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Research Article

Angiotensin II type 2 receptor inhibits expression and function of insulin receptor in rat renal proximal tubule cells

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Abstract

Both renin–angiotensin systems and insulin participate in kidney-involved blood pressure regulation. Activation of angiotensin II type 2 receptor (AT_2R) decreases sodium reabsorption in renal proximal tubule (RPT) cells, whereas insulin produces the opposite effect. We presume that AT_2R has an inhibitory effect on insulin receptor expression in RPT cells, which may affect renal sodium transport and therefore be of physiological or pathological significance. Our present study found that activation of AT_2R inhibited insulin receptor expression in a concentration and time-dependent manner in RPT cells from Wistar-Kyoto (WKY) rats. In the presence of a protein kinase C (PKC) inhibitor (PKC inhibitor peptide 19–31, 10^{-6} mol/L) or a phosphatidylinositol 3 kinase inhibitor (wortmannin, 10^{-6} mol/L), the inhibitory effect of AT_2R on insulin receptor was blocked, indicating that both PKC and phosphatidylinositol 3 kinase were involved in the signaling pathway. There was a linkage between AT_2R and insulin receptor which was determined by both laser confocal microscopy and coimmunoprecipitation. However, the effect of AT_2R activation on insulin receptor expression was different in RPT cells from spontaneously hypertensive rats (SHRs). Being contrary to the effect in WKY RPT cells, AT_2R stimulation increased insulin receptor in SHR RPT cells. Insulin (10^{-7} mol/L, 15 minutes) enhanced Na^+-K^+-ATPase activity in both WKY and SHR RPT cells. Pretreatment with CGP42112 decreased the stimulatory effect of insulin on Na^+-K^+-ATPase activity in WKY RPT cells, whereas pretreatment with CGP42112 increased it in SHR RPT cells. It is suggested that activation of AT_2R inhibits insulin receptor expression and function in RPT cells. The lost inhibitory effect of AT_2R on insulin receptor expression may contribute to the pathophysiology of hypertension. J Am Soc Hypertens 2017;■(■):1–11.

Keywords: Angiotensin II type 2 receptor; hypertension; insulin receptor; renal proximal tubule cells; kidney.

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Introduction

Kidney contributes importantly to the long-term blood pressure control via regulating sodium homeostasis.[1–3] Renal proximal tubule (RPT) is one of the most critical segments for sodium reabsorption within kidney and therefore involved in blood pressure adjustment. This process is regulated by numerous hormones and humoral factors, including renin–angiotensin system (RAS) and insulin/insulin receptor.[4–6]

Angiotensin II (Ang II), as the major effector peptide of RAS, regulates sodium balance via two main receptor subtypes, angiotensin II type 1 receptor (AT_1R) and angiotensin II type 2 receptor (AT_2R).[7,8] AT_1R and AT_2Rs are expressed throughout the vascular and tubule sites in adult kidney. Stimulation of AT_1R increases sodium (Na^+) reabsorption in RPT and induces antinatriuresis. AT_2R, as an antagonist of AT_1R, can oppose or offset this action.[9–11] Activation of AT_2R decreases sodium reabsorption, induces natriuresis, maintains negative Na^+ balance, and lowers blood pressure chronically in angiotensin-dependent hypertension via reducing Na^+ reabsorption in RPT.[1]

Insulin/insulin receptor is another critical signaling pathway involved in sodium balance in RPT. Insulin receptor was expressed throughout the nephron.[12,13] Studies have demonstrated that insulin/insulin receptor has the potential of increasing sodium retention through stimulating sodium transporters Na^+-H^+-exchanger and Na^+-K^+-ATPase in RPT.[14–16]

Some researches have been performed to elucidate the interaction between AT_2R and insulin in different aspects. AT_2R can impact the β-cell to α-cell ratio of the neonate islets, insulin secretory function, and the glucose tolerance in pups.[17] Activation of AT_2R leads to inhibition of insulin-induced extracellular signal-regulated protein kinase 2 (ERK2) activity and cell proliferation in transfected Chinese hamster ovary cells.[18] In rat pheochromocytoma cell line, AT_2R inhibits insulin-associated phosphoinositide 3-kinase and Akt phosphorylation and induces cell apoptosis.[19] Although relationship between AT_2R and insulin receptor has been revealed, the detailed mechanisms remain unclear. Both AT_2R and insulin receptor exist in RPT and they execute the opposite functions in sodium metabolism,[5,6,20] we presume that activation of AT_2R inhibits insulin receptor in RPT. To confirm this hypothesis, we explored the interaction between AT_2R and insulin receptor in RPT cells from Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). We found that activation of AT_2R was capable of decreasing insulin receptor expression and function in RPT cells from WKY rats, whereas this inhibitory effect was disturbed in RPT cells from SHRs. We suggested that an aberrant interaction between AT_2R and insulin receptor may play an important role in the abnormal regulation of sodium excretion and consequently be implicated in hypertension.

Methods

Cell Culture

Immortalized RPT cells from WKY rats and SHRs were cultured as previously described.[4] Briefly, cells were cultured at 37°C in 95% O_2 and 5% CO_2 atmosphere in Dulbecco's Modified Eagle Medium/F-12 containing 10% fetal bovine serum, 5 μg/mL transferrin, 5 μg/mL insulin, 4 μg/mL dexamethasone, and 10 ng/mL epidermal growth factor. The cells were diluted to 1×10^6 cells/mL and plated in different culture dishes according to the specific experimental requirements. Before the treatment of cells, the medium was replaced with serum-free medium for 2 hours.

Immunoblotting

The RPT cells were treated with different reagents or the vehicle (dH_2O) at the indicated concentrations and times according to the specific experimental requirements. Immunoblotting was performed as previously reported except that the transblots were probed with the rabbit anti-insulin receptor antibody (1:400, Santa Cruz Biotechnology, Inc, Santa Cruz, CA), rabbit anti-phospho-ERK1/2 antibody, or rabbit anti-ERK1/2 antibody (1:400, Cell Signaling Technology, Beverly, MA).[4,21] The amount of protein transferred onto the membranes was verified by immunoblotting for α-actin (Santa Cruz Biotechnology Inc) and used for the normalization of the receptor densities.

RNA Isolation, Reverse Transcription, and Real-Time Polymerase Chain Reaction

Total RNA from RPT cells was extracted using Trizol reagent, and 1 ng RNA was used to synthesize cDNA following the manufacturer's instructions. For glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the forward primer was 5′- CAGGGCTGCCTTCTCTTGTG-3′ and the reverse primer was 5′-GGTGATGGGTTTCCCGTTGA-3' (GeneBank Accession No.: NM_017008.4). For insulin receptor, the forward primer was 5′-TTCAGGAAGACCTTC GAGGATTACCTGCAC-3′ and the reverse primer was 5′-AGGCCAGAGATGACAAGTGACTCCTTGTT-3' (GeneBank Accession No.: NM_017071.2). Insulin receptor mRNA expression was normalized for GAPDH mRNA.

Analysis of the Second Messengers Involved in the Regulation of AT_2R on Insulin Receptor Expression

To determine the second messenger(s) involved in the AT_2R-mediated regulation of insulin receptor expression in RPT cells from WKY rats and SHRs, several inhibitors or agonists were used: protein kinase C (PKC) inhibitor (19–31, 10^{-6} mol/L), PKC activator (phorbol