Insulin Signaling in Regulation of -Glutamylcysteine Ligase Catalytic Subunit in Primary Cultured Rat Hepatocytes

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Decreased glutathione (GSH) levels and -glutamylcysteine ligase (GCL) activity have been observed in diabetic patients, and insulin reportedly increases GSH synthesis *via* increased GCL catalytic subunit (GCLC) gene expression. The signaling pathways responsible for mediating insulin effects on GCLC expression and GSH levels, however, are unknown. The signaling pathways involved in the regulation of GSH synthesis in response to insulin were examined in primary cultured rat hepatocytes. GSH levels, GCL activity and GCLC protein and mRNA levels were increased to 140%, 160%,600% and 340% of that monitored in untreated cells, respectively, in hepatocytes cultured with 100 nM insulin. The phosphatidylinositol 3-kinase (PI3K) inhibitors, wortmannin and LY294002, dominant negative Akt, or rapamycin, an inhibitor of mTOR and ribosomal p70 S6 kinase (p70S6K) phosphorylation, inhibited the insulin-mediated increase in GCLC protein and GSH levels. Although the mitogen-activated protein kinases (MAPKs), ERK, p38, and JNK were activated in response to insulin, PD98059, an inhibitor of MEK, SP600125, an inhibitor of JNK and SB203580, an inhibitor of p38MAPK failed to inhibit the insulin-mediated increase in GCLC protein levels. Interestingly, GSH levels were markedly increased by the MEK inhibitor PD98059 or flavone in hepatocytes cultured in the presence or absence of insulin, whereas SP600125 augmented the insulin-mediated increased in GSH levels. This study shows that insulin signaling pathways involving PI3K/Akt/p70S6K are active in the insulin-mediated regulation of GSH synthesis *via*increased expression of GCLC. Moreover, the PD98059-mediated elevation of GSH levels may be responsible for the suppressive effect of PD98059 on antioxidant enzyme induction mediated via oxidative stress