Hemodynamic, Hormonal, and Renal Effects of (Pro)Renin Receptor Blockade in Experimental Heart Failure

Miriam T. Rademaker, PhD; Tim G. Yandle, PhD; Leigh J. Ellmers, PhD; Christopher J. Charles, PhD; M. Gary Nicholls, MD; A. Mark Richards, MD, PhD

Background—The (pro)renin receptor (P)RR is implicated in blood pressure regulation and the pathophysiology of heart failure (HF). The effects of (P)RR blockade in HF have not been previously investigated.

Methods and Results—Eight sheep received on 2 separate days a vehicle control and incremental intravenous boluses of a (P)RR antagonist, ovine handle region peptide (HRP) (1, 5, and 25 mg at 90-minute intervals), both before (normal) and after induction of HF by rapid left ventricular pacing. In normal sheep, HRP reduced heart rate (P<0.001) and hematocrit (P=0.019) compared with time-matched control data, without significantly affecting any other hemodynamic, hormonal, or renal variables. In sheep with HF, HRP treatment induced progressive falls in mean arterial pressure (P<0.001) in association with decreases in left atrial pressure (P<0.001), peripheral resistance (P=0.014), and hematocrit (P<0.001). Cardiac contractility tended to decline (P=0.096), whereas cardiac output was unaltered. HRP administration produced a dose-dependent decrease in plasma renin activity (P=0.004), with similar trends observed for plasma angiotensin II and aldosterone (P=0.093 and P=0.088, respectively). Circulating natriuretic peptides, endothelin-1, and catecholamine levels were unchanged. HRP also induced a reduction in plasma sodium concentrations relative to control (P=0.024), a natriuresis (P=0.046), and a tendency for creatinine excretion and clearance to improve.

Conclusions—(P)RR antagonism in experimental HF resulted in cardiovascular and renal benefits in association with inhibition of the renin-angiotensin-aldosterone system. These findings suggest that (P)RR contributes to pressure/volume regulation in HF and identifies the receptor as a potential therapeutic target in this disease.

Key Words: angiotensin II ■ heart failure ■ hemodynamics ■ (pro)renin receptor ■ renin
were taken into EDTA tubes on ice, centrifuged at 4°C, and stored at either −20°C or −80°C before assay for PRA, AngII, aldosterone, endothelin-1, atrial natriuretic peptide, brain natriuretic peptide, and catecholamines. For each hormone, all samples from individual animals were measured in the same assay to avoid interassay variability. Hematocrit was measured with every blood sample taken. Samples for analysis of plasma sodium, potassium, and creatinine concentrations were drawn into heparin tubes at 90-minute intervals, starting immediately before treatment.

Urine collections were made at 90-minute intervals, starting 1.5 hours before treatment and thereafter for 6 hours for the measurement of volume and sodium, potassium, and creatinine excretion. An additional collection was made overnight. Water intake was measured at the same time intervals as urine collections.

Statistics

Results are expressed as means±SEM. Baseline hemodynamic and hormonal values represent the mean of measurements made within the hour immediately before treatment. Paired Student t tests were used to test for baseline differences between normal and HF sheep (Table 1) and between control and HRP study limbs in each state. To test for the effects of (P)RR blockade, control and HRP arms (in both normal and HF states separately) were compared using 2-way repeated measures ANOVA. In the repeated measures ANOVA, sheep were treated as a random factor and HF and HRP as fixed within-individual effects. In addition, the interaction between the 2 within-individual effects, HF and HRP, was tested. Hormone data were log transformed before analysis. Where significant differences were identified by ANOVA, the level of significance at individual time points in Figures 1 to 3 and Tables 2 and 3 was determined by Fisher protected least-significant difference tests. Significance was assumed at P<0.05.

Table 1. Effects of Rapid Left-Ventricular Pacing (7 days at 225 bpm)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal</th>
<th>Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output, L/min</td>
<td>5.53±0.42</td>
<td>2.32±0.19†</td>
</tr>
<tr>
<td>dP/dt max, mm Hg/s</td>
<td>2001±159</td>
<td>1132±96†</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>89±2</td>
<td>71±3†</td>
</tr>
<tr>
<td>Left atrial pressure, mm Hg</td>
<td>4±1</td>
<td>23±2†</td>
</tr>
<tr>
<td>Total peripheral resistance, mm Hg/L per min</td>
<td>17±2</td>
<td>32±4†</td>
</tr>
<tr>
<td>Plasma atrial natriuretic peptide, pmol/L</td>
<td>20±5</td>
<td>272±46†</td>
</tr>
<tr>
<td>Plasma brain natriuretic peptide, pmol/L</td>
<td>3±1</td>
<td>42±8†</td>
</tr>
<tr>
<td>Plasma renin activity, nmol/L per h</td>
<td>0.25±0.05</td>
<td>1.37±0.43*</td>
</tr>
<tr>
<td>Plasma angiotensin II, pmol/L</td>
<td>9±2</td>
<td>42±10*</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>268±58</td>
<td>1842±629*</td>
</tr>
<tr>
<td>Plasma endothelin-1, pmol/L</td>
<td>2.7±0.4</td>
<td>5.2±0.7*</td>
</tr>
<tr>
<td>Plasma norepinephrine, pmol/L</td>
<td>1647±478</td>
<td>10755±3248*</td>
</tr>
<tr>
<td>Plasma epinephrine, pmol/L</td>
<td>192±28</td>
<td>674±183*</td>
</tr>
<tr>
<td>Urine volume, mL/1.5 h</td>
<td>72±6</td>
<td>49±10</td>
</tr>
<tr>
<td>Urinary sodium, excretion, mmol/h</td>
<td>3.41±0.71</td>
<td>1.04±0.38*</td>
</tr>
<tr>
<td>Urinary potassium excretion, mmol/h</td>
<td>12.2±1.2</td>
<td>5.2±0.9*</td>
</tr>
<tr>
<td>Urinary creatinine excretion, mmol/h</td>
<td>0.45±0.02</td>
<td>0.34±0.03*</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>103±6</td>
<td>70±6*</td>
</tr>
</tbody>
</table>

HRP indicates handle region peptide; bpm, beats per minute.

Mean±SEM measurements in 8 sheep before (normal: mean nonpaced control and HRP baseline data) and after induction of heart failure (mean paced control and HRP baseline data) by 7 days of rapid left ventricular pacing at 225 bpm. Significant differences are shown by *P<0.01, †P<0.001.

Methods

Eight Coopworth ewes (37–67 kg) were instrumented as previously described26 via a left lateral thoracotomy under general anesthesia (induced by IV thiopentone 15 mg/kg and maintained with 2.5% isoflurane/2 L/min NO/2 L/min oxygen) and using approved peri-/postoperative analgesia (intercostal bupivacaine 0.5%/lignocaine 2%: IV carprofen 4 mg/kg; IV buprenorphine 0.005–0.01 mg/kg). Briefly, 2 polyvinyl chloride catheters were inserted in the left atrium for blood sampling and left atrial pressure (LAP) determination; a Konigsberg pressure-tip transducer was inserted into the aorta to record mean arterial pressure (MAP) and into the apex of the LV as an index of contractility; an electromagnetic flow probe was subepicardially to the wall of the LV for subsequent rapid pacing. A bladder catheter was inserted per urethra for urine collections. Animals recovered for 14 days before commencing the study protocol. During the experiments, the animals were held in metabolic cages, fed a standard laboratory diet (500 g sheep pellets and 250 g chaff/day, containing 80 mmol sodium and 200 mmol potassium), and had free access to water.

Study Protocol

The study protocol was approved by the University of Otago, Christchurch Animal Ethics Committee. Each sheep received on 2 separate days a day apart, in a balanced random order, a vehicle control (5 mL 0.9% saline) and the (P)RR antagonist, ovine HRP (NH2-RIFLKKMPSV-COOH; Mimotopes, Australia) at 3 incremental doses (1, 5, and 25 mg at 90-minute intervals), both before (normal) and after induction of HF by rapid LV pacing (7 days at 225 beats per minute).

Using an online data acquisition system (PowerLab Systems, ADInstruments, Dunedin, NZ), hemodynamic recordings (HR, MAP, LAP, CO, LV dP/dt max, and calculated total peripheral resistance [CTPR=MAP/C0]) were performed at 15-minute intervals in the hour before treatment (baseline), at 15, 30, 60, and 90 minutes after each bolus, and at 2, 2.5, 3, and 18 hours after the final bolus. All measurements were made with the sheep standing quietly in the metabolic cage.

Blood samples were drawn from the left atrium 30 minutes and immediately before treatment (baseline), at 30 and 90 minutes after each bolus, and at 3 and 18 hours after the final bolus. Samples were centrifuged at 4°C, and stored at either −20°C or −80°C before assay for PRA, AngII, aldosterone, endothelin-1, atrial natriuretic peptide, brain natriuretic peptide, and catecholamines. For each hormone, all samples from individual animals were measured in the same assay to avoid interassay variability. Hematocrit was measured with every blood sample taken. Samples were log transformed before analysis. Where significant differences were identified by ANOVA, the level of significance at individual time points in Figures 1 to 3 and Tables 2 and 3 was determined by Fisher protected least-significant difference tests. Significance was assumed at P<0.05.

The effects of (P)RR antagonism in HF have not been previously investigated. Research in this area is crucial, given the deleterious downstream effects of receptor activation, the localization of receptors in critical tissues susceptible to end-organ damage (including the heart, kidney, and vasculature), and the increased levels of circulating renin and prorenin found in HF. Furthermore, HF treatment with angiotensin-converting enzyme inhibitors acts to further raise levels of both factors. We, therefore, examined the acute hemodynamic, hormonal, and renal effects of (P)RR blockade in sheep before and after HF induction by rapid LV pacing.
Results
Rapid LV pacing at 225 beats per minute for 7 days produced the hemodynamic, endocrine, and sodium-retaining hallmarks of congestive HF, with reduced CO, MAP, and renal function, elevated LAP and peripheral resistance, and widespread hormonal activation (Table 1). No significant differences between control and HRP baseline data were noted in either normal or HF states for any variable.

Compared with time-matched control data, administration of increasing bolus doses of the (P)RR antagonist, HRP, in normal sheep resulted in a dose-dependent decrease in HR ($P<0.001$), which was sustained up to 4 hours after the high-dose (25 mg) bolus (Table 2). This difference was no longer apparent at 18 hours postbolus. (P)RR blockade also produced an appreciable decrease in hematocrit relative to control ($P=0.019$) (Table 2). No other hemodynamic, hormonal, or renal variables were significantly affected by HRP in normal animals.

In sheep with HF, HRP treatment induced significant progressive falls in MAP ($P<0.001$) in association with graded reductions in LAP ($P<0.001$) and CTPR ($P<0.014$) (Figure 1). These changes were maintained for 3 to 4 hours after administration of the final bolus but were no longer different from control data the following day. $dP/dt_{max}$ also showed a tendency to decline ($P=0.096$) (Figure 1), whereas CO was unaltered (Table 2). As was seen in normal animals, (P)RR blockade decreased hematocrit levels relative to control data in the HF state ($P<0.001$) (Table 2).

HRP treatment additionally had an impact on the RAS in the setting of HF. Whereas PRA levels were stable during the control arm, HRP induced dose-dependent decreases in PRA ($P=0.004$), with similar trends observed for plasma AngII ($P=0.093$) and aldosterone concentrations ($P=0.088$) (Figure 2). Circulating atrial natriuretic peptide, brain natriuretic peptide, endothelin-1, and catecholamine levels were unchanged relative to control data (Table 2).

A significant HRP-induced suppression of plasma sodium levels compared with control was observed ($P=0.024$) (Table 3), together with a natriuresis ($P=0.046$) and visible

![Figure 1. Mean±SEM hemodynamic responses to incremental bolus doses of a vehicle control (0.9% saline) (○) and a (pro)renin receptor antagonist (ovine handle region protein [HRP]) (●) in 8 sheep before (normal; left) and after induction of heart failure (right). Significant differences from time-matched control data are shown by *$P<0.05$, **$P<0.01$, ***$P<0.001$.](http://circheartfailure.ahajournals.org/Downloadedfrom)
trends for creatinine excretion and creatinine clearance to increase (Figure 3; Table 3). Urine volume, urine and plasma potassium, plasma creatinine, and drinking were unaffected (Figure 3; Table 3).

**Discussion**

This is the first study to investigate the effects of (P)RR blockade in HF. We found that although administration of the (P)RR antagonist, HRP, had minimal effect in normal sheep (other than a decrease in HR and hematocrit), in sheep with HF, HRP produced dose-dependent and sustained reductions in CTPR, MAP, and LAP in association with attenuation of the circulating RAS and an improvement in renal function (including a natriuresis and a trend to increase creatinine clearance).

For comparison, the effects of angiotensin-converting enzyme inhibition in this model—the treatment of reference for HF—also induces decreases in MAP, LAP, and CTPR, in conjunction with an increase in CO, rise in PRA, falls in plasma
The mechanism of the observed blood pressure reduction in the present study is presumably due, at least in part, to the inhibitory effect of HRP on the vasoconstrictive RAS, with the declines in circulating PRA and AngII levels seen to precede the falls in MAP and CTPR. The lack of a blood pressure or peripheral resistance effect in normal sheep likely relates to the difference in the pretreatment status of the RAS seen between normal and HF animals, with both PRA and plasma AngII significantly elevated in the latter group (both 4.5-fold higher in HF). In addition, it is possible that (P)RR numbers are upregulated in the HF state, as has been reported in rats with congestive HF,12 and may, therefore, be further contributing to RAS activation and subsequent vasoconstriction in this setting. Indeed, our ovine pacing model of congestive HF shares many of the hemodynamic, hormonal, and organ characteristics evident in the rat ligation model,12 including significant falls in cardiac output and MAP, increases in heart weight and plasma brain natriuretic peptide levels, and pulmonary congestion.19,28 Given these similarities, it is plausible that prorenin receptors are also upregulated in these sheep with HF.

Susac et al13 (mentioned above) also noted a significant HRP-induced reduction in the diastolic time constant (τ) in SHRs fed a high-salt diet, a result that is in keeping with the fall in LAP observed after HRP administration in HF sheep in our study. This decline in LAP and the concomitant trend for dP/dt max to decrease are consistent with the attenuation of AngII’s positive inotropic and negative lusitropic actions29 after falls in circulating concentrations of the peptide. CO was unchanged despite the tendency for contractility to weaken, perhaps because of the counteracting effect of the concurrent fall in cardiac afterload (MAP and CTPR).

Although HRP treatment had minimal hemodynamic impact in normal animals, we did observe a significant decrease in HR in this group. This occurred in the absence of any change in circulating RAS levels. It is possible, given the presence of (P)RRs on cardiomyocytes,8 that receptor antagonism (by HRP) depressed AngII concentrations at the local cardiac tissue level, thereby reducing its chronotropic actions.30 Indeed, several studies have previously shown reduced cardiac AngII immunoreactivity subsequent to HRP administration.10,18 Our results concur with findings in hypertensive salt-loaded rats, where (P)RR blockade also tended to reduce HR (≈15 beats per minute)13 and are consistent with the elevation in HR seen in transgenic rats overexpressing the receptor.8

The attenuation of the RAS after (P)RR blockade demonstrated in the present study in HF sheep has been observed previously in renovascular hypertensive (2 kidneys, 1 clip) rats, where circulating PRA levels were halved in HRP-treated animals compared with controls,15 and in several investigations by Ichihara and colleagues showing HRP-induced reductions in both cardiac and renal AngII concentrations in hypertensive and diabetic rodents.15,17,18 The decreases in plasma AngII with HRP presumably reflect displacement of prorenin and renin from the (P)RR,31,32 thereby preventing activation of the (nonproteolytic) enzymatic action of prorenin, as well as the enhancement (4- to 5-fold) of renin’s catalytic efficiency.5 In keeping with the decrease in plasma AngII, we also noted an ≈1000 pmol/L fall in plasma aldosterone concentrations.


a result concordant with the raised plasma aldosterone levels noted in rats overexpressing (P)RR.8

(P)RR blockade in sheep with HF tended to augment renal function in the present study, including dose-dependent increases in sodium excretion, as well as the maintenance of urine output and creatinine excretion in the face of a reduction in arterial pressure (and, therefore, presumably renal perfusion pressure). Similar improvements in renal function have been demonstrated in salt-loaded hypertensive rats, where HRP administration significantly decreased serum creatinine concentrations, together with trends to reduce urinary protein excretion and induce a natriuresis.13 In diabetic SHRs, (P)RR antagonism significantly reduced protein excretion in association with a decrease in renal AngII content, 18 whereas in rats overexpressing (P)RR, HRP inhibited the development of glomerulosclerosis and proteinuria. 33 The mechanisms underlying the renal effects of HRP in our study conceivably relate to the concomitant suppression of plasma concentrations of the antinatriuretic factors AngII and aldosterone from elevated levels and perhaps inhibition of local renal AngII as demonstrated by Seki et al.18 An improvement in renal hemodynamics may also have contributed, given the trend for the glomerular filtration rate to increase (as evidenced by a rise in creatinine clearance), and observations by others in hypertensive rats showing (P)RR blockade tended to reduce renal vascular resistance (≈10 mm Hg · mL⁻¹ · min⁻¹ · 100 g⁻¹) and improve renal blood flow (≈0.5 mL/min per g).13 In accordance with these results, (P)RRs have been demonstrated in both the glomerular mesangial cells and vascular structures of the kidney.5

A consistent and interesting effect of HRP observed in both normal and HF sheep is the decrease in hematocrit. Whereas our data do not provide a clear explanation for this decline in hematocrit with HRP administration, we speculate that, in the absence of a significant change in water intake or urine output, there was a prompt redistribution of water into
the extracellular compartment. Clearly, additional research is needed to confirm this premise and to clarify underlying mechanisms.

In conclusion, we have shown for the first time in HF that (P)RR blockade produces dose-dependent and sustained reductions in CTPR, MAP, and LAP in association with attenuation of the circulating RAS and a natriuresis. These findings suggest that (P)RR contributes to pressure/volume regulation in HF and identifies the receptor as a potential therapeutic target in this disease.

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