Endothelin stimulates an endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine, in experimental heart failure

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ABSTRACT

Congestive heart failure (CHF) is characterized by increased peripheral vascular resistance. Endothelin-1 (ET-1), a potent endothelium-derived vasoconstrictor, is present at increased concentrations in the plasma and contributes to the regulation of vascular tone in CHF. An endothelium-derived relaxing factor, nitric oxide (NO), also regulates vascular tone, but endothelium-dependent NO-mediated vasodilatation is blunted in CHF. An endogenous NO synthase inhibitor, asymmetric dimethylarginine (ADMA), which inhibits NO production and endothelium-dependent relaxation, is present at increased levels in the plasma and plays a role in impaired endothelial function in CHF. However, at present, the relationship between ET-1 and impaired vascular relaxation in CHF is not well known. We hypothesized that ET-1 inhibits NO-mediated vasodilatation via increased ADMA production in CHF, and that an endothelin receptor antagonist can prevent this increase in plasma ADMA levels. In the present study, we first examined whether circulating ADMA levels were increased in a dog model of CHF induced by 3 weeks of rapid ventricular pacing (n = 5; 270 beats/min) compared with normal dogs (n = 5). After 3 weeks of pacing, cardiac output had decreased significantly (1.56 ± 0.16 compared with 2.93 ± 0.25 litres/min; P < 0.01) and systemic vascular resistance had increased (4653 ± 374 compared with 3227 ± 396 dyn · s · cm⁻⁵; P < 0.01) in dogs with CHF compared with normal dogs. Plasma levels of both ET-1 (4.95 ± 0.83 compared with 2.12 ± 0.39 pg/ml; P < 0.05) and ADMA (3.27 ± 0.49 compared with 1.91 ± 0.25 nmol/ml; P < 0.05) were significantly increased in CHF dogs. A significant positive correlation was observed between plasma ET-1 and ADMA levels (r = 0.72, P < 0.05). Secondly, we chronically administered an ETA receptor antagonist, TA-0201 (0.3 mg/kg; n = 5), to paced CHF dogs. Drug administration started on day 8 of pacing and continued throughout the experiment. TA-0201 significantly increased cardiac output (2.58 ± 0.24 litres/min; P < 0.01) and suppressed the increases in plasma ADMA levels and systemic vascular resistance (2.36 ± 0.30 nmol/ml and 2423 ± 188 dyn · s · cm⁻⁵ respectively; P < 0.05 for each) compared with CHF dogs without TA-0201 treatment. In conclusion, ET-1 contributes to the regulation of vascular tone due, in part, to increased levels of an endogenous NO synthase inhibitor in CHF, and an ETA receptor antagonist can prevent the inhibition of NO production and the increased peripheral vascular resistance observed in CHF.

Key words: dog, endothelin, heart failure, nitric oxide, nitric oxide synthase.

Abbreviations: ADMA, asymmetric dimethylarginine; CHF, congestive heart failure; ET-1, endothelin-1; l-NMMA, N⁰-monomethyl-l-arginine; NOS, nitric oxide synthase; SVR, systemic vascular resistance.

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INTRODUCTION

Congestive heart failure (CHF) is characterized by increased peripheral vascular resistance [1]. The vascular endothelium orchestrates the control of vascular tone through the production of both vasoconstrictor and vasodilator substances, the most important being endothelin-1 (ET-1) and nitric oxide (NO) [2]. ET-1, a potent endothelium-derived vasoconstrictor [3], is increased in the plasma and contributes to the regulation of vascular tone in CHF [4–7]. NO, an endothelium-derived relaxing factor, also regulates vascular tone [8], but endothelium-dependent, NO-mediated vasodilation is blunted in CHF [9,10]. An endogenous NO synthase (NOS) inhibitor, \( \text{N}^\text{G},\text{N}^\text{G}\text{-dimethylarginine (asymmetric dimethylarginine; ADMA)} \), which inhibits NO production and endothelium-dependent relaxation, is increased in plasma [11] and may play a role in impaired endothelial function in CHF. However, at present, the relationship between ET-1 and impaired vascular relaxation in CHF is not well known.

In the present study, we examined whether circulating ADMA levels are increased in a dog model of CHF induced by 3 weeks of rapid ventricular pacing compared with normal dogs, and tested the hypothesis that an ET receptor antagonist can prevent an increase in plasma ADMA levels in CHF.

METHODS

Experiments were carried out using 15 conditioned beagles (10–13 kg). This study was approved by the Animal Research Committee of Shiga University of Medical Science. All surgery to implant the two cardiac pacemaker leads and intravascular catheters was performed as reported previously [12]. After more than 2 weeks of recovery from surgery, we randomly divided the dogs into three groups: (1) normal group; sham controls (n = 5); (2) CHF group; chronic rapid pacing at 270 beats/min for 3 weeks with placebo treatment (n = 5); (3) ETRA group; chronic rapid pacing and ET receptor antagonist treatment (n = 5). The ETRA group was given the ET\(_A\) receptor antagonist TA-0201 (0.3 mg/kg) orally once per day from day 8 until day 22 following the initiation of rapid right ventricular pacing. Mean arterial pressure and cardiac output were recorded with the dogs in a conscious state, as described previously [12].

Blood for the analysis of plasma ET-1 and ADMA levels was drawn from the pulmonary artery through the indwelling thermodilution catheter. ET-1 levels in the sampled blood were measured by RIA as described previously [4,12]. HPLC was used to measure ADMA levels using \( \text{o-phthalaldehyde for fluorescence determination} \), as described by Matsuoka et al. [13], with minor modifications. HPLC was performed on a Hitachi L-7480 system equipped with an F-1080 fluorescence detector for excitation at 348 nm, and emission at 450 nm, with a PEGASIL ODS (4.6 mm × 250 mm; Chemical Inspection & Testing Institute). Samples were eluted with 75 mmol/l aqueous sodium acetate buffer.

All data are presented as means ± S.E.M. Comparisons among the three groups were performed by ANOVA. Correlation analysis was by linear regression. A P value of < 0.05 was considered significant.

RESULTS

The results of haemodynamic and blood analyses are summarized in Table 1. After 3 weeks of pacing, mean arterial pressure and cardiac output were decreased significantly, and systemic vascular resistance (SVR) and plasma levels of ET-1 and ADMA were increased significantly, compared with normal dogs. As shown in Figure 1, a significant positive correlation was observed between plasma ET-1 and ADMA levels (r = 0.72,
DISCUSSION

Increased SVR in CHF results from both excessive vasoconstriction and impaired vasodilation. The former is associated with enhanced levels of neurohumoral factors such as ET-1. Acute administration of ET\(_A\) and ET\(_A/ET\(_B\) receptor antagonists results in decreases in blood pressure and SVR in CHF [6,7]. In the present study, chronic ET\(_A\) receptor antagonism also reduced SVR in paced dogs with CHF. Therefore ET-1 contributes to systemic vasoconstriction in CHF via ET\(_A\) receptor activation. The latter is due mainly to vascular endothelial dysfunction. In the coronary and femoral arteries, and at the level of the resistance vessel in chronically paced dogs, impaired endothelium-dependent but intact endothelium-independent vasodilation has been demonstrated [13–15]. In addition, the vasoconstriction observed after inhibition of NOS is attenuated [16,17], suggesting an impairment of basal endothelial function. Duerrschmidt et al. [18] recently reported that ET-1 augments superoxide anion generation in human endothelial cells. Therefore ET-1 may lead to endothelial dysfunction by this mechanism, as oxygen free radicals may inactivate NO [19].

It is clear that the impairment resides in the NO component. Plasma levels of ADMA are increased in patients with CHF [11] and in rats with CHF induced by coronary ligation [20]. In the present study, we also demonstrated that plasma ADMA levels were significantly increased in dogs with CHF caused by rapid pacing, and there was a significant relationship between plasma ET-1 and ADMA levels. ADMA, which is an endogenous inhibitor of the cellular uptake of l-arginine and its binding to endothelial NOS [21], may reduce the NO synthesis rate. Bogle et al. [22] demonstrated that endothelial cells transport the NOS inhibitor N\(^\text{\textsuperscript{\text{\textcircled{N}}}}\) mono-methyl-l-arginine (L-NMMA) by a carrier-mediated transporter that is shared by l-arginine and other endogenous dimethylarginines such as ADMA. L-Arginine transport via the cationic amino acid transport system \(y^+/\text{CAT}\) is up-regulated in erythrocytes [23] and peripheral blood mononuclear cells [24] from CHF patients. Azuma [25] reported that, in the rabbit carotid artery, an accumulation of the endogenous NOS inhibitors l-NMMA and ADMA in regenerated endothelial cells after endothelial denudation was accompanied by decreased NO generation and increased ET-1 content within the vessel wall, and that l-NMMA and ADMA inhibited NO generation and endothelium-mediated cGMP generation in a dose-dependent manner, which was inhibited by BQ123 (ET\(_A\) receptor antagonist) and ATZ1993 (ET\(_A/ET\(_B\) receptor antagonist), but not BQ788 (ET\(_B\) receptor antagonist) [25]. These results suggest that ET-1 may stimulate the uptake of L-NMMA and ADMA via ET\(_A\) receptor activation. In the present study, TA-0201, a selective ET\(_A\) receptor antagonist, significantly decreased plasma ADMA levels compared with those in untreated dogs with CHF, suggesting that ET-1 may contribute to endothelial dysfunction through ET\(_A\) receptors. Although ET-1 may be involved in this transmembrane transport mechanism, further investigation of this point is required.

In conclusion, ET-1 contributes to the regulation of vascular tone in CHF due, in part, to an increase in the levels of the endogenous NOS inhibitor ADMA, and an ET\(_A\) receptor antagonist can prevent the inhibition of NO production and the increased peripheral vascular resistance that are characteristic of CHF.

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REFERENCES