Title
ACTIVATED NF-KB MAINTAINS HIGH ANGIOTENSINOGEN EXPRESSION IN HUMAN RENAL PROXIMAL TUBULAR CELLS.

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Abstract
Intrarenal angiotensinogen (AGT), which is expressed mainly in renal proximal tubular cells (RPTCs), plays important roles in blood pressure regulation. Rat AGT is regulated by nuclear factor-kappa B (NF-κB) activity and an NF-κB binding site has been identified in the rat AGT promoter region. We have previously reported that an NF-κB inhibitor, parthenolide, decreased human AGT mRNA expression in a human RPTC line, however, an NF-κB binding site has not yet been identified in the human AGT promoter region. Therefore, this study was performed to determine the existence of an NF-κB binding site in the human AGT promoter region and to demonstrate that activated NF-κB augments human AGT expression in human RPTCs. The human AGT promoter region was cloned by PCR method from nucleotides -4,358 to +122 using genomic DNA from a human cell line. Human kidney-2 (HK-2) cells, immortalized human RPTCs, were transfected with pGL4.14 luciferase vectors containing progressive deletion mutants created using the clone (hAGT_-4,358/+122) to determine the expression by dual luciferase reporter assay. Endogenous AGT mRNA expression was determined by real-time RT-PCR. The promoter activity of pGL4.14_hAGT_-4,358/+122 was higher than the promoter-less vector (93.7 ± 2.8, ratio to promoter-less vector). Bioinformatics analysis of the human AGT promoter region indicates a candidate 10-bp region for a possible NF-κB binding site from nucleotides -2,515 to -2,506 and removal of this region in pGL4.14_hAGT_-3,681/+122 decreased the promoter activity (0.54 ± 0.17, ratio to pGL4.14_hAGT_-3,681/+122). Treatment with 10 μM parthenolide reduced basal human AGT mRNA levels at 24 hr (0.39 ± 0.01, ratio to untreated cells); additionally, parthenolide treatment significantly decreased the pGL4.14_hAGT_-3,681/+122 promoter activity (0.47 ± 0.09, ratio to untreated cells). However, there was no change in pGL4.14_hAGT_-2,414/+122 promoter activity, which does not contain the possible NF-κB binding region, by the parthenolide treatment. These data indicate that removal of the possible NF-κB binding region reduces the high basal expression levels of AGT in HK-2 cells. Furthermore, the data shows that the possible NF-κB binding region is required for inhibition of human AGT promoter activity by parthenolide treatment. Together, these data indicate that activated NF-κB plays an important role in the high expression of human AGT in human RPTCs. Thus, targeting the intrarenal NF-κB signaling pathway may provide a new and possibly more effective treatment for patients with hypertension.

NIH/NIDDK (R01DK072408)
NIH/NCRR (P20RR017659)
NIH/NHLBI (R01HL026371)