Enhanced AT₁ receptor-mediated Growth Response of Cultured Mesangial Cells from Stroke-Prone SHR: Role of AT₂ Receptor in Mesangial Cell Growth Regulation

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The AT₂ receptor may mediate growth inhibition and vasorelaxation, but the intracellular signaling mechanisms that are activated by the AT₂ receptor have been still poorly clarified. We previously reported that the AT₂ receptor expression in the whole kidney of fetal and neonatal stroke-prone spontaneously hypertensive rats (SHRSP) was significantly lower than that in age-matched normotensive Wistar-Kyoto rats (WKY), and that the AT₂ receptor expression of cultured mesangial cells (MC) from WKY was faint in growing cells but markedly induced in confluent cells, whereas it was undetectable in MC from SHRSP which were much more proliferative than WKY-MC. In this study, to further explore the role of the AT₂ receptor in MC and implication in hypertensive states, we examined its functional role and signaling mechanism in MC.

MC were prepared from WKY and SHRSP at the age of 20 weeks. DNA synthesis in MC was measured by ³H-thymidine incorporation. The phosphorylation of MAP kinase was analyzed by Western blotting using phospho-specific antibody that detects phosphorylated tyrosine residues of p44 and p42 MAP kinases. The analysis of apoptosis was performed by morphological changes of nuclei and electrophoretic DNA fragmentation.

In WKY-MC, Ang II significantly increased the DNA synthesis by 157%. The increase was completely abolished with AT₁-antagonist CV-11974, but rather significantly augmented with AT₂-antagonist PD123319 by 53% ($P < 0.05$). In contrast, AT₂-agonist CGP42112A attenuated PDGF-stimulated DNA synthesis by 67% ($P < 0.05$), which effect was completely reversed by addition of PD123319. Phosphorylation of p44 and p42 MAP kinases by Ang II was also inhibited with CV-11974 by 52% ($P < 0.05$), but significantly augmented with PD123319 by 76% ($P < 0.05$). On the other hand, Ang II-stimulated DNA synthesis in SHRSP-MC, which was 1.3 times more than that in WKY-MC, was not enhanced with PD123319, and PDGF-induced DNA synthesis was unaffected by CGP42112A. Furthermore, Ang II induced apoptosis in WKY-MC, which was enhanced by CV-11974 but inhibited by PD123319 (Ang II : Ang II + CV : Ang II + PD; $1.0 \pm 0.2 : 1.8 \pm 0.4 : 1.0 \pm 0.3$ units), whereas no induction was observed in SHRSP-MC.

These results indicate that mesangial AT₂ receptor expression exerts antiproliferative and apoptotic effects in part by inhibiting the MAP kinase signaling cascade, suggesting its modulatory role in MC growth. Augmented AT₁ receptor-mediated growth response in SHRSP-MC may suggest its involvement in higher MC proliferation activity. Together with lower AT₂ receptor expression in SHRSP kidney, the AT₂ receptor may be involved in the pathogenesis and/or progression of hypertensive renal diseases.