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Endothelin$_B$ Receptors Are Functionally Important in Mediating Vasoconstriction in the Systemic Circulation in Patients With Left Ventricular Systolic Dysfunction

Peter J. Cowburn, MBBS, MRCP,* John G. F. Cleland, MD, FRCP, FESC, FACC,‡
John D. McArthur, MB, ChB, FRCP,† Margaret R. MacLean, PhD,*
John J. V. McMurray, BSc, MD, FRCP, FESC,* Henry J. Dargie, MB, ChB, FRCP, FESC,*
James J. Morton, PhD*

Glasgow, Scotland, and Kingston-upon-Hull, United Kingdom

OBJECTIVES This study was designed to assess the functional importance of endothelin (ET)$_B$ receptors in patients with left ventricular systolic dysfunction (LVSD) by comparing the hemodynamic effects of ET-1, a nonselective ET$_A$ and ET$_B$ agonist, with ET-3, a selective ET$_B$ receptor agonist.

BACKGROUND Knowledge of the functional importance of ET$_B$ receptors in mediating vasoconstriction in chronic heart failure will help determine whether antagonists at both ET$_A$ and ET$_B$ receptors are required to fully prevent vasoconstriction to endogenously produced ET-1.

METHODS We infused ET-1 (5 and 15 pmol/min) and ET-3 (5 and 15 pmol/min) into two separate groups of eight patients with LVSD with similar baseline hemodynamic indices. Hemodynamics were measured using a pulmonary thermodilution catheter and an arterial line.

RESULTS Endothelin-1 infusion led to systemic vasoconstriction, with a rise in mean arterial pressure (mean ± SEM 100 ± 3 to 105 ± 3 mm Hg, $p < 0.02$) and systemic vascular resistance (1,727 ± 142 to 2,055 ± 164 dyn/s/cm^{-5}, $p < 0.001$) and a fall in cardiac index (2.44 ± 0.21 to 2.22 ± 0.20 liters/min/m^2, $p < 0.01$). Endothelin-3 infusion also led to systemic vasoconstriction, with a rise in mean arterial pressure (99 ± 6 to 105 ± 6 mm Hg, $p < 0.01$) and systemic vascular resistance (1,639 ± 210 to 1,918 ± 245 dyn/s/cm^{-5}, $p < 0.01$) and a fall in cardiac index (2.66 ± 0.28 to 2.42 ± 0.24 liters/min/m^2, $p < 0.05$). Pulmonary hemodynamic measurements did not change significantly in either group.

CONCLUSIONS Both ET-1 and ET-3 infusions led to systemic vasoconstriction; the hemodynamic changes observed were of a similar magnitude at the same molar concentration. This suggests that ET$_B$ receptors are functionally important in mediating vasoconstriction, at least in the systemic circulation, in patients with LVSD. (J Am Coll Cardiol 1999;33:932–8) © 1999 by the American College of Cardiology

The endothelins are a family of potent vasoconstrictor peptides (1,2). Endothelin-1 (ET-1) is the predominant isoform expressed in the human vasculature (3).

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Endothelin-3 is also detectable in human plasma, although its physiologic role remains unclear. The endothelins act via at least two receptor subtypes, denoted ET$_A$ and ET$_B$ (4,5).

From the *Medical Research Council Clinical Research Initiative in Heart Failure, University of Glasgow, Department of Cardiology, †Western Infirmary, Glasgow, Scotland, and ‡The Academic Unit, Department of Cardiology, Kingston-upon-Hull, United Kingdom. This project, Dr. Peter Cowburn and Dr. John Cleland were supported by grants from the British Heart Foundation.

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Endothelin$_A$ receptors have selective affinity for ET-1 (K_i of 0.6 nmol/liter for ET-1 compared with 140 nmol/liter for ET-3 [6]) and are expressed primarily on vascular smooth muscle cells and cardiac myocytes. Endothelin$_B$ receptors, which have similar affinity for each endothelin isoform (K_i values of 0.12 and 0.06 nmol/liter for ET-1 and ET-3, respectively [6]), are expressed on both endothelial cells and vascular smooth muscle. Both receptors have been shown to mediate vasoconstriction (7,8); the endothelial ET$_B$ receptor also mediates vasodilation via nitric oxide and prostaglandins (9).

In patients with moderate to severe chronic heart failure (CHF), plasma concentrations of ET-1 are elevated (10–12), correlate with the symptomatic and hemodynamic severity of CHF (11,12) and independently predict prognosis on multivariate analysis (13). There is therefore consid-

Abbreviations and Acronyms

CHF = chronic heart failure
CI = cardiac index
CO = cardiac output
DCM = dilated cardiomyopathy
ET = endothelin
LVSD = left ventricular systolic dysfunction
MPAP = mean pulmonary artery pressure
PCWP = pulmonary capillary wedge pressure
PVR = pulmonary vascular resistance
RAP = right atrial pressure
SNP = sodium nitroprusside
SVR = systemic vascular resistance

erable interest in the therapeutic potential of endothelin receptor antagonists in CHF (14). Uncertainty about the role of the ET_B receptor in CHF has led to controversy as to whether selective ET_A or nonselective ET_A/ET_B antagonists would be the most appropriate therapeutic agent in CHF. Enhanced ET_B-mediated vasoconstriction has been demonstrated in coronary arteries in a canine model of CHF using sarafatoxin S6c, a selective ET_B agonist (15). Studies of the forearm circulation in CHF patients have also shown enhanced ET_B-mediated vasoconstriction, but attenuation of the vasoconstrictor response to ET-1, compared to control subjects (16). Sarafatoxin S6c was again used as the selective ET_B agonist in this study. This suggests that a nonselective ET_A/ET_B receptor antagonist may be required to completely prevent vasoconstriction to endogenously produced ET-1 in CHF.

Endothelin-3 is an endogenous, relatively selective ET_B receptor agonist in humans and is a less potent vasoconstrictor of normal forearm resistance vessels than ET-1, a nonselective ET_A and ET_B receptor agonist (8). The hemodynamic effects of ET-3 have yet to be described in patients with left ventricular systolic dysfunction (LVSD). To investigate the contribution of ET_A and ET_B receptors to ET-mediated vasoconstriction in patients with LVSD, we compared the hemodynamic effects of ET-1 and ET-3 in two separate groups of patients with LVSD, with or without overt heart failure.

METHODS

Patient selection. Patients with chronic LVSD, defined as a left ventricular ejection fraction of <40%, measured by echocardiography using Simpson's biplane method or radionuclide ventriculography, were eligible for study. Patients with unstable angina, known critical coronary stenoses at angiography, valvular heart disease, atrial fibrillation, insulin-dependent diabetes, uncontrolled hypertension and chronic renal impairment (creatinine >200 μmol/liter) were excluded.

Study protocols. Studies were conducted with the approval of the local ethics committee and with the written, informed consent of each patient. Cardiac medications were withheld for a minimum of 24 h before the study. Patients were fasted for 4 h before the study. Studies took place in the cardiac catheterization laboratory. Heart rate was recorded electrocardiographically. Hemodynamics were measured by pulmonary thermodilution catheter and femoral arterial line. Systemic arterial, right atrial (RAP), pulmonary arterial and pulmonary capillary wedge pressure (PCWP) measurements were made simultaneously. Cardiac output (CO) was measured, in triplicate, at each time point. Cardiac index (CI), and systemic (SVR) and pulmonary (PVR) vascular resistance were calculated from standard formulae (17). Heparin (2,500 U) was given as standard prophylaxis against thrombus formation.

Baseline hemodynamic measurements were obtained at a minimum of 15 min postinstrumentation and repeated at 5-min intervals until stable. Sodium nitroprusside (SNP) was then infused centrally at 0.56 and 1.12 μg/kg/min to assess vasodilator reserve. After 5 min of each dose a complete set of hemodynamic measurements was taken. After hemodynamic values had returned to baseline (approximately 30 min), either ET-1 or ET-3 (Clinalfa, Switzerland) were infused at 5 and 15 pmol/min. Each dose was infused for 20 min with hemodynamic measurements being made at 5 and 15 min. Further measurements were taken 5 and 15 min after the infusion was complete.

Measurement of plasma endothelin concentrations. In six patients from the ET-1 group, and in all eight patients from the ET-3 group, blood samples were taken from the femoral artery at baseline before SNP infusion, after reestablishment of a baseline before ET infusion, at the end of each dose of ET and at 5 and 15 min of recovery. Blood was collected into chilled tubes containing 4% ethylenediaminetetraacetic acid. Samples were kept on ice and were then centrifuged at 4°C. Separated plasma samples were immediately stored at −20°C.

Endothelin-1 and big ET-1 were assayed directly (and separately) using enzyme immunoassays (Biomedica). The kits incorporate an immunoaffinity purified polyclonal capture antibody and a monoclonal detection antibody, both highly specific for endothelin (1–21) or big endothelin (1–38). Samples were assayed in duplicate and averaged.

Endothelin (1–28) assay characteristics include measuring range: 0.1 to 15.6 fmol/ml; cross-reactivity—ET-1: 100%, ET-2: 100%, ET-3: <5%, big endothelin (1–38): <1%, big endothelin (22–38): <1%.

Big endothelin (1–38) assay characteristics include measuring range: 0.025 to 6.25 fmol/ml; cross-reactivity—big endothelin (1–38): 100%, big endothelin (22–38): <1%, ET-1: <1%, ET-2: <1%, ET-3: <1%.

Statistics. Baseline values are reported as mean ± SD and values relating to an intervention are reported as mean ± SEM. The primary measures of interest were the changes in PVR and SVR from baseline to the peak (15 pmol/min) doses of ET-1 and ET-3. Repeated measures analysis of variance examining the effects of duration of infusion, ET-1 versus ET-3 and dose was applied to the data. Significant

Table 1. Patient Characteristics

	ET-1 Study	ET-3 Study
Sex, n	7M/1F	7M/1F
Age, years (mean ± SD)	62 ± 10	64 ± 11
Etiology	5 IHD, 3 DCM	6 IHD, 2 DCM
NYHA class		
I	2	2
II	3	4
III	3	2
LVEF (mean ± SD)	28 ± 7%	26 ± 7%
Drug therapy*		
ACE-I	6	6
Diuretic	4	4
Digoxin	1	2
Calcium antagonist	1	1
Oral nitrate	3	2
Beta-blocker	3	3
Aspirin	4	6

*Drug therapy withheld for 24 h.
ACE-I = angiotensin-converting enzyme inhibitor; DCM = dilated cardiomyopathy; ET = endothelin; F = female; IHD = ischemic heart disease; LVEF = left ventricular ejection fraction; M = male; NYHA = New York Heart Association.

differences were further explored using Student paired *t* test (two tailed) with a Bonferroni correction for multiple comparisons. Values were considered significantly different if $p < 0.05$ after correction for multiple comparisons.

RESULTS

Patient characteristics. Eight patients with similar baseline hemodynamic indices and baseline characteristics took part in each study (i.e., eight patients received ET-1 and eight patients received ET-3). Six patients had symptomatic heart failure and two patients had asymptomatic left ventricular dysfunction in each group. One patient had a history of systemic hypertension and one patient had non–insulin-dependent diabetes in each group. Further characteristics are given in Table 1.

Sodium nitroprusside infusion. Table 2 demonstrates the hemodynamic effects of SNP in these patient groups. As reported in previous studies SNP infusion led to systemic and pulmonary vasodilation. Mild flushing was reported by a minority of patients.

Endothelin-1 infusion. Endothelin-1 infusion (15 pmol/min) caused systemic vasoconstriction with a rise in mean arterial pressure (6%) and SVR (20%), and a fall in CO (9%) (Table 3). There were nonsignificant trends toward a rise in PCWP (and hence trends to a fall in transpulmonary gradient) and, therefore, PVR remained unchanged despite the fall in CO. No side effects were noted with ET-1 infusion.

Endothelin-3 infusion. Endothelin-3 infusion (15 pmol/min) caused systemic vasoconstriction with a rise in mean arterial pressure (7%) and SVR (18%), and a fall in CO (8%) (Table 4). There were nonsignificant trends toward a rise in mean pulmonary artery pressure (MPAP), PCWP and PVR. One patient (DCM, New York Heart Association class III) became breathless during the 15 pmol/min dose, with a rise in PCWP (14 to 19 mm Hg) and a rise in MPAP (24 to 29 mm Hg, i.e., transpulmonary gradient was unchanged). A diuretic was given, and the symptoms resolved quickly on stopping ET-3. There were no other adverse events.

Figure 1 shows changes in SVR in response to ET-1 and ET-3 infusion.

Plasma ET-1 concentrations. Figure 2 shows the femoral plasma ET-1 concentrations during ET-1 (six patients) and ET-3 infusion (seven patients). Plasma concentrations of ET-1 rose significantly during ET-1 infusion (baseline pre-SNP to 15 pmol/min ET-1, $p < 0.01$), whereas plasma ET-1 did not change with ET-3 infusion. Femoral plasma big ET concentrations did not change significantly during the studies: baseline pre-SNP: 0.63 ± 0.10 and 0.83 ± 0.14 fmol/ml, ET 15 pmol/min: 0.64 ± 0.12 and 0.88 ± 0.10

Table 2. Hemodynamic Responses to Sodium Nitroprusside

	SNP/ET-1 Group		SNP/ET-3 Group		Comparison: Baseline to End of SNP Infusion (ET-1 and ET-3 Groups Combined)
	Baseline	Mean Change	Baseline	Mean Change	
HR	76 ± 17	+10 ± 2	67 ± 15	+13 ± 3	$p < 0.0001$
MAP	100 ± 9	−24 ± 4	101 ± 18	−27 ± 5	$p < 0.0001$
RAP	5 ± 2	−1 ± 1	6 ± 2	−2 ± 1	$p < 0.01$
MPAP	20 ± 4	−9 ± 1	20 ± 5	−10 ± 1	$p < 0.0001$
PCWP	12 ± 3	−8 ± 1	12 ± 4	−8 ± 1	$p < 0.0001$
TPG	7.5 ± 1.5	−1.1 ± 0.9	8.0 ± 2.3	−1.6 ± 0.7	$p < 0.05$
CI	2.63 ± 0.62	+0.16 ± 0.10	2.68 ± 0.64	+0.22 ± 0.11	$p < 0.05$
SVR	1,617 ± 287	−464 ± 68	1,615 ± 495	−505 ± 83	$p < 0.0001$
PVR	126 ± 24	−27 ± 14	132 ± 44	−35 ± 11	$p < 0.01$

Baseline values: mean ± SD; mean change given as mean ± SEM; p values are for both endothelin ET-1 and ET-3 groups combined.
CI = cardiac index (liters/min/m^2); HR = heart rate (beats/min); MAP = mean arterial blood pressure (mm hg); MPAP = mean pulmonary artery pressure (mm Hg); PCWP = pulmonary capillary wedge pressure (mm Hg); PVR = pulmonary vascular resistance (dyn/s/cm^{-5}); RAP = right atrial pressure (mm Hg); SNP = sodium nitroprusside; SVR = systemic vascular resistance (dyn/s/cm^{-5}); TPG = transpulmonary gradient (mm Hg).

Table 3. Hemodynamic Responses to Endothelin-1

	Baseline	ET-1 (5 pmol/min)	ET-1 (15 pmol/min)	Comparison: Baseline to End of ET-1 (15 pmol/min) Infusion
HR	73 ± 18	72 ± 6	73 ± 6	NS
MAP	100 ± 8	102 ± 2	105 ± 3	$p < 0.02$
RAP	6 ± 1	5 ± 1	5 ± 1	NS
MPAP	21 ± 6	21 ± 2	22 ± 2	NS
PCWP	13 ± 6	14 ± 2	15 ± 2	NS
TPG	8 ± 1	7 ± 1	7 ± 1	NS
CI	2.44 ± 0.58	2.34 ± 0.16	2.22 ± 0.20	$p < 0.01$
SVR	1,727 ± 403	1,849 ± 105	2,055 ± 164	$p < 0.01$
PVR	152 ± 44	140 ± 21	149 ± 15	NS

Baseline values: mean ± SD; ET-1 (5 and 15 pmol/min) given as mean ± SEM. Abbreviations as in Table 2.

fmol/ml for ET-1 and ET-3, respectively. One patient in the ET-3 group had very high baseline plasma ET-1 concentrations, which also did not change during ET-3 infusion (baseline pre-SNP infusion: 12.5 fmol/ml, ET-3 15 pmol/min: 12 fmol/ml). This outlying data set is not included in Figure 1 to avoid misrepresentation of results. The patient's hemodynamic response was broadly similar to other group members.

DISCUSSION

This study shows that equal molar concentrations of ET-1 and ET-3 cause a similar degree of systemic vasoconstriction in patients with LVSD, with or without overt heart failure. As ET-1 acts as a nonselective ET_A/ET_B receptor agonist and ET-3 acts as a selective ET_B receptor agonist at the infused concentrations (8), this suggests that ET_B receptors are functionally important in mediating vasoconstriction, at least in the systemic circulation, in patients with LVSD. We did not see systemic vasodilation before vasoconstriction as seen in forearm studies, though, as this phenomenon has only been seen transiently with high dose local infusion (60 pmol/min) (8), we would not have expected to see this effect. We have previously reported the lack of pulmonary vasoconstriction in response to exogenous ET-1 in patients with LVSD (18). Similarly, in this study, we saw no significant changes in MPAP or PVR with ET-3 infusion.

To our knowledge, the pulmonary and cardiac hemodynamic effects of ET-3 have not previously been described in humans in vivo. Inoue et al. first reported the effects of the ET-1 and ET-3 in anesthetized rats and found ET-1 to be the more potent vasoconstrictor (3). In a study of rabbits, both ET-3 and ET-1 caused modest vasoconstriction in the pulmonary vascular bed (ET-1 > ET-3), but both caused systemic vasodilation (19). The authors noted that the limited effects of ET-1 and ET-3 on the pulmonary bed were in marked contrast to the potent vasoconstriction seen with isolated pulmonary conductance vessels in vitro (19). In human pulmonary resistance arteries, in vitro, ET_B receptor-mediated vasoconstriction predominates at physiologically relevant concentrations (20). In the only in vivo study of ET-3 reported in humans, ET-3 vasoconstricted forearm resistance vessels of healthy volunteers, although to a lesser extent than ET-1 (8). This suggests that both ET_A and ET_B receptors mediate vasoconstriction in vivo, at least in the normal forearm vascular bed. The less potent vasoconstriction observed with ET-3 (8) may reflect a greater effect on endothelial ET_B receptors, the resultant vasodila-

Table 4. Hemodynamic Responses to Endothelin-3

	Baseline	ET-3 (5 pmol/min)	ET-3 (15 pmol/min)	Comparison: Baseline to End of ET-3 (15 pmol/min) Infusion
HR	67 ± 14	67 ± 6	68 ± 6	NS
MAP	99 ± 17	101 ± 6	105 ± 6	$p < 0.01$
RAP	6 ± 2	5 ± 1	5 ± 1	NS
MPAP	19 ± 4	20 ± 2	21 ± 2	NS
PCWP	12 ± 4	12 ± 2	13 ± 2	NS
TPG	7 ± 2	7 ± 1	8 ± 1	NS
CI	2.66 ± 0.79	2.49 ± 0.25	2.42 ± 0.24	$p < 0.05$
SVR	1,639 ± 593	1,782 ± 222	1,918 ± 245	$p < 0.01$
PVR	124 ± 34	133 ± 13	132 ± 14	NS

Baseline values: mean ± SD; ET-3 (5 and 15 pmol/min) given as mean ± SEM. Abbreviations as in Table 2.

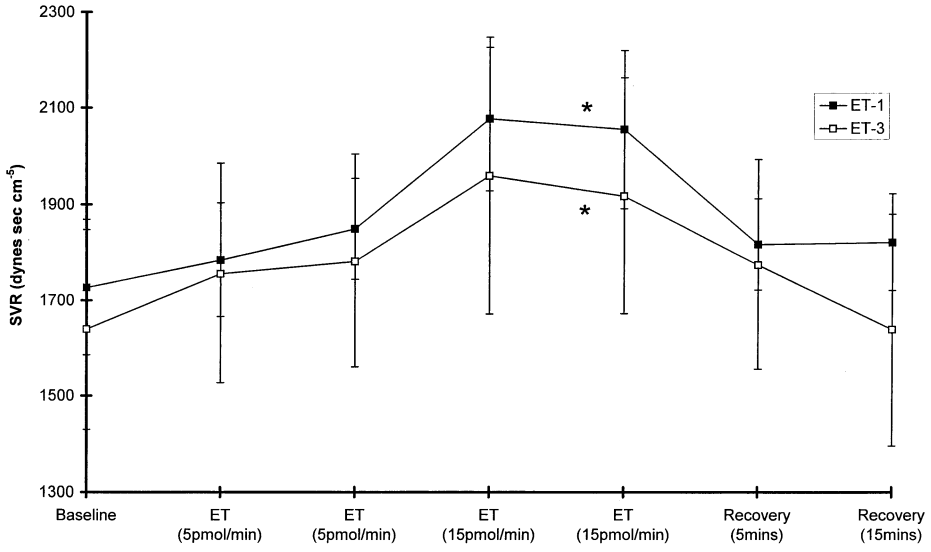

Figure 1. Sequential changes in systemic vascular resistance (SVR) in response to endothelin-1 (ET-1) and endothelin-3 (ET-3) infusion. Compared with baseline measurements, the rise in SVR was significant at a 15 pmol/min dose of ET-1 and ET-3 respectively, *$p < 0.01$. Repeated measures analysis of variance failed to demonstrate differences between ET-1 and ET-3 or between 5 and 15 min of infusion at each dose.

tion offsetting the smooth muscle ET_A- and ET_B-receptor–mediated vasoconstriction.

In contrast to studies in healthy volunteers, we found that ET-1 and ET-3 have very similar hemodynamic effects at the same molar concentration in patients with LVSD, with or without overt heart failure. This observation adds to the growing evidence that endothelin receptor function is disturbed in heart failure. Love et al. demonstrated enhanced forearm vasoconstriction in CHF patients to sarafatoxin S6c, a highly selective ET_B receptor agonist in CHF, but attenuation of the vasoconstrictor response to ET-1, compared to control participants (16). There is also evidence for impaired vasoconstriction to ET-1 in human CHF vessels in vitro (21). Our data are consistent with enhanced ET_B-mediated vasoconstriction, possibly due to down-regulation of endothelial ET_B receptors. Alternatively, or in addition, there may be attenuated ET_A-mediated vasoconstriction in the systemic circulation in LVSD/CHF.

It is possible, however, that the vasoconstriction observed during ET-3 infusion is not ET_B mediated. An alternative explanation is that the ET_B receptor agonist displaces ET-1 from the receptor and this causes unopposed vasoconstriction at the ET_A receptor. Similarly, the ET_B receptor has been demonstrated to act as a clearance receptor for endo-

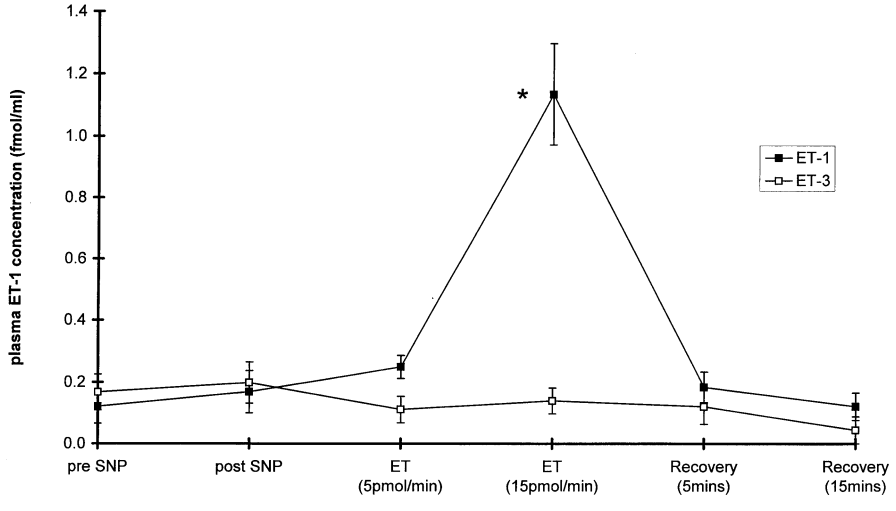

Figure 2. Sequential changes in mean plasma endothelin-1 (ET-1) concentration in response to endothelin-1 (ET-1) and endothelin-3 (ET-3) infusion. Compared with baseline values, the rise in plasma ET-1 concentration observed during ET-1 infusion (15 pmol/min) was significant, *$p < 0.01$. SNP = sodium nitroprusside.

thelin in animals (22,23), and therefore an ET_B receptor agonist could lead to increased ET-1 concentrations by blocking ET-1 clearance. An early report has suggested that, in dogs, plasma ET-1 concentrations rise with administration of both an ET_B-selective agonist, sarafatoxin S6c, and antagonists of the ET_B receptor (24). We found that circulating plasma concentrations of ET-1 did not change with ET-3 infusion, in contrast to the rise seen with ET-1 infusion (Fig. 2). This makes the above hypotheses unlikely, though it is still possible that changes in ET-1 concentrations occur at a tissue, but not at a plasma level. Endothelin-3 could also mediate vasoconstriction via a putative ET_C receptor (ET-3 selective), situated on smooth muscle cells. However, although there is evidence from binding and functional studies to support the existence of an ET-3 selective receptor in the vasculature (25–27), and a potential candidate has been identified in *Xenopus laevis* melanophores (28), and more recently in chickens (29), such a receptor has not yet been identified in humans.

Clarification of the functional importance of ET_B receptors in CHF, in mediating both vasodilation and vasoconstriction, is necessary to determine whether nonselective ET_A/ET_B receptor or selective ET_A receptor antagonists are likely to be the more effective vasodilator class in CHF. Our data suggest that a nonselective ET_A/ET_B receptor antagonist may be necessary to fully inhibit the vasoconstrictor effects of endogenous ET-1. Indeed, bosentan, which is such an agent, caused pulmonary and systemic vasodilation in CHF patients (30). However, a study in a canine model (31) and an early report from human forearm studies (32) found that parenteral administration of selective ET_B receptor antagonists caused vasoconstriction in CHF, suggesting that ET_A-selective antagonists might be more potent vasodilators than nonselective agents in this syndrome. The reasons for this discrepancy have yet to be elucidated.

Conclusions. Endothelin-1 and ET-3, when infused into patients with LVSD, led to systemic vasoconstriction, with little or no effect in the pulmonary vasculature. The hemodynamic changes observed were of a similar magnitude at the same molar concentration. This suggests that ET_B receptors are functionally important in mediating vasoconstriction, at least in the systemic circulation, in patients with LVSD. Studies of the pulmonary and systemic effects of selective endothelin antagonists in patients with CHF are required before concluding that nonselective ET_A/ET_B receptor antagonists have the greater potential as vasodilating agents in CHF.

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Reprint requests and correspondence: Dr. John G. F. Cleland, Castle Hill Hospital, Castle Road, Cottingham Hull, HU16 5JQ, United Kingdom. E-mail: J.Cleland@bio.gla.ac.uk.

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