Effects of Combined Angiotensin II and Endothelin Receptor Blockade With Developing Heart Failure

Effects on Left Ventricular Performance

R. Brent New, MD; Angela C. Sampson, BS; Mary K. King, DVM; Jennifer W. Hendrick, BA; Mark J. Clair, MS; James H. McElmurray III, BS; Jeffrey Mandel, MD; Rupak Mukherjee, PhD; Marc de Gasparo, MD; Francis G. Spinale, MD, PhD

Background—The goal of this study was to determine the comparative effects of angiotensin II type 1 (AT_1) receptor inhibition alone, endothelin-1 (ET) receptor blockade alone, and combined receptor blockade on left ventricular (LV) function, contractility, and neurohormonal system activity in a model of congestive heart failure (CHF).

Methods and Results—Pigs were randomly assigned to each of 5 groups: (1) rapid atrial pacing (240 bpm) for 3 weeks (n=9), (2) concomitant AT_1 receptor blockade (valsartan, 3 mg/kg per day) and rapid pacing (n=8), (3) concomitant ET receptor blockade (bosentan, 50 mg/kg BID) and rapid pacing (n=8), (4) concomitant combined AT_1 and ET receptor inhibition and rapid pacing (n=8), and (5) sham-operated control (n=9). LV stroke volume was reduced from the control value after rapid pacing, was unchanged with either AT_1 or ET receptor blockade alone, but was improved with combination treatment. LV peak wall stress was reduced in both groups with ET receptor blockade compared with the rapid pacing group. Plasma norepinephrine levels were increased by >3-fold after rapid pacing, remained increased in the monotherapy groups, but were reduced after combination treatment. LV myocyte velocity of shortening was reduced after rapid pacing–induced CHF, remained reduced after AT_1 receptor blockade, increased after ET receptor blockade (compared with rapid pacing–induced CHF values), and returned to within control values after combined blockade.

Conclusions—Combined AT_1 and the ET receptor blockade in this model of CHF improved LV pump function, and contributory factors included the effects of LV loading conditions, neurohormonal system activity, and myocardial contractile performance. Thus, combined receptor blockade may provide a useful combinatorial therapeutic approach in CHF. **(*Circulation*. 2000;102:1447-1453.)**

Key Words: ventricles ■ norepinephrine ■ hemodynamics ■ heart failure ■ angiotensin ■ endothelin

The progression of congestive heart failure (CHF) is accompanied by left ventricular (LV) pump dysfunction and neurohormonal system activation. Specifically, increased circulating levels of catecholamines, angiotensin II (Ang II), and endothelin-1 (ET) have all been identified to occur with the development of LV dysfunction and are related to the severity of the CHF process. Studies have demonstrated that Ang II type 1 (AT_1) receptor blockade can be successfully instituted in the setting of CHF and may provide favorable effects.[1,2] Activation of the ET receptor has been demonstrated to modulate a wide variety of biological processes, including vascular tone and myocardial contractile function.[3–6] Chronic activation of the ET receptor system occurs with CHF and therefore may contribute to the progression and severity of LV dysfunction in the setting of CHF.[3,5–8] There is experimental evidence to suggest that interactions exist between the Ang AT_1 and ET receptor transduction pathways.[9,10] The central hypothesis of this project was that compared with either treatment alone, combined AT_1 and ET receptor blockade would provide additional effects on LV function and contractility in the setting of developing CHF.

Methods

Dose Determination Studies

The selective AT_1 receptor antagonist valsartan (Novartis) and the mixed ET_A/ET_B receptor antagonist bosentan (Actelion LTD) were used.[11,12] Yorkshire pigs (20 kg, male, Hambone Farms, Orangeburg, SC; n=10) were instrumented for measurement of aortic blood pressure in the conscious state.[13–15] Ang II (10 μg, Sigma Chemical Co) or ET (10 μg, Sigma) was infused, and aortic pressure was recorded. After the control measurements, peritoneal osmotic

Received November 1, 1999; revision received April 13, 2000; accepted April 30, 2000.

From the Division of Cardiothoracic Surgery (R.B.N., A.C.S., M.K.K., J.W.H., M.J.C., J.H.M., J.M., R.M., F.G.S.), Medical University of South Carolina, Charleston, and the Pharmaceutical Division (M.d.G.), Novartis, Basel, Switzerland.

Guest Editor for this article was Wilson S. Colucci, MD, Boston Medical School, Boston, Mass.

Correspondence to Francis G. Spinale, MD, PhD, Cardiothoracic Surgery, Room 625, Strom Thurmond Research Building, 770 MUSC Complex, Medical University of South Carolina, 114 Doughty St, Charleston, SC 29425.

Circulation **is available at http://www.circulationaha.org**

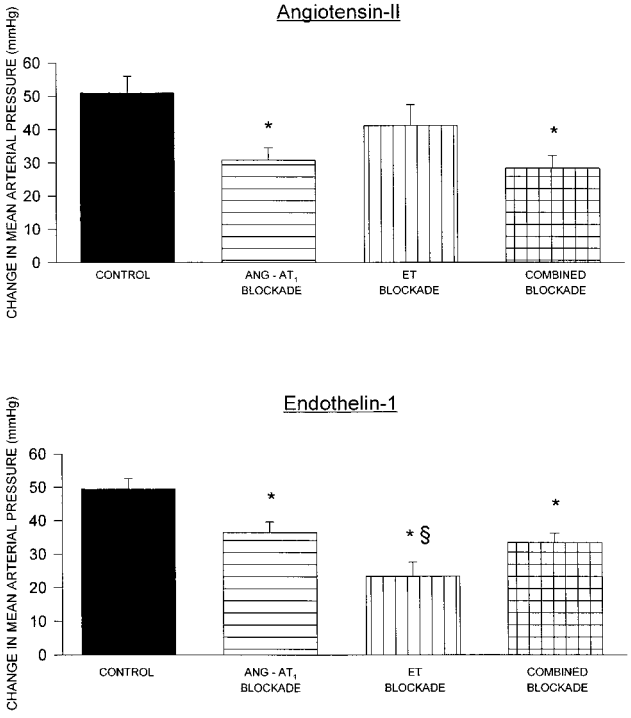

Figure 1. Percent change in mean arterial pressure after infusion of 10 μg of Ang II (top) or ET (bottom) in conscious pigs. AT_1 receptor blockade (valsartan, 3 mg/kg per day) caused a significant reduction in the Ang II pressor response. Combined AT_1 and ET receptor blockade (bosentan, 50 mg/kg BID) for 3 days also blunted the Ang II pressor response. Treatment with AT_1 or ET receptor blockade as well as combined treatment reduced the ET pressor response from untreated control values. However, the greatest reduction was observed with ET receptor blockade alone. *$P<0.05$ vs untreated control; §$P<0.05$ vs AT_1 receptor blockade alone.

minipumps (2 ML1, Alza Corp; n=5) containing valsartan were implanted to maintain a dose of 3 mg/kg per day of the AT_1 receptor antagonist.[13] This route of delivery provided a more consistent plasma level for this AT_1 receptor antagonist.[13] In a second group (n=5), bosentan was administered in an oral formulation at a dose of 50 mg/kg BID. The chemical formulation of bosentan and the quantity required to be delivered daily prevented osmotic pump delivery. This dosing regimen for bosentan caused a small but significant fall in resting blood pressure from control values (91±2 versus 101±2 mm Hg, $P<0.05$). In preliminary studies, higher doses of bosentan caused a significant fall in systemic pressure and tachycardia. At 5 days of treatment, AT_1 receptor blockade reduced the Ang II pressor response, and ET receptor blockade reduced the ET pressor response (Figure 1). Pigs with implanted minipumps were then administered bosentan (50 mg/kg BID) for 5 days. With this combination treatment, resting blood pressure was reduced from control values (88±4 mm Hg, $P<0.05$) but was not different from values measured after ET receptor blockade only. Combined AT_1 and ET receptor blockade significantly blunted both the Ang II and ET pressor responses (Figure 1).

Experimental Design

Forty-two pigs were instrumented with a vascular access port and a modified pacemaker to induce CHF by rapid pacing.[13–16] The animals were assigned to the following treatment protocols: (1) rapid atrial pacing (240 bpm) for 3 weeks (n=9), (2) concomitant AT_1 receptor blockade (3 mg/kg valsartan per day by osmotic infusion pump) and rapid pacing (n=8), (3) concomitant ET receptor blockade (50 mg/kg bosentan BID by oral route) and rapid pacing (n=9), (4) concomitant combined AT_1 and ET receptor inhibition and rapid pacing (n=8), and (5) sham-operated control (n=9). The drug treatment protocols were begun at the initiation of pacing and were continued for the entire 21-day pacing protocol. All animals were treated and cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health (National Research Council, Washington, 1996).

LV Function and Neurohormonal Measurements

Two-dimensional echocardiographic studies were used to image the LV for the measurement of LV dimensions, wall thickness, and fractional shortening.[13–15] Systemic aortic pressure was simultaneously measured to determine LV peak wall stress.[13–15] After which, blood was collected for neurohormonal assay.[13–15] After the LV echocardiographic studies, the pigs were anesthetized for a more comprehensive study of LV function and hemodynamics.[14] A precalibrated microtipped transducer (7.5F, Millar Instruments Inc) was placed in the LV apex. Four piezoelectric crystals (2 mm, Sonometrics) were positioned on the LV anterior free wall to obtain orthogonal myocardial dimensions.[14] LV preload was altered by sequential occlusion and release of the inferior vena cava. LV myocardial velocity of circumferential fiber shortening, corrected for heart rate, was computed from the digitized LV crystal and pressure data as described previously.[17]

LV Morphometry and Myocyte Studies

The region of the LV free wall constituting the left anterior descending artery was perfusion-fixed with 2.5% glutaraldehyde solution at 50 mm Hg, and myocardial sections were prepared to measure myocyte cross-sectional area.[15,16] The posterior region of the LV free wall (3×3 cm) was snap-frozen in liquid nitrogen for subsequent analysis of Ang II and ET content.[10,16] The cannulated coronary artery was perfused with a collagenase solution to obtain viable LV myocytes for study.[15,16] Isolated myocyte function was examined as previously reported by this laboratory.[15,16] Myocytes were also examined in 1 of 3 ways: (1) after β-adrenergic receptor stimulation with 25 nmol/L (−)isoproterenol (Sigma), (2) after exposure to 200 pmol/L ET (Sigma), or (3) with increased extracellular Ca^{2+} (8 mmol/L).

Data Analysis

LV function, systemic hemodynamics, neurohormonal profiles, and contractility were compared by ANOVA, and pairwise tests of individual group means were performed by use of Bonferroni probabilities. All LV measurements, morphometric analyses, and myocyte contractility studies were performed in a blinded fashion with respect to treatment. Results are presented as mean±SEM.

Results

LV Function and Hemodynamics

LV function and hemodynamics are summarized in Table 1. One pig in the ET blockade group died during the terminal catheterization study, and the final sample size is reported. LV end-diastolic dimension increased, and fractional shortening decreased in all rapid pacing groups compared with the control group. LV end-diastolic dimension was lower, and fractional shortening was higher in the combination treatment group. Cardiac output was reduced in the rapid pacing group and both monotherapy groups but was normalized in the combination group. Compared with the untreated rapid pacing value, LV peak wall stress was reduced in both ET receptor blockade groups. Systemic and pulmonary vascular resistances were increased after rapid pacing and returned to within control values after ET receptor blockade and combination treatment. The slope of the relation of the velocity of circumferential fiber shortening, corrected for heart rate, to end-systolic wall stress was reduced in all rapid pacing

TABLE 1. Systemic Hemodynamics With Chronic Rapid Pacing: Effects of Ang II Inhibition, ET Inhibition, or Combined Treatment

	Control	Rapid Pacing	Rapid Pacing/AT_1 Blockade	Rapid Pacing/ET Blockade	Rapid Pacing/AT_1/ET Blockade
Resting heart rate, bpm	87±5	144±9*	137±10*	145±8*	128±8*
LV geometry and pump function					
End-diastolic dimension, cm	3.8±0.1	5.6±0.2*	5.4±0.2*	5.3±0.2*	5.2±0.1*†
Fractional shortening, %	41.7±2.5	20.0±2.2*	25.5±2.2*	25.0±2.2*	27.4±2.3*†
Stroke volume, mL	33.85±2.81	14.89±1.29*	15.27±1.60*	17.93±1.86*	24.87±2.16*†§¶
Cardiac output, L/min	2.83±0.13	2.08±0.15*	2.03±0.19*	2.51±0.22	3.11±0.20†§¶
Pressures					
Mean arterial pressure, mm Hg	88±2	80±3*	82±3	77±5*	80±3*
LV peak systolic pressure, mm Hg	122±4	106±4*	112±4	99±5*§	103±3*
LV end-diastolic pressure, mm Hg	8±1	15±2*	17±4*	19±1*	15±2*
PA mean pressure, mm Hg	14±2	26±2*	23±3*	28±2*	22±2*
Peak+dP/dt, mm Hg/s	1875±161	1379±107*	1449±111*	1272±136*	1358±103*
LV peak systolic wall stress, g/cm^2	149.9±5.6	343.2±29.4*	330.6±28.4*	248.7±17.0*†§	257.8±13.4*†§
Vcf_c–end-systolic stress slope, $\times 10^{-3} \cdot s^{-0.5} \cdot cm^2 \cdot g^{-1}$	−17.6±3.6	−3.4±1.8*	−4.9±1.2*	−7.3±1.6*	−8.9±1.4*†§
Resistances					
Systemic, dyne · s · cm^{-5}	2534±123	3186±206*	3391±319*	2541±185†§	2116±149*†§
Pulmonary, dyne · s · cm^{-5}	244±67	442±86*	256±51†	292±49	233±28†
Sample size, n	9	9	8	8	8

Values are mean±SEM.

Rapid pacing indicates 21 days of rapid pacing at 240 bpm; rapid pacing/AT_1 blockade, 60 mg/d valsartan (osmotic pump); rapid pacing/ET blockade, 1 g/d PO bosentan BID; rapid pacing/AT_1/ET blockade, 60 mg/d valsartan (osmotic pump) and 1 g/d PO bosentan BID; PA, pulmonary artery; and Vcf_c, heart rate–corrected velocity of circumferential fiber shortening.

*$P<0.05$ vs control; †$P<0.05$ vs rapid pacing only; §$P<0.05$ vs rapid pacing/AT_1 blockade; and ¶$P<0.05$ vs rapid pacing/ET blockade.

groups. The slope of this relation increased in the combination treatment group.

Plasma Neurohormones and Drug Levels

Plasma norepinephrine increased by 3-fold after rapid pacing and remained elevated after chronic AT_1 receptor blockade (Figure 2). In the combined treatment group, plasma norepinephrine levels returned to control values. Plasma renin activity was reduced from AT_1 receptor blockade values after ET receptor blockade. Plasma Ang II levels were highest in the groups with AT_1 receptor blockade only and with combined treatment. Plasma ET increased in all rapid pacing groups and was increased further in both ET receptor blockade groups. Plasma levels of valsartan were 220±37 nmol/L in the AT_1 receptor blockade group and 328±69 nmol/L in the combined treatment group ($P=0.21$).

LV Myocardial ET and Ang II Content

LV myocardial ET content was increased in the untreated rapid pacing group compared with the control group (Figure 3). LV myocardial ET was increased in AT_1 receptor blockade group compared with the control group and was lower in both ET receptor blockade groups. Myocardial Ang II levels were increased in all pacing groups. Myocardial Ang II levels were lower in the AT_1 receptor blockade only group than in the untreated rapid pacing group.

LV Myocyte Geometry and Contractility

LV myocyte cross-sectional area was decreased from control values after chronic rapid pacing (252±3 versus 269±12 μm^2, $P<0.05$). Compared with the control value, LV myocyte cross-sectional area remained reduced in the AT_1 receptor blockade group and the ET receptor blockade group (235±18 and 238±10 μm^2, respectively; both $P<0.05$). In the combination treatment group, LV myocyte cross-sectional area was not different from control values (272±25 μm^2). LV isolated myocyte resting length was increased in the rapid pacing group compared with the control group (163±4 versus 132±3 μm, $P<0.05$). Resting myocyte length remained increased in all receptor blockade groups, with values similar to those in the untreated rapid pacing group.

LV Myocyte Contractility

Steady-state myocyte contractile function was examined in >500 myocytes from each group (minimum of 75 myocytes per pig), with representative contraction profiles shown in Figure 4 and results summarized in Table 2. In the untreated rapid pacing group, myocyte percentage and velocity of shortening were reduced to 50% of the control values. In the AT_1 receptor blockade and rapid pacing group, indices of LV myocyte contractile function were similar to untreated rapid pacing values. In the ET receptor blockade and rapid pacing group, myocyte velocity of shortening was increased com-

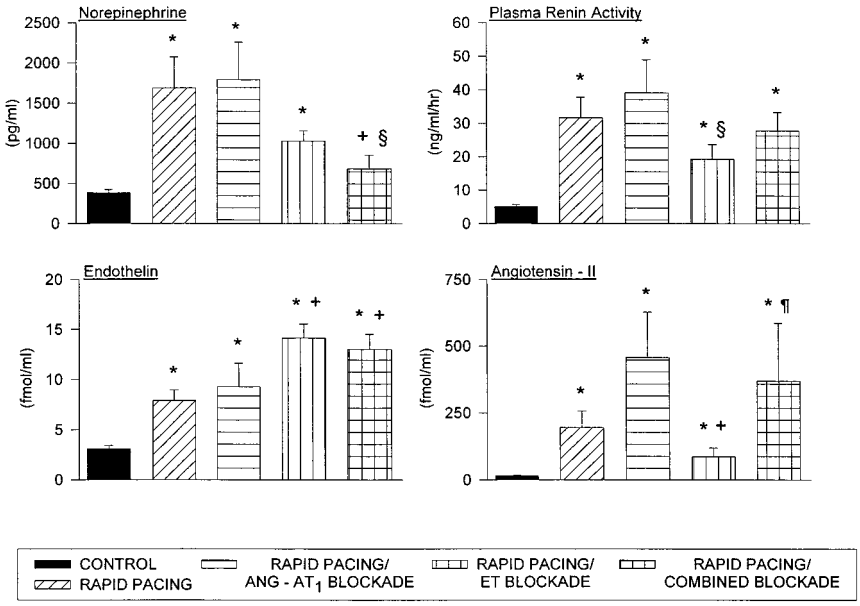

Figure 2. Plasma norepinephrine levels increased by 3-fold with pacing-induced CHF and remained elevated after chronic AT_1 receptor blockade. Concomitant treatment with ET receptor blockade during pacing reduced plasma norepinephrine levels compared with levels after pacing-induced CHF ($P=0.0568$). In combined treatment group, norepinephrine level was not different from control. Plasma renin activity was reduced from AT_1 receptor blockade values after ET receptor blockade. Plasma Ang II levels were increased in all rapid pacing groups, with highest values observed in AT_1 receptor blockade group and combination treatment group. Plasma ET increased in all rapid pacing groups and was increased further in both ET receptor blockade groups. *$P<0.05$ vs control values; +$P<0.05$ vs rapid pacing only; §$P<0.05$ vs AT_1 receptor blockade alone; and ¶$P<0.05$ vs ET receptor blockade.

pared with rapid pacing values. In the combined AT_1 and ET receptor blockade group, indices of myocyte contractile function were increased compared with the untreated rapid pacing values and with the monotherapy treatment values.

The absolute change in myocyte velocity of shortening was computed for myocytes after either β-receptor stimulation, exposure to increased extracellular Ca^{2+}, or ET blockade (Figure 5). LV myocyte inotropic response to β-receptor stimulation was significantly blunted in the untreated rapid pacing group and the AT_1 receptor blockade only group compared with the control group. In the ET receptor blockade group and the combined treatment group, β-adrenergic response was significantly improved compared with untreated pacing values. Myocyte inotropic response to extracellular Ca^{2+} was reduced in all rapid pacing groups compared with the control group but was higher in the ET receptor blockade group and the combined treatment group than in the untreated rapid pacing group. In all rapid pacing groups, ET caused a significant negative effect on myocyte contractile function compared with control values.

Discussion

The development and progression of CHF is accompanied by increased circulating levels of Ang II and ET. Inhibition of the AT_1 receptor has been demonstrated to provide beneficial effects in studies of CHF.[1,2,13] ET receptor inhibition has also been demonstrated to provide effects on LV function and hemodynamics in the setting of CHF.[3,6] The overall goal of the present study was to determine the effects of combined AT_1 and ET receptor blockade in a model of pacing-induced CHF. The important and unique findings of the present study were 3-fold. First, combined AT_1 and ET receptor blockade improved the indices of LV ejection performance to a greater degree than was obtained with either receptor antagonist alone. Second, combined receptor blockade with chronic rapid pacing reduced systemic vascular resistance and plasma catecholamines to a greater degree than was obtained by treatment with either receptor antagonist alone. Third, LV myocyte function was increased with combined receptor blockade, indicating an endogenous improvement in LV myocardial contractility. Thus, combined AT_1 and ET receptor blockade in CHF may provide beneficial effects that are due to improved LV loading conditions and contractility.

There is evidence to suggest that cross talk occurs between the AT_1 and ET receptor systems.[9,10,18,19] Combined AT_1 and ET receptor blockade reduced systemic vascular resistance to a greater degree than was achieved by either receptor antagonist alone, and combined receptor blockade reduced plasma norepinephrine to a greater degree than was achieved by either receptor antagonist alone. This observation suggests that a synergistic effect occurred with AT_1 and ET receptor blockade with respect to sympathetic efferent activity. Plasma renin activity and Ang II levels were increased with pacing-induced CHF and appeared to increase further with AT_1 receptor blockade, consistent with a pharmacological interruption of the renin-angiotensin system. ET plasma levels were increased in the ET receptor blockade groups; this increase was likely due to inhibition of the receptor-mediated clearance of circulating ET. Myocardial Ang II levels were increased with pacing-induced CHF. The reduction in myocardial Ang II levels in the AT_1 receptor blockade group likely reflect reduced uptake of Ang II into myocardial cells as well as potentially reduced synthesis. Combined AT_1 and ET receptor blockade may have prevented chronic activation of both of these receptor systems within the myocardial compartment with pacing-induced CHF, which in turn would provide a protective effect on myocyte contractile performance in vivo.

The underlying basis for the changes in LV geometry and function with pacing-induced CHF include structural remodeling of the myocardium and intrinsic defects in contractility.[5,6,13–16] A structural basis for the LV dilation and subsequently increased wall stress with pacing CHF is reduced myocyte cross-sectional area and increased length. AT_1 or ET receptor blockade alone did not significantly reduce the degree of LV dilation after chronic pacing and did not alter

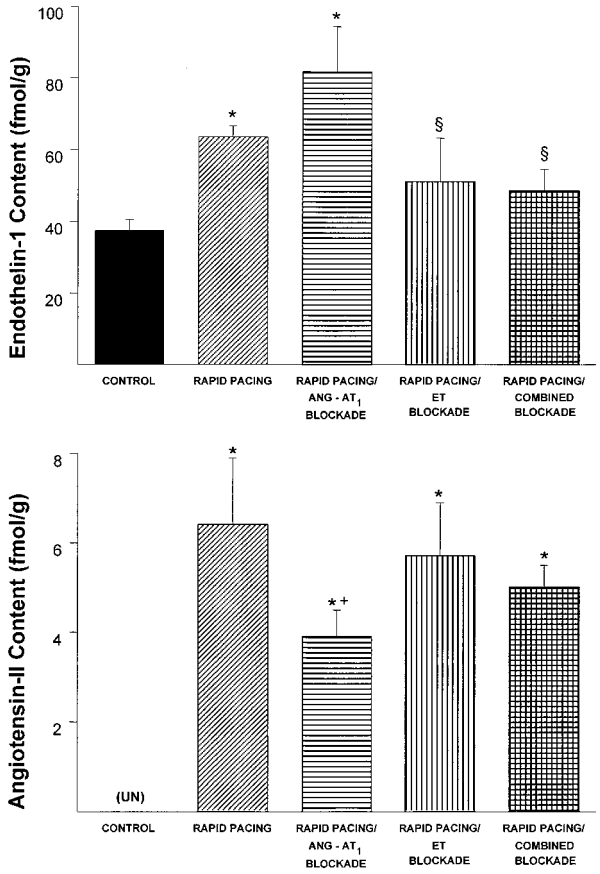

Figure 3. Top, LV myocardial ET was increased in untreated rapid pacing groups compared with control group. LV myocardial ET content remained increased in AT_1 receptor blockade group compared with control group. In both ET receptor blockade groups, myocardial ET levels were lower than those measured in AT_1 receptor blockade group and were not different from control values. Bottom, LV myocardial Ang II levels were below detection limits (UN) in control myocardial samples but were increased in all pacing groups. In AT_1 receptor blockade group, myocardial Ang II levels were reduced from pacing only values. *$P<0.05$ vs control; +$P<0.05$ vs rapid pacing only; §$P<0.05$ vs AT_1 receptor blockade.

isolated LV myocyte geometry compared with untreated pacing-induced CHF levels. Combined AT_1 and ET receptor blockade reduced the degree of LV dilation, albeit to a modest degree after pacing-induced CHF. These changes in LV geometry in the combination blockade group were paralleled by a normalization of LV myocyte cross-sectional area.

LV ejection performance was significantly improved in the combination treatment group compared with the untreated rapid pacing group. However, significant changes in LV geometry and neurohormonal systems occurred in this model of CHF, with and without treatment; therefore, the in vivo indices of myocardial contractility are difficult to interpret. Accordingly, LV isolated myocyte contractile performance was examined in all treatment groups. Combined ET and AT_1 receptor blockade improved the indices of steady-state myocyte contractile function to a greater degree than was achieved with either receptor antagonist alone. A fundamental component of severe CHF is depressed inotropic responsiveness, particularly to β-receptor stimulation. Concomitant monotherapy by AT_1 receptor blockade with chronic rapid pacing did not significantly influence myocyte β-adrenergic responsiveness. In contrast, ET receptor blockade, with and without AT_1 receptor blockade, improved myocyte β-adrenergic responsiveness. Myocyte inotropic response to extracellular Ca^{2+} was improved in the ET receptor blockade group and in the combination treatment group. Thus, the improved myocyte β-adrenergic response was likely due to improvements in Ca^{2+} homeostasis and/or improved myofilament sensitivity to Ca^{2+}. One likely contributory factor for the favorable effects on myocyte contractility that were observed in the combination treatment group was the significant reduction in circulating plasma norepinephrine levels.

The negative inotropic effect of ET in the setting of CHF is probably due to alterations in intracellular transduction pathways. Exposure and activation of myocyte ET receptors have been demonstrated to influence a number of intracellular events that ultimately result in modulating Ca^{2+} exposure to the contractile apparatus.[19,20] Thus, the reduction in contractile function after exposure of CHF myocytes to ET was likely due to exacerbation of these abnormalities in Ca^{2+} homeostatic processes. ET receptor blockade may prevent the

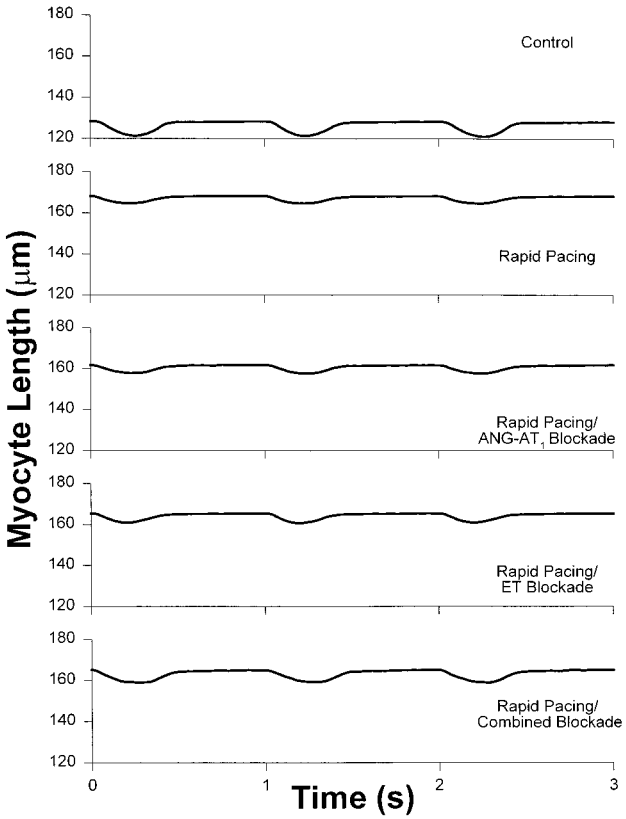

Figure 4. Representative isolated myocyte contraction profiles for control myocytes and myocytes after 3 weeks of chronic rapid pacing with and without concomitant AT_1 receptor blockade, ET receptor blockade, or combination treatment. Isolated myocyte length increased with chronic rapid pacing, and extent of shortening was reduced. In ET receptor blockade and combined treatment groups, myocyte length remained increased from control, but extent of shortening appeared improved from untreated rapid pacing values. Summary data obtained from a large number of myocyte contraction profiles in each treatment group are presented in Table 2.

TABLE 2. Steady-State Isolated Myocyte Contractile Function: Effects of Ang II Inhibition, ET Inhibition, or Combined Treatment

	Control	Rapid Pacing	Rapid Pacing/AT_1 Blockade	Rapid Pacing/ET Blockade	Rapid Pacing/AT_1/ET Blockade
Percent shortening, %	4.98±0.17	2.21±0.12*	2.56±0.13*	2.47±0.09*	3.52±0.32*†§¶
Velocity of shortening, μm/s	64.18±3.59	31.14±2.16*	36.05±1.31*	42.79±2.03*†§	60.79±7.30†§¶
Time to peak contraction, ms	231.87±11.83	242.92±7.10	238.40±10.37	223.15±7.23	227.15±7.38
Total duration, ms	470.81±13.53	496.53±9.51	479.12±15.92	474.58±16.72	482.36±11.02
Myocytes, n	797	552	553	840	809

Values are mean±SEM. Rapid pacing indicates 21 days of rapid pacing at 240 bpm; rapid pacing/AT_1 blockade, 60 mg/d valsartan (osmotic pump); rapid pacing/ET blockade, 1 g/d PO bosentan BID; and rapid pacing/AT_1/ET blockade, 60 mg/d valsartan (osmotic pump) and 1 g/d PO bosentan BID.
*$P<0.05$ vs control; †$P<0.05$ vs rapid pacing only; §$P<0.05$ vs rapid pacing/AT_1 blockade; and ¶$P<0.05$ vs rapid pacing/ET blockade.

negative inotropic effects of increased ET after pacing-induced CHF and, in turn, improve LV function.

There are 2 predominant subtypes of the ET receptor system, ET_A and ET_B. The ET_A receptor subtype is predominant on systemic smooth muscle vasculature and cardiac myocytes and, when activated, causes vasoconstriction and changes in contractility. ET_B receptor activation has been demonstrated to result in NO production and may be an important factor in modulating pulmonary vascular resistance as well as a clearance mechanism for circulating ET.[21] In the present study, the mixed ET receptor antagonist, bosentan, which possesses binding affinity for both receptor subtypes, was used.[12] This ET receptor antagonist was chosen because it has been the best characterized and because clinical studies have been described.[3,22] Blockade of the ET_B receptors may have reduced endothelium-dependent vasodilation.[21] In the present study, treatment with bosentan alone did not reduce pulmonary vascular resistance to a degree similar to that found with AT_1 receptor blockade or with combined treatment. This would suggest that nonselective ET receptor blockade may influence ET_B-mediated pulmonary vascular relaxation. The relative contribution of ET_A and ET_B receptor activation to the progression of the CHF process and whether and to what degree selective versus nonselective ET receptor blockade provides differential effects with developing CHF warrant further study.

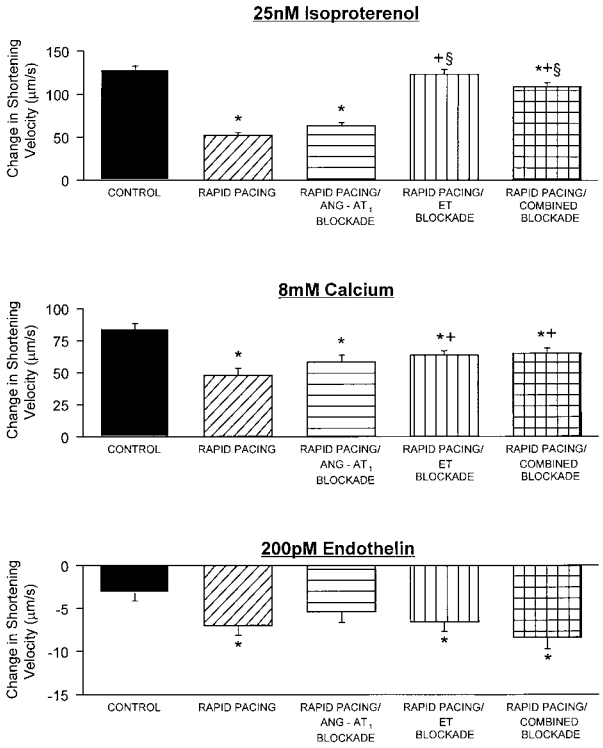

Figure 5. Absolute change in myocyte velocity of shortening was computed in all treatment groups after β-adrenergic receptor stimulation with isoproterenol, with increased extracellular Ca^{2+}, or after exposure to ET. Myocyte β-adrenergic response was blunted in rapid pacing group and AT_1 receptor blockade group. In both ET receptor blockade groups, LV myocyte β-adrenergic responsiveness was increased compared with the rapid pacing only group. In ET receptor blockade only group, absolute increase in LV myocyte shortening velocity after β-receptor stimulation was not different from control value. Myocyte contractile response to increased extracellular Ca^{2+} was decreased in all rapid pacing groups but was higher than untreated rapid pacing values in the ET receptor blockade group and the combined treatment group. Exposure to ET induced a significant negative effect on contractile function compared with control values. This negative influence on myocyte contractile function after exposure to ET was unaffected in all treatment groups. *$P<0.05$ vs control; +$P<0.05$ vs rapid pacing, and §$P<0.05$ vs AT_1 receptor blockade.

Chronic rapid pacing in animals causes an invariable and time-dependent progression to CHF.[5,6,13–16] Thus, the pacing model can be used to examine the effects of interventional strategies on the progression of CHF. However, it must be recognized that any animal model will not fully represent the complex clinical spectrum of CHF. Because receptor blockade was instituted before the induction of CHF, then extrapolation of these findings directly to the clinical presentation of CHF should be performed with caution. The effects of both AT_1 receptor and ET receptor blockade produce dose-dependent effects.[11,12] The present study was performed with the use of a single dosing regimen, and although the doses of receptor antagonists chosen demonstrated pharmacological activity, the potential dose-dependent effects of these antagonists in this model of CHF were not addressed. The doses of AT_1 and ET receptor antagonists used in the present study were the highest that could be used without causing systemic hypotension in the normal porcine preparation. It must be recognized that these doses did not completely abolish the respective Ang II or ET pressor response and that the plasma levels of the AT_1 receptor antagonist were higher in the combination group. Thus, combined treatment may have resulted in a greater degree of AT_1 receptor blockade, which

prevents assessment of additive and/or synergistic effects. Nevertheless, the findings from the present study suggest that both the AT_1 and the ET receptor systems contribute to the progression of the CHF process and that combined receptor blockade may be a useful combinatorial therapeutic approach in CHF.

Acknowledgments

This study was supported in part by National Institutes of Health grants HL-57952 and HL-59165 (Dr Spinale) and a Basic Research Grant from Novartis (Dr Spinale). Dr Spinale is an Established Investigator of the American Heart Association. The authors wish to thank Dr Martine Clozel (Actelion, Ltd, Alschwil, Switzerland) for providing the source of bosentan and Steve Whitebread (Novartis, Basel, Switzerland) for performing the valsartan plasma assays.

References

1. Gottlieb SS, Dickstein K, Fleck E, et al. Hemodynamic and neurohormonal effects of the angiotensin II antagonist losartan in patients with congestive heart failure. *Circulation*. 1993;88(pt 1):1602–1609.
2. Pitt B, Segal R, Martinez FA, et al, on behalf of the ELITE Study Investigators. Randomised trial of losartan versus captopril in patients over 65 with heart failure (Evaluation of Losartan in the Elderly Study, ELITE). *Lancet*. 1997;349:747–752.
3. Kiowski W, Sutsch G, Hunziker P, et al. Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. *Lancet*. 1995; 346:732–736.
4. Seo B, Oemar BS, Siebenmann R, et al. Both ET_A and ET_B receptors mediate contraction to endothelin-1 in human blood vessels. *Circulation*. 1994;89:1203–1208.
5. Thomas PB, Liu ECK, Webb ML, et al. Evidence of an endothelin-1 autocrine loop in cardiac myocytes: relation to contractile function with congestive heart failure. *Am J Physiol*. 1996;271:H2629–H2637.
6. Spinale FG, Walker JD, Mukherjee R, et al. Concomitant endothelin receptor subtype A blockade during the progression of congestive heart failure in rabbits has direct and beneficial effects on left ventricular and myocyte function. *Circulation*. 1997;95:1918–1929.
7. Teerlink JR, Loffler BM, Hess P, et al. Role of endothelin in the maintenance of blood pressure in conscious rats with chronic heart failure: acute effects of the endothelin receptor antagonist Ro 47–0203 (bosentan). *Circulation*. 1994;90:2510–2518.
8. Mulder P, Richard V, Derumeaux G, et al. Role of endogenous endothelin in chronic heart failure: effect of long-term treatment with an endothelin antagonist on survival, hemodynamics, and cardiac remodeling. *Circulation*. 1997;96:1976–1982.
9. Fareh J, Touyz RM, Schiffrin EL, et al. Endothelin-1 and angiotensin II receptors in cells from rat hypertrophied heart: receptor regulation and intracellular Ca^{2+} modulation. *Circ Res*. 1996;78:302–311.
10. Moreau P, d'Uscio L, Shaw S, et al. Angiotensin II increases tissue endothelin and induces vascular hypertrophy: reversal by ET_A receptor antagonist. *Circulation*. 1997;96:1593–1597.
11. Criscione L, de Gasparo M, Bühlmayer P, et al. Pharmacological profile of valsartan: a potent, orally active, nonpeptide antagonist of the angiotensin II AT_1 receptor subtype. *Br J Pharmacol*. 1993;110:761–771.
12. Clozel M, Breu V, Bray GA, et al. Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. *J Pharmacol Exp Ther*. 1994;270:228–235.
13. Spinale FG, de Gasparo M, Whitebread S, et al. Modulation of the renin-angiotensin pathway through enzyme inhibition and specific receptor blockade in pacing induced heart failure, I: effects on left ventricular performance and neurohormonal systems. *Circulation*. 1997; 96:2385–2396.
14. Kribbs SB, Merrit WM, Clair MJ, et al. Amlodipine monotherapy, angiotensin converting enzyme inhibition, and combination therapy with pacing induced heart failure. *Hypertension*. 1998;31:755–765.
15. Spinale FG, Coker ML, Thomas CV, et al. Time-dependent changes in matrix metalloproteinase activity and expression during the progression of congestive heart failure: relation to ventricular and myocyte function. *Circ Res*. 1998;82:482–495.
16. Spinale FG, Mukherjee R, Iannini JP, et al. Modulation of the renin-angiotensin pathway through enzyme inhibition and specific receptor blockade in pacing induced heart failure, II: effects on myocyte contractile processes. *Circulation*. 1997;96:2397–2406.
17. Colan SD, Borrow KM, Neuman A. Left ventricular end-systolic wall stress-velocity of fiber shortening relation: a load independent index of myocardial contractility. *J Am Coll Cardiol*. 1984;4:715–724.
18. Dascal D, Nirula V, Lawus K, et al. Shared determinants of receptor binding for subtype selective and dual endothelin-angiotensin antagonists on the AT1 angiotensin II receptor. *FEBS Lett*. 1998;423:15–18.
19. Jones LG, Rozich JD, Tsutsui H, et al. Endothelin stimulates multiple responses in isolated adult ventricular cardiac myocytes. *Am J Physiol*. 1992;263:H1447–H1454.
20. James AF, Xie LH, Fujitani Y, et al. Inhibition of the cardiac protein kinase A-dependent chloride conductance by endothelin-1. *Nature*. 1994; 370:297–300.
21. Gellai M, DeWolf R, Fletcher T, et al. Contribution of endogenous endothelin-1 to the maintenance of vascular tone: role of nitric oxide. *Pharmacology*. 1997;55:299–308.
22. Sutsch G, Kiowski W, Yan XW, et al. Short term endothelin receptor antagonist therapy in conventionally treated patients with symptomatic severe chronic heart failure. *Circulation*. 1998;98:2262–2268.