AT₁ receptor-mediated stimulation by angiotensin II of rat aortic fibronectin gene expression *in vivo*

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Fibronectin plays an important role in various vascular diseases. A subpressor (200 ng kg⁻¹ min⁻¹) or pressor (1000 ng kg⁻¹ min⁻¹) dose of angiotensin II was continuously infused into rats by osmotic minipump for various times, to investigate the effects on aortic fibronectin gene expression. In rats infused with a subpressor dose of angiotensin II in which blood pressure was normal for 3 days, aortic fibronectin mRNA levels started to increase by 1.4 fold at 12 h and reached the maximal levels (increased by 3.1 fold) at 3 days. Treatment with TCV-116 (3 mg kg⁻¹ day⁻¹), a non-peptide selective AT₁ receptor antagonist, completely inhibited the angiotensin II-induced increase in aortic fibronectin mRNA, while hydralazine (10 mg kg⁻¹ day⁻¹) did not block this effect. Similar results were also obtained for a pressor dose of angiotensin II. Thus, angiotensin II directly stimulates aortic fibronectin gene expression *in vivo*, which is mediated by the AT₁ receptor but not by blood pressure.

Keywords: angiotensin II; fibronectin; AT₁ receptor; TCV-116; gene expression; blood pressure; transforming growth factor- β 1; collagen

Introduction Recent studies suggest that angiotensin II (AII) may be involved not only in hypertension but also in various vascular diseases (Griendling *et al.*, 1993). However, the mechanism by which AII is responsible for vascular diseases *in vivo* remains to be elucidated. Fibronectin, the main extracellular matrix protein, influences cell adhesion and migration, and modulates vascular smooth muscle cells from a contractile to a synthetic phenotype, thus being implicated in vascular hypertrophy and neointimal formation (Ruoslahti, 1988; Glukhova *et al.*, 1989; Takasaki *et al.*, 1990). In this study, we have shown the importance of AII for aortic fibronectin gene expression *in vivo*.

Methods Eight-week-old male Wistar rats (Clea Japan, Japan), anaesthetized with ether, were implanted subcutaneously with an Alzet osmotic minipump (model 1003D or 2001, Alza Corp, USA) containing AII (Peptide Institute Inc., Japan) in saline or saline alone, and received a subpressor (200 ng kg⁻¹ min⁻¹) or pressor (1000 ng kg⁻¹ min⁻¹) infusion of AII for various times. For both doses, rats were separated into 4 groups: (1) saline-infused (control), (2) AII-infused, (3) AII-infused and TCV-116 (3 mg kg⁻¹ day⁻¹)-treated, and (4) AII-infused and hydralazine (10 mg kg⁻¹ day⁻¹)-treated groups. TCV-116 (($\pm$)-1-(cyclohexyloxycarbonyloxy)-ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylate) (Takeda Chemical Industries, Ltd., Japan) (Shibouta *et al.*, 1993), a non-peptide selective AT₁ receptor antagonist, was given to rats by gastric gavage 24 h before implantation of the osmotic minipump to the end of AII infusion once a day. Hydralazine (Wako Pure Chemical Industries, Ltd., Japan), in the drinking water, was given to rats from 24 h before the start of AII infusion up to the end of AII infusion. At the indicated times, each group ($n = 4$) of rats was decapitated and the thoracic aortae were immediately excised, frozen in liquid nitrogen, pooled and stored at -80°C until use.

RNA isolation and northern blot hybridization The detailed method was described in our previous paper (Kim *et al.*, 1994).

Results *Effect of AII infusion on blood pressure* For 200 ng kg⁻¹ min⁻¹ of AII, blood pressure (mean $\pm$ s.e.mean) of control, AII-infused, AII-infused and TCV-116-treated, and AII-infused and hydralazine-treated groups was 122 ± 1 , 126 ± 2 , 119 ± 2 and 120 ± 2 mmHg, respectively, at 2 days, and was 122 ± 2 , 133 ± 2 , 115 ± 5 and 118 ± 6 mmHg, respectively, at 3 days. As judged by ANOVA and Duncan's multiple range test, no statistically significant increase in blood pressure was produced by the infusion of 200 ng kg⁻¹ min⁻¹ of AII for 3 days, being consistent with the previous report by Simon & Altman (1992). In contrast, an infusion of 1,000 ng kg⁻¹ min⁻¹ increased blood pressure to 157 ± 8 mmHg ($P < 0.05$) on day 2, an effect which was completely inhibited by TCV-116 treatment.

Effects of AII infusion on aortic fibronectin mRNA levels As shown in Figures 1 and 2a, with a subpressor dose of AII, aortic fibronectin mRNA began to increase by 1.4 fold at 12 h after the infusion, gradually increased and reached a peak (3.1 fold) at 3 days. TCV-116 completely inhibited this increase in fibronectin mRNA at all time points examined, while hydralazine produced little inhibition. There was no significant increase in aortic TGF- β 1, or type I or III collagen mRNA throughout AII infusion (Figure 1). The same experiments were repeated and similar results were obtained, thereby revealing that our observations were reproducible. A pressor dose of AII increased aortic fibronectin mRNA with a similar pattern to a subpressor dose (Figure 2), but failed to increase aortic TGF- β 1 and types I and III collagen mRNAs throughout the infusion (data not shown).

Discussion We found that AII stimulated aortic fibronectin gene expression *in vivo* via the AT₁ receptor, but not via blood pressure. Fibronectin, the major extracellular matrix protein, modulates vascular smooth muscle cells from a contractile to a synthetic phenotype, causing cell proliferation (Ruoslahti, 1988). Fibronectin expression is increased in the hypertensive rat aorta (Takasaki *et al.*, 1990) and balloon-injured artery (Glukhova *et al.*, 1989), thereby supporting the contribution of fibronectin to vascular disease. Furthermore, Viswanathan *et al.* (1992) reported an increased AT₁ receptor expression in injured rat aorta. Therefore, our observations suggest that AII may participate in the development of vascular diseases by stimulating fibronectin expression.

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Figure 1 Representative autoradiograms of aortic mRNAs at 24 h, 3 days and 7 days after a subpressor ($200 \text{ ng kg}^{-1} \text{ min}^{-1}$) angiotensin II (AII) infusion. Lane 1, control rat; lane 2, AII-infused rat; lane 3, AII-infused and TCV-116-treated rat; lane 4, AII-infused and hydralazine-treated rat. In each group, aortic tissues were pooled from 4 rats. The size of mRNA was 2.5 kb for transforming growth factor- β 1 (TGF- β 1), 7.9 kb for fibronectin, 4.7 and 5.7 kb for α 1(I) collagen (collagen I), 5.9 kb for α 1(III) collagen (collagen III) and 1.4 kb for glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

In this study, we also measured aortic TGF- β 1 mRNA, because TGF- β 1 stimulates fibronectin and collagen expressions (Border & Ruoslahti, 1992). Interestingly, in contrast to fibronectin, aortic TGF- β 1 and types I and III collagen mRNAs were not stimulated by a subpressor or a pressor AII infusion. Thus, the AII-induced stimulation of aortic fibronectin expression *in vivo* seems to be a rather specific action and is not mediated by TGF- β 1.

In conclusion, AII stimulates aortic fibronectin gene expression *in vivo*, via the AT $_1$ receptor but not via its pressor effect. Our present observations may partially explain the mechanism of the beneficial effects of inhibitors of the renin-angiotensin system on vascular diseases. However, further study is needed to elucidate the molecular mechanism of AII-induced vascular injury.

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Figure 2 Aortic fibronectin mRNA levels at 6, 12, and 24 h, and 3 and 7 days after a subpressor ($200 \text{ ng kg}^{-1} \text{ min}^{-1}$) (a) and a pressor ($1000 \text{ ng kg}^{-1} \text{ min}^{-1}$) (b) angiotensin II (AII) infusion. The ordinate scale indicates aortic fibronectin mRNA levels, corrected for GAPDH mRNA levels. The value in the control group at each time point is represented as 1. Aortae were pooled from 4 rats in each group, and total RNA was extracted from the pooled aortae and subjected to northern blot analysis. Each column indicates control (open column), AII-infused (solid column), AII-infused and TCV-116-treated (hatched column) and AII-infused and hydralazine-treated groups (stippled column).

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