Effect of endothelin-1 on endothelium-derived vascular responsiveness in man

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ABSTRACT

1. Endothelium-dependent vasodilatation via nitric oxide in response to muscarinic stimulation is decreased in chronic heart failure while basal release of nitric oxide may be increased. As production of the endothelium-derived vasoconstrictor endothelin-1 is increased in chronic heart failure, endothelin-1 may act in an autocrine manner to modulate these effects.

2. To test this, we determined whether prolonged endothelin infusion in normal subjects would reproduce the alterations in basal and stimulated nitric oxide release observed in patients with chronic heart failure. Basal nitric oxide production was determined by measurement of forearm blood flow using strain gauge venous occlusion plethysmography before and after brachial artery infusion of a nitric oxide synthase inhibitor (\textit{N}G\textit{-}monomethyl-L-arginine). Stimulated nitric oxide production was determined by brachial artery infusion of acetylcholine. As metabolic vasodilatation is thought to be mediated in part via nitric oxide and is decreased in chronic heart failure, forearm blood flow during peak reactive hyperaemia was also measured. Studies were then repeated during brachial artery infusion of endothelin-1 and a non-specific vasoconstrictor, noradrenaline.

3. Neither basal nor stimulated nitric oxide production was altered by endothelin-1 and noradrenaline infusion. However, absolute forearm blood flow responses to peak reactive hyperaemia were decreased during infusion of endothelin-1 in comparison to noradrenaline. These data suggest that increased endothelin-1 may not contribute greatly to altered basal and stimulated nitric oxide production in patients with chronic heart failure but may contribute to impaired metabolic vasodilatation, by mechanisms presumably unrelated to altered nitric oxide production.

INTRODUCTION

Chronic heart failure is associated with impaired vasodilator responsiveness to physiological and pharmacological stimuli in the peripheral circulation [1]. This impairment may contribute to the exercise intolerance that is a major clinical manifestation of this condition. The cause of the vasodilator impairment of chronic heart failure is not certain, but one possibility is the endothelial dysfunction that accompanies this condition. Chronic heart failure is associated with increased plasma levels of the endothelium-derived vasoconstrictor peptide, endothelin-1 [2]. In addition, there is impairment of endothelium-dependent vasodilatation in response to muscarinic stimulation by acetylcholine. Controversy exists in the literature regarding basal release of the endothelium-dependent relaxation factor, nitric oxide, in chronic heart failure. Some studies suggest that basal

Key words: endothelin-1, endothelium, nitric oxide, vascular tone.
Abbreviations: ET, endothelin; \textit{t}\textit{-}NMMA, \textit{N}^\omega\textit{-}monomethyl\textit{-}l-arginine.
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release of nitric oxide is preserved or even enhanced in this condition, while in others basal release appears to be impaired [3,4]. Thus, endothelial dysfunction may contribute to impaired vasodilator responsiveness via impaired endothelium-dependent vasodilatation or increased endothelin production limiting counter-regulatory vasodilator mechanisms (or both).

In relation to endothelin-1, we have recently observed that plasma levels of endothelin-1 measured during maximum exercise were inversely correlated with maximal exercise capacity in patients with chronic heart failure [5]. This association was not observed with circulating vasoconstrictor factors such as noradrenaline.

As endothelin-1 has paracrine and autocrine effects, we hypothesized that the peptide may contribute directly to endothelium-dependent vasodilator alterations in patients with chronic heart failure, by acting in an autocrine manner to decrease stimulated endothelium-dependent vasodilatation. In addition, we sought to determine whether endothelin-1 would selectively alter basal production of nitric oxide, as endothelin-1 has been shown to increase nitric oxide release (at least transiently) via stimulation of a vasodilator endothelin receptor (the ET$_B$ receptor) located on the endothelial cell.

Infusion of endothelin (and other vasoconstrictors) was conducted in normal subjects in the present study in order to reproduce the milieu of increased circulating endothelin in chronic heart failure.

**METHODS**

The study population comprised eight normal subjects (three males and five females) with a mean age of 39 ± 6 years. All subjects were free of cardiovascular disease and taking no medication. No smoking or alcohol was permitted from midnight of the day of the study.

Vasodilator and vasoconstrictor responsiveness was assessed in the forearm using venous occlusion plethysmography, as previously described in detail. Briefly, with the arm resting comfortably above the right atrium, a mercury-in-silastic strain gauge was placed around the widest portion of the upper third of the forearm. A blood pressure cuff placed above the elbow was rapidly inflated to suprasystolic levels to assess whether infusion of agents

# As described below was associated with systemic effects on blood pressure and heart rate.

The plethysmographic tracings were analysed in a blinded manner. Study data were divided into sections, coded, then reference to infusate removed from the plethysmographic tracings. Coded segments of data were then analysed in a semi-automated manner by the investigator (H.K.) to derive measures of forearm blood flow. Measured values comprise the mean of five consecutive plethysmographic recordings.

Blood flow was assessed before (after 30 min supine rest) and after brachial artery infusion of, in order: acetylcholine, 50 µg/min, to measure stimulated endothelium-dependent vasodilatation; the nitric oxide synthesis inhibitor N$\text{^\text{-}}$monomethyl-L-arginine (L-NMMA), infused at 2 µmol/min to block nitric oxide production and thus assess basal endothelium-dependent vasodilatation; and after peak reactive hyperaemia (4 min of proximal cuff inflation to suprasystolic levels) to assess metabolic vasodilatation to ischaemia, which is thought to be mediated, at least in part, via endothelium-dependent mechanisms.

The effects on forearm blood flow of acetylcholine, L-NMMA and peak reactive hyperaemia were assessed in the absence of a background vasoconstrictor agent as well as during background continuous intra-arterial infusion of noradrenaline (non-endothelium-derived, circulating vasoconstrictor) and endothelin-1 (endothelium-derived, autocrine/paracrine vasoconstrictor). Endothelin-1 was infused at 50 ng/min to achieve approximately a one-third reduction in forearm blood flow. The dose of noradrenaline infused (240 pmol/min) was chosen to match the reduction in forearm blood flow achieved by endothelin-1. Infusion of noradrenaline always preceded that of endothelin-1 because of the long-lasting vasoconstrictor actions of the latter.

Infusion of each agent took place for at least 10 min before plethysmographic recording to establish new steady-state levels of forearm blood flow in the setting of the infusate. Furthermore, periods of up to 20 min after cessation of infusion were required to re-establish basal levels of forearm blood flow.

Statistical analysis was by one-way analysis of variance (pre versus post acetylcholine, L-NMMA, peak reactive hyperaemia) with pairwise comparison of significant values by Student’s paired t-test. The primary comparison was between interventions during endothelin-1 and noradrenaline infusion. Initial sample size calculations were performed to test the hypothesis that endothelin would reduce forearm blood flow by 20% compared with the non-endothelium-derived vasoconstrictor, noradrenaline. With a within-day coefficient of variation of forearm blood flow of 5% and based on a two-sided test, six subjects would be required to demonstrate these differences should they be present. All values are described as means ± S.E.M.
RESULTS

During endothelin-1 infusion there was a transient increase noted in forearm blood flow after 4 min (23 ± 6 %) followed by sustained vasoconstriction which reached a steady state at 10 min.

Steady-state infusion of vasoconstrictor agents (noradrenaline and endothelin-1) resulted in significant reductions in forearm blood flow compared with baseline (Figure 1). The magnitude of these reductions was similar between the two vasoconstrictor agents (27 ± 3 % for noradrenaline and 29 ± 2 % for endothelin-1, \( P < 0.05 \)).

There was a significant reduction in forearm blood flow with the nitric oxide synthase inhibitor, L-NMMA, in the absence of background vasoconstrictor infusion (Table 1), and the magnitude of this reduction was similar with both noradrenaline and endothelin-1 infusions (Figure 2). There was no statistically significant difference in blood flow reduction due to L-NMMA between noradrenaline and endothelin-1 infusions.

The effect of vasoconstrictor agents on stimulated endothelium-dependent vasodilatation was examined by infusion of acetylcholine. There was a significant increase in forearm blood flow with acetylcholine in the absence of vasoconstrictor agent (Table 1), and this was similar in the presence of background noradrenaline and endothelin-1 infusion, with no statistical differences between vasoconstrictor agents (Figure 3).

In contrast to these findings, metabolic vasodilatation (as determined by absolute level of forearm blood flow) in forearm blood flow with acetylcholine in the absence of vasoconstrictor agent (Table 1), and this was similar in the presence of background noradrenaline and endothelin-1 infusion, with no statistical differences between vasoconstrictor agents (Figure 3).

Table 1 Forearm blood flow during pharmacological perturbation in normal subjects

<table>
<thead>
<tr>
<th>Vasoconstrictor</th>
<th>Baseline</th>
<th>L-NMMA (2 µmol/min)</th>
<th>Acetylcholine (50 µg/min)</th>
<th>Peak reactive hyperaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>2.98 ± 0.41</td>
<td>2.06 ± 0.48</td>
<td>7.4 ± 1.7</td>
<td>24.6 ± 5.9</td>
</tr>
<tr>
<td>Endothelin-1 (50 ng/min)</td>
<td>2.04 ± 0.17</td>
<td>1.49 ± 0.21</td>
<td>5.1 ± 2.1</td>
<td>15.4 ± 1.6</td>
</tr>
<tr>
<td>Noradrenaline (240 pmol/min)</td>
<td>2.27 ± 0.36</td>
<td>1.49 ± 0.27</td>
<td>5.9 ± 1.6</td>
<td>20.8 ± 2.4</td>
</tr>
</tbody>
</table>

\( * P < 0.05 \)

The present study examined the role of endothelin-1 in contributing to derangements of endothelium-derived vasodilator function in man. Assessment was also made of responsiveness to vasodilator stimuli such as peak reactive hyperaemia (mediated in part via nitric oxide) in the setting of endothelin-1 infusion.

The effect of endothelin-1 on endothelium-dependent vasodilator responsiveness, both basal (measured by reduction in forearm blood flow in response to L-NMMA) and stimulated (measured by increase in forearm blood flow in response to acetylcholine), was not different compared with either the absence of background vasoconstrictor or the presence of noradrenaline. Thus, endothelin-1 does not appear to significantly alter basal or stimulated endothelium-dependent vasodilatation during steady-state vasoconstriction in man.

The transient increase in blood flow seen early on in endothelin-1 infusion is thought to be related to increased basal release of nitric oxide [6]; however, this increase in basal nitric oxide production is not sustained during prolonged infusion of endothelin-1 in man, as determined by L-NMMA infusion in the present study. This finding is of considerable clinical interest as endothelin receptor antagonists have recently been developed. Whereas ET₁ receptors on the vascular smooth muscle cell mediate vasoconstriction, ET₂ receptors mediate both vasoconstriction (via vascular smooth muscle cell receptors) and vasodilation (via endothelial cell receptors), the latter predominantly via release of nitric oxide and vasodilator prostaglandins. The finding in the present study, that prolonged stimulation of the endothelial cell ET₂ receptor during endothelin-1 infusion does not result in sustained increases in nitric oxide release, suggests that endothelial cell ET₂ receptor blockade should not substantially reduce the vasodilator benefits of endothelin receptor antagonism.

Another finding of the present study was that the absolute response of metabolic vasodilatation to peak reactive hyperaemia was significantly impaired during background endothelin-1 infusion in comparison to noradrenaline. Given the absence of a selective effect of endothelin-1 on either basal or stimulated nitric oxide production, this impairment of metabolic vasodilator responsiveness would appear to be via a nitric-oxide-
independent mechanism. This observation may also underlie the initial finding of a selective inverse relationship between endothelin-1 and maximal exercise capacity in normal subjects and patients with chronic heart failure.

The present study was not able to further elucidate the mechanism of this vasodilator impairment with endothelin-1, and further studies are required to address this issue. However, it is of interest that we have recently observed that decreased endothelial production of prostaglandins (but not nitric oxide) may limit metabolic vasodilatation in patients with chronic heart failure [7]. The contribution of endothelin-1 to the vasodilatory prostaglandin deficiency of heart failure has not as yet been determined. These issues may be best addressed by physiological studies involving the use of specific endothelin receptor antagonists in relevant disease states.

In summary, steady-state infusion of endothelin-1 in man does not appear to alter basal or stimulated endothelium-dependent vasodilator responsiveness, a finding that may have implications regarding choice of endothelin receptor antagonist in the management of cardiovascular disease. In addition, a selective impairment of vasodilatation in response to peak reactive hyperaemia was seen with endothelin-1 infusion, and in this way endothelin-1 may contribute to vasodilator impairment in chronic heart failure.

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