 ± 1%, $P < 0.001$) and a significant increase in resting levels of CSNA burst incidence (from 56 ± 11 to 87 ± 2 bursts / 100 heart beats, $P < 0.01$). Infusion of tezosentan for 60 minutes significantly decreased resting MAP in both normal and HF sheep (-8 ± 4 mmHg and -4 ± 3 mmHg respectively; $p<0.05$). This was associated with a significant decrease in CSNA (by 25 ± 26% of control) in normal sheep, but there was no change in CSNA in HF sheep. Calculation of spontaneous baroreflex gain indicated significant impairment of the baroreflex control of HR after intravenous tezosentan infusion in normal animals, but no change in HF animals. These data suggest that endogenous levels of ET-1 contribute to the baseline levels of CSNA in normal animals, but this effect is absent in HF.
Introduction

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide hormone, produced predominantly by vascular tissue (12), that acts on vascular ET-1 receptors to increase blood pressure in normal animals (4). In addition to the direct vasoconstrictor actions of ET-1, there is good evidence that ET-1 can increase blood pressure via sympathoexcitatory actions (14). Indeed, ET-1 receptors have been observed in central cardiovascular brain regions such as the area postrema (AP), subfornical organ and hypothalamic areas (12). In support of a central role for ET-1, intravenous infusion of ET-1 excites the majority of neurons in the area postrema (8) and the subfornical organ (40). More importantly, injection of ET-1 into either the area postrema (7) or the subfornical organ (40) of urethane-anaesthetised rats increased arterial blood pressure indicating the pressor effects of ET-1 can be mediated by central actions.

In heart failure (HF), ventricular impairment leads to reduced organ perfusion that compromises organ function (27). One of the central features of HF is neurohormonal activation, including increased levels of ET-1 (10, 31) as well as a differential increase in sympathetic nerve activity to various organs (34). Experimental animal and clinical evidence suggests that overactivity of the sympathetic nervous system (17, 37), and increased levels of ET-1, are directly correlated with progression of the disease (6). Recent clinical trials indicate that administration of tezosentan had no additional benefit in patients with acute heart failure (22, 26). However, more than half of the patients in both of these trials were also being treated with ACE inhibitors or angiotensin receptor antagonists, making interpretation of the effects of ET-1 on sympathetic drive difficult. In addition, it is
impossible to ascertain if blockade of ET receptors results in alterations in CSNA in clinical studies. Given that the increase in cardiac sympathetic nerve activity (CSNA) during the progression of HF leads to arrhythmias and sudden death (34, 42), this remains an important unanswered question.

In this study we investigated whether baseline circulating levels of ET-1 contribute to the resting levels of CSNA in normal animals. We also tested the hypothesis that the high circulating levels of ET-1 in HF drive the increase in CSNA, by examining whether infusion of tezosentan (a non-selective $\text{ET}_A$ and $\text{ET}_B$ receptor antagonist) reduced the increased CSNA in ovine HF.
Methods

Studies were carried out in conscious adult (30-40kg) Merino ewes. All procedures were approved by the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee (AEC No. 07-097) and conformed to the National Health & Medical Research Council’s Australian code of practice for the care and use of animals for scientific purposes. The sheep were fed 800 g/day oaten chaff, and had access to water *ad libitum*.

Surgical Procedures

Briefly, each animal underwent two surgical procedures under general anaesthesia prior to experimentation (30). Anaesthesia was induced with 5% sodium pentothal (0.4 mg/kg, IV) and maintained with a 2% isoflurane/air/O₂ mixture. The first procedure was to construct a carotid artery loop and to insert a pacing lead into the right ventricle. The control groups only had the carotid artery loop constructed.

After the first surgery, sheep were given >7 days to recover from the pacing lead implantation before baseline ejection fraction was measured with echocardiography. Thereafter, pacing of the heart commenced from a normal resting heart rate of ~70 bpm to 200 - 220 bpm. Echocardiograph measurements were performed weekly. Once ejection fraction fell below 40%, sheep were considered to be in HF. Generally, the sheep reached HF status after 8-12 weeks of pacing.

When HF had developed, or at least 2 weeks after the first surgery for the control group, recording electrodes were inserted into the cardiothoracic sympathetic nerves.
Sheep in HF were anaesthetised with isoflurane alone since they are more susceptible to anaesthetic death. Sheep were given antibiotic injections (15mg/kg, IM; Ilium Propercillin, Troy Laboratories, N.S.W., Australia) at the start of the surgery and daily for 2 days after surgery. Additionally, to provide analgesia sheep were given flunixin meglumine (1 mg/kg IM; Troy Laboratories) at the start of surgery and 4 and 16 h post-surgery.

The carotid artery and jugular vein were cannulated to measure arterial blood pressure and for drug infusion, respectively, one day before experimentation. Experiments were started at least 3 days after implantation of nerve recording electrodes, with the cardiac pacing switched off. In conscious standing sheep a 30 min baseline recording of arterial pressure, heart rate and CSNA was obtained. Tezosentan (8 mg/kg/hr at 30 ml/hr) was then infused intravenously for 60 min. The dose and time period of tezosentan infusion were chosen based on pilot studies that demonstrated that an infusion of 8 mg/kg/h completely blocked the pressor response (9 ± 3 mmHg) to an i.v. bolus of ET-1 (1200pmol in 1 mL saline). Furthermore, there was no further decrease in MAP or CSNA when the infusion was continued for a further hour. Tezosentan was a generous gift from Actelion Pharmaceuticals.

Analysis of cardiac sympathetic nerve activity

CSNA was recorded from the pair of electrodes with the best signal-to-noise ratio (19, 42). The nerve signal was displayed on an oscilloscope, amplified (x 20,000) and filtered (band pass 300-1000 Hz). The signal was recorded on a desktop computer with a CED micro 1401 interface and a data acquisition program (Spike 2). The signals were full-wave rectified and integrated with a low-pass time constant of
20 ms. The rectified and integrated area that was between the diastolic pressures of
the smallest burst was chosen as the minimum area of a burst. Consequently, a
burst was determined when the rectified and integrated area between diastolic
pressures was higher than the minimum area. Burst incidence (bursts per 100 heart
beats), burst frequency (bursts per minute), burst amplitude (average area under the
curve for each burst) and the mean level of sympathetic nerve activity (product of the
burst incidence and burst amplitude) were determined. For amplitude and total
CSNA, the control periods (-15 and 0 min) were chosen to be 100%, and changes
within the drug infusion period were expressed as a percentage of those periods.

Spontaneous baroreflex analysis

For construction of spontaneous baroreflex curves, the systolic and diastolic blood
pressures, heart rate values and CSNA burst incidence values for 10 minutes of
baseline and the last 10 minutes post tezosentan infusion were used. The baseline
blood pressures were sorted from the lowest to the highest pressures and put into
bins of 10 beats each. The mean systolic blood pressure of each bin was plotted
against the mean heart rate. The mean diastolic blood pressures of each bin were
plotted against the mean CSNA burst incidence.

Statistical Analysis

All data are expressed as mean ± SEM, except where indicated. Unpaired Students
t-tests were used to determine the effects of HF on the baseline levels of MAP, HR
and CSNA. For analysis of the effect of time and group on hemodynamic parameters
(MAP, HR and CSNA) in HF and normal sheep, an ANOVA design was utilised. The
sum of squares was completely partitioned to account for all of the variability in the
data. Data were analysed using the statistical package Statistica (Version 10,
StatSoft Inc.). Data were considered significant if P < 0.05.
Results

Comparison of normal and HF groups

During the 8 -12 weeks of right ventricular pacing, weekly echocardiogram recordings in conscious paced sheep revealed a gradual decline in ejection fraction and fractional shortening. At the end of the pacing period, ejection fraction and fractional shortening were significantly lower in the HF animals compared with pre-pacing values (P < 0.001) (Table 1).

Mean arterial pressure (MAP) was significantly lower in the HF sheep compared with normal sheep (84 ± 2 vs. 99 ± 4 mmHg, P < 0.05) (Figures. 1A, 2A and 3), while diastolic blood pressures between normal and HF animals were not different. Mean burst incidence of CSNA was significantly higher in the HF compared with the normal animals (Fig. 3).

Effects of intravenous tezosentan infusion in the normal and HF groups

In normal sheep, intravenous infusion of tezosentan (8 mg/kg/h) for 60 min significantly reduced MAP by 8 ± 4 mmHg (P<0.001) and increased HR by 8 ± 3 beats/min (Figures 1 and 3). The decrease in arterial pressure was associated with a reduction in CSNA burst incidence (-13 ± 4 bursts/100 heartbeats; P<0.05) (Figure 3) and CSNA burst amplitude (to 91 ± 5 % of baseline; p<0.05). In sheep in HF, tezosentan significantly decreased MAP by 4 ± 3 mmHg (P < 0.05; Figure 3), but did not change HR (Figures 2 and 3). In contrast to the normal animals, tezosentan caused no change in CSNA burst incidence (Figure 3) or CSNA burst amplitude.
Spontaneous baroreflex

There was a significant reduction in the slope of the systolic BP-HR relationship in the HF compared with the normal group (Figure 4). Tezosentan infusion resulted in a significant blunting of the slope in the normal animals, but there was no change in the HF animals. There was no difference in the slope of the diastolic BP-CSNA relationship between the normal and the HF animals (Figure 4). Blockade of ET-1 receptors did not change the slope of the baroreflex control of CSNA in the normal animals or the animals with HF.
Discussion

The level of CSNA in sheep with HF was higher than in normal sheep, consistent with our previous results and clinical studies measuring cardiac norepinephrine spillover in HF patients. Non-selective blockade of $\text{ET}_A$ and $\text{ET}_B$ receptors, with an intravenous infusion of tezosentan, cause small decreases in MAP in both normal and HF sheep. Tezosentan significantly decreased CSNA in normal sheep, but not in sheep with HF. Our findings using direct recordings indicate that ET-1 plays an important role in regulating the level of CSNA in normal animals, but this effect on CSNA was absent in HF animals.

Effect of intravenous tezosentan on systemic hemodynamics and cardiac sympathetic nerve activity in normal sheep

Previous studies have demonstrated that in anaesthetized rats intravenous infusion of ET-1 increased blood pressure (44) and blockade of $\text{ET}_A$ receptors decreased systolic blood pressure (2). In another study, infusion of bosentan, a dual $\text{ET}_A$ and $\text{ET}_B$ receptor antagonist, decreased diastolic blood pressure in patients with essential hypertension (14). Together these studies support a tonic vasoconstrictor role for ET-1. Similarly, in normal sheep, ET-1 is a potent vasoconstrictor (20) and in this study -tezosentan, a non-selective $\text{ET}_A$ and $\text{ET}_B$ receptor, decreased MAP.

Our study using direct recordings of CSNA indicate that non-selective blockade of $\text{ET}_A$ and $\text{ET}_B$ receptors decreased CSNA. These results suggest that baseline endogenous levels of ET-1 tonically stimulates the baseline level of CSNA in normal animals. In terms of the site of action of ET-1, previous studies have reported the presence of ET-1 receptors in many brain regions, including regions that are considered important in the regulation of the cardiovascular system such as the area
Importantly the area postrema and SFO are circumventricular organs and can be accessed by ET-1 and tezosentan, which do not cross the blood-brain barrier. In this context, intravenous infusion of ET-1 excites neurons in the area postrema (8) and injection of ET-1 into the area postrema (7) of urethane-anaesthetised rats increased arterial blood pressure indicating the pressor effects of ET-1 can be mediated by central actions of ET-1 on the area postrema. ET receptors are also present in the subfornical organ and injection of ET-1 into the subfornical organ can also increase blood pressure (40). Taken together these results suggest that the actions of tezosentan on CSNA may be mediated by its actions on ET receptors in the circumventricular organs.

Effect of intravenous tezosentan on systemic hemodynamics and cardiac sympathetic nerve activity in heart failure sheep

The levels of ET-1 are elevated in HF patients and animal models of HF (6, 13, 18) and there is evidence that the increase in ET-1 levels contributes to the sympathoexcitation in HF. Inhibition of the ET-1 receptor in dogs with pacing-induced HF reduced plasma NE levels compared with placebo infusion (21). Interestingly, infusion of an ET-1 receptor antagonist reduced directly recorded RSNA in pacing-induced HF in rabbits (16) and in the myocardial infarction model of HF in rats (38). These results led to clinical evaluation of blockade of ET receptors in patients with heart failure. In the case of tezosentan, the clinical trials indicated that administration had no additional benefit in patients with acute heart failure (22, 26). Interpretation of clinical trials can be difficult given that more than half of the patients in both of these trials were on concomitant ACE inhibitors or angiotensin receptor antagonists.
Consistent with previous studies in humans and animal models of HF (9, 33), intravenous infusion of tezosentan decreased MAP in sheep with HF. However, contrary to our hypothesis, there was no change in the high levels of CSNA in animals with HF. These data indicate that circulating ET-1 does not have a role in driving the increased CSNA in HF. Our findings, made in the absence of other medications, indicate that in HF circulating ET does not mediate the high level of CSNA and offer one explanation for lack of a beneficial effect of tezosentan in clinical trials. It must be noted that other studies have indicated a role for ET in mediating the high levels of RSNA in HF, which is consistent with our previous studies showing that different mechanisms drive CSNA and RSNA in HF (3, 5, 35, 39, 43).

Our data regarding spontaneous baroreflex curves indicates a blunted arterial baroreflex control of HR during HF compared with normal animals, consistent with our previous results using a modified Oxford technique (29, 41). In contrast, the baroreflex control of CSNA was not altered, which is also consistent with our previous data in ovine HF (41). The present findings indicate an important role for ET-1 in mediating the arterial baroreflex control of heart rate in normal animals. Previous studies have indicated that blockade of ET-1 is associated with depressed control of heart rate in normal animals (32). There is good evidence that ET receptors are present at multiple loci that affect baroreflex control including the carotid sinus, NTS, area postrema and RVLM (24, 28). Our data appear consistent with the possibility that ET can modulate baroreflex function in normal animals, but this ability appears to be absent during HF.

Possible roles of Endothelin-1 in Heart Failure
ET-1 does not appear to play an important role in setting the elevated level of CSNA in ovine HF, in contrast to its role in maintaining CSNA in the normal state. The reason for this reduced sympathoexcitatory effect of ET-1 in HF is unclear, but could be due to down regulation of ET-1 receptor levels in central cardiovascular regions in HF. To the best of our knowledge, no studies have investigated ET-1 receptor density in the brain during HF. In the periphery there is evidence for both increases and decreases in ET receptor levels. In the aorta and pulmonary arteries of patients with heart disease, ETA receptor density was reduced (15), whereas the density of myocardial ET-1 receptors was increased in a rat model of HF (23).

Conclusion

Blockade of ET-1 receptors with tezosentan decreased MAP in both normal and HF sheep. Despite this fall in arterial pressure, there was no reflex increase in directly recorded CSNA in sheep with HF while there was a decrease in CSNA in normal sheep. These data suggest that endogenous levels of ET-1 contribute to the baseline levels of CSNA in normal animals, but this effect is absent in HF.

Perspectives and Significance

HF is associated with a large increase in cardiac sympathetic nerve activity (CSNA) that has been shown to lead to arrhythmias and sudden death. Our findings indicate an important role for ET-1 in maintaining baseline levels of CSNA in normal animals. In contrast to normal animals, the lack of effect of tezosentan on CSNA in HF suggests that treatment with ET receptor antagonists will not reduce the detrimental, high level of sympathetic nerve activity in HF. These findings, made in the absence of other medications, offer one explanation for the lack of a beneficial effect of
tezosentan in clinical trials of HF. The site of action of ET-1 to modulate CSNA in the normal animals remains unknown and future studies that examine putative changes in endothelin receptors in the brain are clearly needed.
References


Acknowledgements

The authors acknowledge the expert technical assistance of Alan McDonald and Tony Dornom. Tezosentan was a generous gift from Dr. Mark Iglarz of Actelion Pharmaceuticals.

Grants

This work was supported by National Health and Medical Research Council of Australia Grant 628573, and the Victorian Government's Operational Infrastructure Support Program. R. Ramchandra was the recipient of National Health and Medical Research Council / National Heart Foundation Postdoctoral Fellowship 07M 3293, and C. N. May was supported by a National Health and Medical Research Council Research Fellowship 566819.

Disclosures

No conflicts of interest are declared by the author(s).
Figure Legends

Figure 1. Representative arterial blood pressure and cardiac sympathetic nerve activity (CSNA) in a conscious normal sheep. Direct recordings show arterial pressure (top panels) and burst incidence (bottom panels) before and after 60 min of IV tezosentan infusion.

Figure 2. Representative arterial blood pressure and cardiac sympathetic nerve activity (CSNA) in a conscious sheep in heart failure. Direct recordings show arterial pressure (top panels) and burst incidence (bottom panels) before and after 60 min of IV tezosentan infusion.

Figure 3. Effect of intravenous tezosentan on hemodynamic parameters and CSNA in conscious normal and heart failure sheep. Changes in (A) Mean arterial pressure (MAP), (B) heart rate (HR) and (C) total cardiac sympathetic nerve activity (CSNA) before (0 min) and after 60 min of tezosentan (8 mg/kg, IV). * P < 0.05; ** P < 0.001, different from baseline (0 min). Bars represent mean values. Individual sheep responses are illustrated with symbols.

Figure 4. Effect of intravenous tezosentan on spontaneous baroreflex control of heart rate (left panels) and CSNA (right panels) in conscious normal sheep (top panels) and sheep in heart failure (bottom panels). The left panels denote the average baroreflex gain before (solid line) and after 60 min of tezosentan (8 mg/kg, IV; dashed line) in the normal (top) and HF (bottom) animals.
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Table 1. Resting values for hemodynamic parameters between conscious normal and HF sheep.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Heart Failure</th>
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<tbody>
<tr>
<td>Ejection Fraction, %</td>
<td>74±2</td>
<td>38±1 **</td>
</tr>
<tr>
<td>Fractional Shortening, %</td>
<td>46±3</td>
<td>18±1 **</td>
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<td>Heart Rate, beats/min</td>
<td>80±9</td>
<td>81±6</td>
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<td>Diastolic BP, mmHg</td>
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<td>60±3</td>
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<tr>
<td>Systolic BP, mmHg</td>
<td>98±5</td>
<td>85±2 *</td>
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</tbody>
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BP, blood pressure. * P < 0.05, ** P < 0.001