EXPERIMENTAL STUDIES

Comparison of the Effects of Selective Endothelin ET\(_A\) and ET\(_B\) Receptor Antagonists in Congestive Heart Failure

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**Objectives.** This study was designed 1) to determine the extent to which endogenous endothelin (ET) affects hemodynamic, hormonal and body fluid balance through ET\(_A\) and ET\(_B\) receptors in congestive heart failure (CHF); and 2) to assess the therapeutic benefits and adverse effects of ET receptor antagonists for ET\(_A\) and ET\(_B\) on cardiorenal and neurohormonal variables.

**Background.** ET has two receptors, ET\(_A\) and ET\(_B\), both of which are distributed in various tissues and cells. In vascular beds, ET\(_A\) receptors mediate vasoconstriction, whereas ET\(_B\) receptors mediate vasorelaxation. However, ET\(_B\) receptors also exist in smooth muscle and mediate vasoconstriction.

**Methods.** We administered either the ET\(_A\) receptor antagonist FR139317 (FR [n = 8], 1 and 10 mg/kg body weight) or the ET\(_B\) receptor antagonist RES-701-1 (RES [n = 8], 0.2 and 1.5 mg/kg) to dogs with CHF induced by rapid ventricular pacing. The effects of both antagonists on cardiorenal and hormonal functions were studied.

**Results.** FR decreased cardiac pressures and the plasma atrial natriuretic peptide (ANP) level and increased cardiac output (CO). Urinary flow rate and urinary sodium excretion increased in association with an increase in the glomerular filtration rate and renal plasma flow (RPF). In contrast, RES increased cardiac pressures and decreased CO. It also decreased the plasma aldosterone level and RPF. Neither antagonist affected plasma norepinephrine levels.

**Conclusions.** Endogenous ETs increase cardiac pressures and the retention of body fluid through ET\(_A\) receptors in CHF. The vasodilative action through ET\(_B\) receptors is overall functionally more important than the constrictive action through ET\(_A\) receptors. ETs may regulate the secretion of ANP and aldosterone. Our findings suggest that selective ET\(_A\) receptor antagonists have potential therapeutic benefits affecting both hemodynamic variables and diuresis, whereas ET\(_B\) receptor antagonists have adverse hemodynamic effects, with the possibility of preventing fluid retention through suppression of aldosterone secretion in dogs with CHF.

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Abbreviations and Acronyms

ANP = atrial natriuretic peptide
CHF = congestive heart failure
CO = cardiac output
ET = endothelin
FR = FR139317
GFR = glomerular filtration rate
MAP = mean arterial pressure
PCWP = pulmonary capillary wedge pressure
RES = RES-701-1
RPF = renal plasma flow

onists for ET receptors have been identified (13,14): FR139317 (FR) for ETA receptors, and RES-701-1 (RES) for ETB receptors. In the present study, we investigated the extent to which endogenous ETs affect hemodynamic, hormonal and renal variables through each of the ET receptors using FR and RES in dogs with CHF induced by right ventricular rapid pacing. We also assessed the therapeutic benefits and adverse effects of these ET antagonists on cardiorenal and neurohormonal functions.

Methods

Animal preparation. Conditioned male or female beagles, weighing 10 to 13 kg, were used for all of the experiments, as previously described (15). This study was approved by the Animal Research Committee of Shiga University of Medical Science. After anesthesia was induced with pentobarbital sodium (25 mg/kg body weight), the dogs were ventilated. Through a left thoracotomy, the heart was exposed, and two cardiac pacemaker leads (Matsuda M-23) were sutured onto the right ventricular apex. After the incision was closed, the leads were tunneled to the back and connected to an external pacemaker (Seamed 540). The left femoral vein was then exposed, and a thermodilution catheter (Goodtec T-047-03) was placed in the descending aorta (17).

Study 1: inhibitory effects of FR and RES in response to ET-1. The selective nature of FR and RES have been well demonstrated. FR (Fujisawa Pharmaceutical Co., Ltd), showed a high affinity for ET A receptors, and RES in response to ET-1. The selective nature of FR and RES blocked the binding of ET-1 to bovine cerebellum, in which ETB receptors are predominant (14), and inhibited ET-3-induced endothelium-dependent relaxation in isolated rat aorta (17).

To confirm the in vivo effects of FR and RES as ETA and ETB receptor antagonists, we observed the inhibitory effects of these antagonists on the responses of MAP, CO and systemic vascular resistance to exogenously administered human ET-1 (Peptide Institute, Japan). Twelve dogs were randomly selected and studied when they had completely recovered from the effects of surgery. After the hemodynamic status had stabilized, ET-1 (0.75 nmol/kg, n = 4) was injected as a bolus. FR (1 mg/kg, n = 4) or RES (0.2 mg/kg, n = 4) was administered 5 min before ET-1 injection. The change in MAP was recorded for 30 min in each group. CO was measured before and 30 min after ET-1 injection and systemic vascular resistance was calculated.

Study 2: cardiorenal and hormonal effects of FR and RES in dogs with CHF. To evaluate the effects of endogenous ETs mediated by ETA and ETB receptors in CHF, we separated the dogs into three groups: The FR group (n = 8) was given FR, the RES group (n = 8) was given RES and the vehicle group (n = 5) received only saline solution as a control. All subsequent measurements were recorded with ongoing rapid ventricular pacing. As previously reported, the urinary bladder was catheterized with a CLINY balloon tube (Create Medic Co.) under brief thiopental sodium anesthesia (4 mg/kg), and 45 min later a priming dose of creatinine (50 mg/kg) and para-aminomhippurate (8 mg/kg) dissolved in 10 ml of saline solution was infused over a period of 10 min (15). This infusion was followed by a constant infusion (0.75 ml/min) of creatinine (1.0 mg/kg per min) and para-aminomhippurate (0.3 mg/kg per min) throughout the experimental period. After a 60-min equilibration period, the first of two clearance periods was performed, each of which lasted for 20 min. After two sequential baseline periods, in the FR group, the first bolus dose of FR at 1 mg/kg was administered intravenously. After 30 min, the second dose of 10 mg/kg was injected, and two 30-min clearances were performed for each dose. In the RES group, RES was administered at 0.2 and 1.5 mg/kg at intervals of
30 min in the same way. In the vehicle group, saline solution was administered at intervals of 30 min to exclude any temporal effects. During each clearance period, the cardiac pressure data were recorded every 5 min, and CO and blood sampling were performed at the end of each period.

**Analysis of blood and urine samples.** The plasma ET-1 concentration was determined by radioimmunoassay as previously described (18). Blood for aldosterone and ANP assay was measured by radioimmunoassay as previously described (15,19). Plasma norepinephrine concentration was performed by high performance liquid chromatography as previously reported (15). Serum and urinary creatinine, para-aminohippurate and sodium concentrations were measured as previously reported (15). Creatinine clearance and para-aminohippurate clearance were calculated by using standard formulas, and were equated with glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively. Filtration fraction was calculated as GFR/RPF. Fractional excretion of sodium was calculated by the standard formula.

**Statistical analysis.** All data are presented as mean value ± SEM. Analysis of variance for repeated measurements was used to determine the significance of changes during multiple time-dependent observations. Comparisons with baseline values were analyzed by Dunnett’s test after analysis of variance for repeated measurements. The Student t test was used to analyze the significance of single comparisons. A p value < 0.05 was considered significant.

**Results**

**Blocking effects of FR and RES on ET-1 injection.** The effects of FR and RES on ET-1–induced changes are shown in Figure 1, A to D. ET-1 produced an initial transient reduction in MAP followed by a sustained increase in MAP that was maximal within 18 min of injection. Corresponding to changes in elevated MAP, systemic vascular resistance increased and CO decreased. Pretreatment with FR significantly blocked ET-1–induced hypertension for about 30 min after ET-1 injection and inhibited the reduction in CO and elevation of systemic vascular resistance compared with values obtained with ET-1 administration alone. In contrast, RES significantly inhibited the initial transient reduction in MAP and augmented the reduction in CO and the significant elevation of systemic vascular resistance.

**Characteristics of CHF.** Baseline hemodynamic and endocrine characteristics of CHF induced by rapid right ventricular pacing are summarized in Table 1 and Figure 2. In the FR, RES and vehicle groups, PCWP was significantly increased but MAP and CO were decreased relative to the respective baseline values. Although right atrial pressure was increased, these changes were not significant. Individual plasma ET-1 levels were significantly increased compared with those in normal control dogs, even though there was heterogeneity in this model (Fig. 2). Plasma ANP, aldosterone and norepinephrine concentrations were significantly increased in all three groups. In addition to the deteriorated hemodynamic status and the
activated hormonal secretion, CHF was considered present in all of the dogs on the basis of evidence of anorexia and signs of exertional dyspnea. When the hemodynamic and hormonal baseline data of all experimental animals were pooled, the differences among the three groups were not significant.

**Hemodynamic effects of FR and RES.** The hemodynamic responses to the two incremental doses of FR or RES were compared (Fig. 3, A to F). FR dose-dependently decreased MAP and PCWP in association with significant reductions in systemic and pulmonary vascular resistance. FR significantly increased CO. However, RES caused significant elevation of systemic and pulmonary vascular resistance. FR significantly decreased MAP and PCWP in association with significant reductions in concentrations of ET-1. FR significantly decreased plasma ANP levels, but RES did not alter ANP levels, despite significant elevation of PCWP. RES significantly decreased plasma aldosterone levels, but FR did not affect them. FR tended to increase, whereas RES slightly reduced plasma norepinephrine levels, but these changes were not significant. None of these variables changed significantly in the vehicle group.

**Renal effects of FR and RES.** The effects of FR and RES on renal functions are shown in Table 2. FR significantly increased the urinary flow rate and absolute urinary sodium excretion over average basal values. FR also significantly increased GFR and RPF, but it did not affect fractional sodium excretion or filtration fraction. However, RES significantly decreased RPF and increased the urinary flow rate. RES tended to reduce urinary sodium excretion and GFR, but these changes were not significant. RES significantly increased filtration fraction but did not affect fractional sodium excretion. There were no significant changes in the vehicle group only.

**Discussion**

The present study shows that FR, an ET\(A\) receptor antagonist, significantly reduced blood pressures and plasma ANP level and increased CO. FR also increased the urinary flow rate and sodium excretion associated with increasing GFR and RPF. In contrast, RES, an ET\(B\) receptor antagonist, elevated cardiac pressures and decreased CO. RES also decreased the plasma aldosterone level and RPF. Neither antagonist affected plasma norepinephrine levels. These results suggest that endogenous ETs increase vascular resistance and retain body fluids to maintain tissue perfusion through ET\(A\) receptors in CHF. The vasodilative action through ET\(B\) receptors is overall functionally more important than the constrictive action through ET\(B\) receptors. The secretion of ANP and aldosterone is regulated by endogenous ETs to modulate the vascular tone and body fluid balance in CHF.

In study 1, pretreatment with FR did not show any inhibitory effect on the initial transient depressor response to ET-1, but it inhibited the subsequent pressor response. In contrast,
RES blunted the initial hypotension and augmented the increase in systemic vascular resistance and decrease in CO observed with ET-1 administration alone. Because the initial transient depressor response has been reported (5) to be mediated by ETB receptors, mainly through the release of relaxing factors from vascular endothelium, the inhibition of the initial depressor response must be the result of selective antagonism of ETB receptors in vivo. As ETs act through autocrine and paracrine mechanisms at local targets, plasma ET-1 concentrations do not precisely reflect true ET activity and there is no adequate index in intact animals for ET activity. We considered the most overt index to be hemodynamic changes, and we administered antagonists at doses that significantly affected blood pressure. FR and RES sufficiently inhibited the effects of the pharmacologic dose of ET-1 in vivo. Therefore, both antagonists were suitable for examining the effects mediated by each ET receptor in CHF.

**Hemodynamic changes.** It is well known that ETA receptors mediate vasoconstriction, whereas ETB receptors mediate vasodilation through the release of nitric oxide and prostacyclin (5). However, because ETA and ETB receptors exist in vascular smooth muscle cells and both receptors are involved in the pressor response (9), the extent to which endogenous ETs actually contribute to the regulation of blood pressures through each receptor type has not yet been clarified in CHF. In the present study, FR reduced MAP in association with a decrease in systemic vascular resistance and increased CO, whereas RES impaired hemodynamic status. Although a mixed ETA/B receptor antagonist lowers blood pressure (11), there has been no report that the systemic administration of a selective ETB receptor antagonist induces vasoconstriction but not vasodilation in CHF. Our results indicate that ETA receptors exert a pathophysiologic vasoconstrictive effect and that ETB receptors generally mediate vasodilative action rather than vasoconstriction in CHF. A selective ETA antagonist may have a beneficial hemodynamic effect and the blockade of ETB receptors may produce hemodynamic deterioration by inhibiting vasodilative actions. Although ET-1 has a positive inotropic effect on cardiac muscle (20), the potent coronary vasoconstrictive effect of ET-1 caused myocardial ischemia resulting in
decreased CO (6,7). FR might elicit a negative inotropic effect (10), but this effect was overcome by the marked reduction of vascular resistance.

Changes in plasma hormones. As for changes in plasma ET-1 levels in response to ET antagonists, RES led to a twofold increase in plasma ET-1 levels, whereas FR did not significantly change plasma ET-1 levels. It was reported (21) that intravenous ET-1 in rats was trapped by lungs, kidneys and liver and that the retention of ET-1 was markedly reduced by an ETB receptor but not by an ETA receptor antagonist. The secretion of ET-1 into the cultured human endothelial cells was enhanced by an ETB receptor antagonist without activating preproET-1 messenger ribonucleic acid (mRNA) synthesis (22). These results suggest that the increased ET-1 may be displaced from ETB receptors of the endothelial cell layers or that ETB receptors may mediate the clearance of ET-1 in plasma.

ANP is a potent vasodilator and has effects opposite to
those of ET-1. We (18) previously reported that endogenous ANP inhibited the secretion of ET-1 through a cyclic guanosine monophosphate (cGMP) pathway using an ANP receptor antagonist. In rat atrial myocytes, an ETA receptor antagonist, BO-123, dose-dependently blocked ET-stimulated ANP secretion, whereas an ETB receptor agonist, IRL-1620, had no effect (23). In vitro, ET-1 may directly stimulate ANP secretion through ETA receptors. However, in the present study, the reduction in ANP with FR may reflect hemodynamic improvement, expressed as a lowering of PCWP. Because ANP is secreted in proportion to the severity of CHF and is a marker for the prognosis of patients with CHF (24), the decline in ANP levels with FR may indicate that an ETA receptor antagonist can attenuate the aggravation of CHF.

ET-1 directly stimulates aldosterone secretion in the adrenal zona glomerulosa (25), and the administration of ET-1 increases the plasma aldosterone concentration in dogs (6,7). Recently Belloni et al. (26) demonstrated that ET-1 enhanced aldosterone secretion in dispersed zona glomerulosa cells, and an ETB antagonist, but not an ETA antagonist, markedly reduced this secretory response. As our findings are consistent with these previous results, endogenous ETs may regulate aldosterone secretion through ETB receptors in CHF.

Changes in renal function. In vitro, ET-1 has direct diuretic and natriuretic actions that inhibit Na\(^+\)-K\(^-\) adenosine triphosphatase activity (27) and reduce antidiuretic hormone-stimulated water permeability in collecting ducts and results in the excretion of hypotonic urine (28). However, the systemic administration of ET-1 caused a profound increase in renal vascular resistance and a reduction in urinary and sodium excretion, associated with a significant decrease in RPF and GFR (6,29). The roles of endogenous ETs in the kidney seem to be very complex. In the present study, the increase in urinary and sodium excretion induced by FR appear to be the consequence of increased GFR and RPF. However, RES increased urinary excretion despite reducing RPF. The exact mechanism of these effects is unclear, and further investigation is needed. As RES increased filtration fraction, RES might produce a proportionally greater vasoconstriction in efferent rather than afferent arterioles and increase the pressure within the downstream glomerular capillary (29). Elevation of the net filtration pressure might overcome the decrease in RPF and preserve GFR after RES administration and increase rather than decrease the urinary flow rate (30). In fact, administration of an NO synthase inhibitor and a prostaglandin inhibitor elevated MAP and increased the urinary flow rate despite decreasing RPF (31,32). Therefore, we consider that the increase in urinary flow produced by RES is a transient effect and the long-term inhibition of ETs receptors decreases RPF and results in decreased urinary excretion. Although Brooks et al. (33) reported that the ETs receptor may inhibit sodium reabsorption in a normal canine model, RES did not affect absolute or fractional sodium excretion in the present study. The direct tubular effects of endogenous ETs seemed to be slight in CHF.

Limitations of the study. Because the development of tachycardia-induced CHF is reversible and the termination of rapid pacing results in improved cardiac and hormonal functions (34), we performed these experiments with ongoing rapid pacing even though it is very important to assess the effects of the antagonists on changes in heart rate. Furthermore, it is possible that a selective ET receptor antagonist blocking effects mediated by one receptor may simultaneously augment the effects mediated by the other receptor. Therefore, the observed effects of ET antagonists may be in part the result of endogenous ETs acting to stimulate the other ET receptor. However, we could not accurately distinguish direct inhibitory effects from actions augmented through other ET receptors in our study.

Conclusions. For the treatment of CHF, a selective ET receptor antagonist may offer therapeutic benefits improving for both hemodynamic status and diuresis, whereas the inhibition of ETs receptors may have adverse effects on cardiac and renal hemodynamics. However, it is possible that long-term administration of ETs blockers may prevent volume retention through the inhibition of aldosterone secretion in dogs with CHF.

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References