Unique Regulation of VEGF Synthesis in Renal Epithelial Cells

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Angiotensin II (ANG II) is a mediator of glomerular and tubular hypertrophy in diabetic renal disease. We have reported a possible role for VEGF in hypertrophy of the kidney in type 1 or 2 diabetes (Kidney Int. 64:468, 2003). As both ANG II and VEGF induce renal hypertrophy, we examined whether ANG II regulated VEGF synthesis in renal proximal tubular epithelial (MCT) cells as they form the bulk of renal cortex that undergoes hypertrophy in diabetes. Quiescent MCT exposed to ANG II (1 nM) increased VEGF protein expression within 5 min, peaking at 15-30 min; VEGF mRNA was unchanged by both northern analysis and RT-PCR.

Transcriptional inhibitors, DRB or actinomycin-D, did not affect ANG II-stimulation of VEGF synthesis, suggesting a post-transcriptional mechanism. Accordingly, we studied regulation of initiation phase of protein translation. Translation of capped mRNAs with complex 5′ untranslated regions, e.g., VEGF mRNA, is dependent on eukaryotic initiation factor 4E (eIF4E), the mRNA cap binding protein. In resting cells eIF4E is held in an inactive state by its binding protein 4E-BP1. Upon stimulation, phosphorylation of 4E-BP1 occurs and the complex dissociates, releasing eIF4E to bind the mRNA cap and facilitate its translation. ANG II-induced VEGF expression was paralleled by increased phosphorylation of 4E-BP1 and eIF4E. ANG II failed to increase VEGF protein in cells stably expressing phosphorylation mutant of 4E-BP1, indicating that ANG II acts at initiation phase of VEGF translation. This was further confirmed by polyribosomal assay. Segregated and aggregated ribosomes were fractionated into four fractions using a sucrose gradient and total RNA was isolated from each fraction; RT-PCR was performed using primers for VEGF and GAPDH. Selective enrichment of heaviest ribosomal fraction (containing polyribosomes) with VEGF mRNA but not GAPDH mRNA was seen in ANG II-treated cells compared to control. Total RNA from the heavy ribosomal fractions was employed in an in vitro translation assay using rabbit reticulocyte lysate. ANG II selectively augmented synthesis of a 23-kd protein that was confirmed to be VEGF by immunoblotting. This effect was inhibited by LY294002, a PI 3-kinase inhibitor and by expression of dominant negative Akt phosphorylation mutant or 4E-BP1 phosphorylation mutant. The rapidity of VEGF synthesis induction by ANG II suggested possible mediation by reactive oxygen species (ROS).

ANG II stimulated ROS production rapidly in MCT cells and inhibition of ROS by N-acetyl cysteine or DPI abolished ROS generation, Akt phosphorylation, 4E-BP1 phosphorylation and VEGF synthesis induced by ANG II. In conclusion, (1) ANG II rapidly increases expression of VEGF protein in MCT cells by regulation of its ribosomal translation rather than by transcription. (2) ANG II regulation of VEGF synthesis is dependent on ROS generation by ANG II. ANG II-VEGF axis may be a novel pathway of renal injury in diabetes.

References

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