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

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Abukar Y, May CN, Ramchandra R. Role of endothelin-1 in mediating changes in cardiac sympathetic nerve activity in heart failure. Am J Physiol Regul Integr Comp Physiol 310: R94–R99, 2016. First published October 14, 2015; doi:10.1152/ajpregu.00205.2015.—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 nonselective ETA and ETB receptor antagonist) (8 mg·kg−1·h−1) 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 heartbeats, P < 0.01). Infusion of tezosentan for 60 min significantly decreased resting mean arterial pressure (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.

METHODS

Studies were carried out in conscious adult (30–40 kg) 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 and 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 anesthesia before experimentation (30). Anesthesia was induced with 5% Pentothal Sodium (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 to 200–220 beats/min. 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 wk of pacing.

When HF had developed, or at least 2 wk after the first surgery for the control group, recording electrodes were inserted into the cardiothoracic sympathetic nerves (19, 42). Sheep in HF were...
anesthetized with isoflurane alone since they are more susceptible to anesthetic death. Sheep were given antibiotic injections (15 mg/kg im; Ilum Propercillen, Troy Laboratories, NSW, 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 postsurgery.

The carotid artery and jugular vein were cannulated to measure arterial blood pressure (MAP) and for drug infusion, respectively, 1 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 (HR), and CSNA was obtained. Tezosentan (8 mg·kg⁻¹·h⁻¹ at 30 ml/h) 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⁻¹·h⁻¹ completely blocked the pressor response (9 ± 3 mmHg) to an intravenous bolus of ET-1 (1,200 pmol in 1 ml saline). Furthermore, there was no further decrease in MAP or CSNA when the infusion was continued for an additional 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 (∗20,000), and filtered (band pass 300–1,000 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 heartbeats), 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.

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

Table 1. Resting values for hemodynamic parameters between conscious normal and heart failure 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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<tr>
<td>Heart rate, beats/min</td>
<td>80 ± 9</td>
<td>81 ± 6</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>70 ± 4</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>98 ± 5</td>
<td>85 ± 2*</td>
</tr>
</tbody>
</table>

BP, blood pressure. *P < 0.05, †P < 0.001.

Spontaneous baroreflex analysis. For construction of spontaneous baroreflex curves, the systolic and diastolic blood pressures, HR values, and CSNA burst incidence values for 10 min of baseline and the last 10 min of 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 HR. The mean diastolic blood pressures of each bin were plotted against the mean CSNA burst incidence.

Statistical analysis. All data are expressed as means ± SE, except where indicated. Unpaired Student’s 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 utilized. The sum of squares was completely partitioned to account for all of the variability in the data. Data were analyzed using the statistical package Statistica (Version 10, StatSoft). Data were considered significant if P < 0.05.

RESULTS

Comparison of normal and HF groups. During the 8–12 wk 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 prepping values (P < 0.001) (Table 1).
MAP was significantly lower in the HF sheep compared with normal sheep (84 ± 2 vs. 99 ± 4 mmHg, P < 0.05) (Figs. 1A, 2A, and 3), whereas 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⁻¹·h⁻¹) for 60 min significantly reduced MAP by 8 ± 4 mmHg (P < 0.001) and increased HR by 8 ± 3 beats/min (Figs. 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) (Fig. 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; Fig. 3) but did not change HR (Figs. 2 and 3). In contrast to the normal animals, tezosentan caused no change in CSNA burst incidence (Fig. 3) or CSNA burst amplitude.

**Spontaneous baroreflex.** There was a significant reduction in the slope of the systolic blood pressure (BP)-HR relationship in the HF compared with the normal group (Fig. 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 (Fig. 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. Nonselective blockade of ET_{A} and ET_{B} receptors, with an intravenous infusion of tezosentan, caused 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 anesthetized rats intravenous infusion of ET-1 increased BP (44) and blockade of ET_{A} receptors decreased systolic BP (2). In another study, infusion of bosentan, a dual ET_{A} and ET_{B} receptor antagonist, decreased diastolic BP 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 nonselective ET_{A} and ET_{B} receptor, decreased MAP.

Our study using direct recordings of CSNA indicate that nonselective blockade of ET_{A} and 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 postrema, SFO and paraventricular nucleus (1, 11, 12, 25, 36). 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-anesthetized rats increased arterial BP 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 SFO, and injection of ET-1 into the SFO can also increase BP (40).

![Fig. 2. Representative AP and CSNA in a conscious sheep in heart failure. Direct recordings show AP (top) and burst incidence (bottom) before and after 60 min of intravenous tezosentan infusion.](https://www.ajpregu.org)
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 renal sympathetic nerve activity (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 HF. In the case of tezosentan, the clinical trials indicated that administration had no additional benefit in patients with acute HF (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 HR in normal animals. Previous studies have indicated that blockade of ET-1 is associated with depressed control of HR in normal animals (32). There is good evidence that ET receptors are present at multiple loci that affect baroreflex control including the carotid sinus, nucleus tractus solitarius (NTS), area postrema, and rostral ventrolateral medulla (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 ET-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 downregulation 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).

In 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 since 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 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.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Y.A. and R.R. performed experiments; Y.A. and R.R. analyzed data; Y.A., C.N.M., and R.R. interpreted results of experiments; Y.A. and R.R. prepared figures; Y.A. and R.R. drafted manuscript; Y.A., C.N.M., and R.R. approved final version of manuscript; C.N.M. and R.R. conception and design of research; C.N.M. and R.R. edited and revised manuscript.
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