IDENTIFYING ANGIOTENSIN II EFFECTS ON PROTEIN KINASE B ACTIVATION IN INSULIN METABOLIC SIGNALING

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Inappropriate activation of the Renin-Angiotensin-Aldosterone System has been linked to oxidative stress and insulin resistance. Whereas insulin binds to its receptor and triggers tyrosine phosphorylation of Insulin Receptor Substrate 1 (IRS-1) for downstream signaling through the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt) cascade leading to glucose uptake, Ang II and aldosterone stimulate NADPH oxidase production of reactive oxygen species (ROS). These ROS are postulated to activate redox-sensitive serine kinases that inappropriately phosphorylate IRS-1 at serine moieties, subsequently decreasing Akt activation/phosphorylation which translates into impaired glucose uptake and decreased insulin sensitivity.

The crucial insulin metabolic signaling event of Akt activation as it relates to Ang II alone was investigated. C2C12 mouse skeletal muscle cells were cultured in 60mm dishes (DMEM with 10% FBS, 1% penicillin/streptomycin) at 5% CO2 and 37 °C until ?80% confluent, then differentiated to myotubules (DMEM containing 2% horse serum, 1% penicillin/streptomycin, 0.1% amphotericin B) with characteristic morphological alignment, elongation, and fusion confirmed using light microscopy. Time course treatments with 100nM Ang II with or without 100nM insulin stimulation for 5 min before harvest followed. Western blot analysis using anti-rabbit antibodies to serine phosphorylated (Ser473) Akt or total Akt (1:1000) provided immunoblot bands that were quantified on the Quantity One software (Bio-Rad).

Ang II and insulin, respectively, signal through Akt with fluctuations in the level of phosphorylated Akt observed within the tested time intervals. Although our preliminary data shows a trend favoring diminished Akt activation in the presence of Ang II, lack of statistical significance warrants further investigation.