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Contrasting effects of intervention with ET_A and ET_B receptor antagonists in angiotensin II-high salt diet induced hypertension

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Abstract

Endothelin receptor antagonists are antihypertensive and renoprotective in angiotensin II (AngII)-induced hypertension when treatment begins at the commencement of AngII infusion, but their effects on established hypertension are not well understood. We therefore tested the effect of ET_A receptor blockade (ABT-627) commencing on day 8 of 14-day treatment of rats with AngII (65ng/min s.c.) plus 8% NaCl diet (AngII-HS). Robust hypertension was observed in both AngII-HS and AngII-HS+ABT-627 groups (mean arterial pressure, MAP 170±5 and 165±5mmHg compared to 110±3mmHg in normal salt control rats at day 7, $P<0.05$). Treatment with ABT-627 from day 8 prevented any further rise in MAP (168±5 and 191±3mmHg at day 13 in AngII-HS+ABT-627 and AngII-HS, $P<0.001$), but did not blunt the significant increases in urinary protein (19-fold), albumin (25-fold) or MCP-1 excretion (6 to 8-fold). Separate groups of rats received AngII-HS alone or with ET_B receptor blockade (A-192621) commencing on day 8. A-192621 only mildly exacerbated AngII-HS induced hypertension (by approximately 12mmHg on day 13; $P<0.05$) without significantly worsening proteinuria or albuminuria. These results indicate the importance of ET_A receptor signaling in the progression of hypertension in the AngII-HS model, and may suggest that ET_B receptor function is depressed in this model.

Introduction

Since being identified in 1988 as a highly potent vasoconstrictor, there has been much interest in the contribution of endothelin to hypertension. During the past 20 years, endothelin has been implicated in multiple models of hypertension, including both angiotensin II (AngII)-dependent hypertension (d'Uscio et al. 1997; Moreau et al. 1997; Herizi et al. 1998; Muller et al. 2000; Rothermund et al. 2003) and models classically thought of as being “low renin” such as the Dahl salt-sensitive rat (Barton et al. 1998) and the deoxycorticosterone-acetate salt model (Allcock et al. 1998).

The chronic AngII infusion model, either with or without high dietary salt intake, is one of the most widely studied models of hypertension. Elevations of vascular (Moreau, d'Uscio et al. 1997) and renal endothelin levels (Alexander et al. 2001; Sasser et al. 2002) have been reported in rats chronically treated with AngII. Further solidifying the importance of endothelin in AngII-dependent hypertension, treatment of rats with endothelin ET_A or combined ET_A and ET_B receptor antagonists, when initiated at the same time as or prior to commencing AngII infusion, has been shown to markedly attenuate (Moreau, d'Uscio et al. 1997; Barton et al. 1998) or completely prevent (Herizi, Jover et al. 1998; Alexander, Cockrell et al. 2001) AngII-induced hypertension and renal injury. Little is known, however, regarding whether ET_A receptor blockade is effective in reducing arterial pressure and renal

injury if treatment is initiated after the onset of AngII-induced hypertension, a scenario more similar to clinical situations. Likewise, the consequences of specifically blocking the ET_B receptor in this model has received little attention, despite numerous studies in this and other models of hypertension employing combined ET_A and ET_B receptor antagonists. The current study therefore tested the effects of selective ET_A or ET_B receptor blockade on AngII-high salt diet-induced hypertension and renal injury, with treatment initiated after induction of hypertension.

Materials and Methods

Effect of ET_A or ET_B receptor blockade on AngII-high salt diet-induced hypertension

Experiments were performed on adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, USA). All animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals*, and all procedures were approved in advance by the Medical College of Georgia Institutional Animal Care and Use Committee. Rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.; Abbott Laboratories, North Chicago, USA) and underwent surgery for implantation of radiotelemetry transmitters (PA-C40, Data Sciences International, St Paul, USA) for recording of conscious blood pressure via a catheter inserted into the abdominal aorta according to the manufacturer's specifications. Data was collected for 10 s every 10 min and used to calculate 24 h average mean arterial pressures (MAP). During a 7–10 day recovery period and 2-day baseline recording period rats were maintained on regular 0.4% NaCl chow and tap water, before being randomized to one of three treatment groups: two groups received AngII s.c. at 65 ng/min (Alzet 2002 miniosmotic pump, DURECT, Cupertino, USA, implanted under isoflurane anesthesia, Baxter Healthcare, Deerfield, USA), and one group received 0.9% NaCl vehicle s.c. for 14 days. Rats receiving AngII were placed on an 8% NaCl chow for the 14-day infusion period (AngII-HS treatment). On day 8 of AngII infusion, one group of the AngII-HS rats commenced treatment with the ET_A receptor antagonist ABT-627 (5 mg/kg/d p.o. in drinking water; Abbott Laboratories). All rats were housed in metabolic cages for the final 24 h of the 14-day AngII/vehicle infusion period to enable collection of urine for analysis. MAP data was not collected during this time. Rats were then anesthetized (sodium pentobarbital, 60 mg/kg i.p.), blood collected from the abdominal aorta, centrifuged and the plasma collected, and the rat killed by thoracotomy. Plasma and urine samples were stored at –80°C until analysis.

In a separate series of experiments, two groups of AngII-HS rats were prepared as described above, with one group receiving the ET_B receptor antagonist A-192621 (10 mg/kg/d p.o. in powdered food; Abbott Laboratories) from day 8 of AngII infusion. Blood pressure was monitored and plasma and urine collected as described above.

Biochemical analyses

Plasma and urinary concentrations of monocyte chemoattractant protein (MCP-1) were measured by ELISA (GE Healthcare Bio-Sciences, Piscataway, USA), creatinine concentrations were measured by the picric acid method adapted for microtitre plates (Allcock, Venema et al. 1998), and urinary concentrations of protein and albumin were measured respectively by standard Bradford assay and ELISA (Cayman Chemical, Ann Arbor, USA).

Statistical analyses

Data are presented as mean ± SE for n = 6–9 rats per group. MAP data were analyzed by repeated measures ANOVA followed by (Bonferroni's) post hoc contrasts between AngII-HS and AngII-HS plus endothelin receptor antagonist-treated groups. Data from plasma and

urine obtained at the end of the 14-day infusion period was analyzed by one-factor ANOVA and Bonferroni post hoc tests where appropriate. $P \leq 0.05$ was considered statistically significant.

Results

ET_A receptor blockade prevents further increases in blood pressure during AngII-HS hypertension, but does not significantly attenuate proteinuria or albuminuria

AngII-HS treatment produced robust hypertension, with MAP reaching 170 ± 5 and 165 ± 5 mmHg on day 7 of AngII-HS treatment in groups without and with later treatment with ABT-627 respectively, compared to 110 ± 3 mmHg in vehicle-treated normal salt diet control rats at day 7 ($P < 0.05$; Figure 1). Intervention with ABT-627 treatment, commencing on day 8, blunted any further rise in MAP such that by day 13, MAP of ABT-627-treated rats was approximately 23 mmHg lower than rats treated with AngII-HS alone (Figure 1). AngII-HS treatment impaired renal function (reduced creatinine clearance) and induced renal injury, as indicated by substantial elevation of urinary excretion of protein, albumin and MCP-1 compared to control rats (Table 1). Treatment with ABT-627 did not significantly ameliorate these effects of AngII-HS. Plasma MCP-1 concentration was not significantly different between the three groups (data not presented).

ET_B receptor blockade exacerbates AngII-HS hypertension, but does not significantly worsen proteinuria or albuminuria

The effect of ET_B receptor blockade with A-192621 on the progression of AngII-HS hypertension was studied in a separate series of experiments. Treatment with A-192621, commencing on day 8, mildly enhanced the severity of AngII-HS-induced hypertension (Figure 2). Treatment of AngII-HS rats with A-192621 did not produce any further increases in urinary excretion of protein, albumin or MCP-1, nor did it further reduce creatinine clearance compared to what was seen in rats treated with AngII-HS alone (data not shown).

Discussion

Our findings are consistent with and extend those of previous studies reporting beneficial effects of ET_A or combined ET_A and ET_B receptor antagonism on angiotensin II-dependent models of hypertension. Most previous studies commenced treatment with endothelin receptor antagonists at the onset of AngII infusion, and found that development of hypertension is either completely prevented (Herizi, Jover et al. 1998; Alexander, Cockrell et al. 2001) or is significantly attenuated (d'Uscio, Moreau et al. 1997; Moreau, d'Uscio et al. 1997). One study by Ballew and Fink (Ballew and Fink 2001) initiated treatment with ABT-627 5 days after commencing AngII infusion, with and without high salt treatment. In contrast to our study, Ballew and Fink found that ABT-627 normalized MAP in AngII-HS rats, although the hypertension in that study was milder (increment of approximately 20 mmHg at day 5) than in our model (increment of approximately 40 mmHg at the same time point). These data all support the potential utility of ET_A receptor blockade in interventional approaches towards treating established hypertension.

Whether treatment with endothelin receptor antagonists following the onset of AngII-induced hypertension can ameliorate proteinuria or albuminuria has not previously been addressed. Our model was associated with renal injury, with significant increases in urinary protein and albumin excretion observed, and impaired renal function indicated by a reduction in creatinine clearance. We also observed an increase in urinary excretion of MCP-1, which has been shown in a previous study to correlate with renal monocyte/macrophage infiltration in this model (Elmarakby et al. 2006) and production of which is

stimulated by AngII in a wide range of cell types including vascular smooth muscle (Chen et al. 1998), tubular and mesangial cells (Tanifuji et al. 2005). Despite the significant attenuation of hypertension by treatment with an ET_A receptor antagonist, none of the markers of renal injury or dysfunction studied were significantly ameliorated. This contrasts with the study by Alexander et al (2001) where ABT-627 treatment, when initiated prior to AngII infusion, was able to completely prevent the rise in arterial pressure and fall in glomerular filtration rate associated with AngII-HS induced hypertension. Combined ET_A and ET_B receptor blockade in AngII-dependent hypertension (Herizi, Jover et al. 1998) and ET_A receptor antagonists in other models of hypertension (Montezano et al. 2005) or diabetes (Sasser et al. 2007) have previously been shown to ameliorate albuminuria or histological evidence of renal injury when treatment was initiated at the onset of the disease model. This apparent lack of effect on renal injury of either ET_A or ET_B receptor blockade in the current study could in both cases be due to the relatively short period of treatment (7 days), or perhaps the attenuated hypertension in the case of ABT-627-treated rats was still of sufficient magnitude to maintain renal injury.

The question of whether or not to block ET_B receptors along with ET_A receptors in the treatment of hypertension is a complex issue. On the one hand, ET_B receptors exert anti-hypertensive effects, such as NO production (Fujitani et al. 1993), natriuresis (Yukimura et al. 1994) and clearance of endothelin (Fukuroda et al. 1994), but vasoconstrictive vascular smooth muscle ET_B receptors may be upregulated in disease states (Cardillo et al. 1999; Black et al. 2003) and there is some evidence that activation of ET_B receptors on sympathetic nerves (Lau et al. 2006) produces superoxide anion, which may enhance nerve activity. ET_B blockade in our study and the previous study (Ballew and Fink 2001) exacerbated the hypertension, but in our study this did not significantly worsen proteinuria and albuminuria, or further reduce creatinine clearance, possibly due to the relatively short time-frame of our study. Interestingly, the magnitude of the increase in MAP during A-192621 treatment of AngII-HS rats in the current study was much smaller than observed by our group previously in rats receiving HS alone (Williams et al. 2004). From this observation it is tempting to speculate that a downregulation of ET_B receptor function may occur in AngII-dependent hypertension.

In conclusion, our data support the continued involvement of ET_A receptor activation in the progression and maintenance of AngII-dependent hypertension, and caution against inclusion of ET_B receptor blockade in therapeutic approaches to treating hypertension.

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Figure 1.

Effect of ET_A receptor blockade on established AngII-HS-induced hypertension. Data shown are 24 h average mean arterial pressure (MAP) before (days B1 and B2) and during 14-day AngII-HS or vehicle-normal salt (NS) treatment. Treatment with ABT-627 (5 mg/kg/d in drinking water) was initiated in the AngII-HS+ABT group where indicated. $*P < 0.05$ between AngII-HS with and without ABT-627 treatment.

Figure 2.

Effect of ET_B receptor blockade on established AngII-HS-induced hypertension. Data shown are 24 h average mean arterial pressure (MAP) before (days B1 and B2) and during 14-day AngII-HS treatment. Treatment with A-192621 (10 mg/kg/d in food) was initiated in the AngII-HS+A-192621 group where indicated. * $P < 0.05$ versus AngII-HS.

Table 1

Markers of renal injury, assessed at day 14 of AngII or vehicle treatment

	Vehicle-NS	AngII-HS	AngII-HS + ABT-627
Urinary Protein Excretion Rate (mg/d)	22 ± 6	431 ± 33 [*]	408 ± 44 [*]
Urinary Albumin Excretion Rate (mg/d)	13 ± 5	348 ± 39 [*]	326 ± 42 [*]
Urinary MCP-1 Excretion Rate (ng/d)	10 ± 2	89 ± 12 [*]	59 ± 10 [*]
Creatinine Clearance (ml/min)	1.07 ± 0.05	0.33 ± 0.05 [*]	0.40 ± 0.07 [*]

NS = 0.4% NaCl diet. HS = 8% NaCl diet.

^{*} $P < 0.05$ versus Vehicle-NS.

Values are mean ± SE for n = 6–8 for all measurements.