

Supplementary Data

Figure S1

Role of PKC and PI3K in the effect of AT $_2$ R on insulin receptor expression in RPT cells from SHRs. (A) Effect of CGP42112 and the PKC inhibitor 19–31 on insulin receptor protein expression in SHR cells. The cells were incubated with CGP42112 (10 ⁻⁶ mol/L) or PKC inhibitor 19–31 (10 ⁻⁶ mol/L) for 24 hours. Results are expressed as the ratio of insulin receptor to α -actin densities (n = 3; ** P < 0.01 vs. control). (B) Effect of CGP42112 and the PI3K inhibitor wortmannin on insulin receptor protein expression in SHR cells. The cells were incubated with CGP42112 (10 ⁻⁶ mol/L) or wortmannin (10 ⁻⁶ mol/L) for 24 hours. Results are expressed as the ratio of insulin (10 ⁻⁶ mol/L) for 24 hours. Results are expressed as the ratio of insulin receptor to α -actin densities (n = 3, ** P < 0.01 vs. control). (B) Effect of CGP42112 (10 ⁻⁶ mol/L) or wortmannin (10 ⁻⁶ mol/L) for 24 hours. Results are expressed as the ratio of insulin receptor to α -actin densities (n = 3, ** P < 0.01 vs. control). AT $_2$ R, angiotensin II type 2 receptor; PI3K, phosphatidylinositol 3 kinase; PKC, protein kinase C; RPT, renal proximal tubule; SHRs, spontaneously hypertensive rats.



Figure S2

The linkage between AT ₂ and insulin receptors in SHR RPT cells. (A) The confocal microscopy of AT ₂ and insulin receptors in SHR RPT cells with or without CGP42112 (10 ⁻⁶ mol/L, 24 h). The cells were washed, fixed, and immunostained for AT ₂ and insulin receptors, as described in the <u>Methods (sec2)</u>. Colocalization appears as yellow after merging the images of fluorescein isothiocyanate-tagged insulin receptor (green) and Alexa 568-tagged AT ₂ receptor (red). (B) Effect of the AT ₂ R agonist, CGP42112, on AT ₂ /insulin receptor coimmunoprecipitation in RPT cells from SHRs. Samples were immunoprecipitated with anti-AT ₂ R antibody and immunoblotted with anti-insulin receptor antibody. Results are expressed as relative density units (DUs) (n = 5). AT ₂, angiotensin II type 2; SHR, spontaneously hypertensive rats; RPT, renal proximal tubule; AT ₂ R, angiotensin II type 2 receptor.