Insulin activates Glycogen Synthase by a novel PI 3-kinase/p70^6k dependent pathway in 3T3-L1 adipocytes

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Insulin causes the activation of a tyrosine kinase activity in the intracellular domain of its receptor, the major substrate of this kinase being the multifunctional docking protein IRS-1. Phosphorylation of specific tyrosine residues on IRS-1 allows this protein to interact with and activate a number of downstream signalling molecules including phosphoinositide 3-kinase (PI 3-kinase), SH-PTP2 and the Grb-2/Sos/ras system (and hence the MAP kinase cascade) [1]. Insulin also causes the activation of ribosomal protein S6-kinase (p70^6k) by unknown mechanisms [2]. However it is not clear how insulin stimulation of these signalling pathways exerts control over metabolic pathways.

We have used rapamycin, which specifically blocks growth factor activation of p70^6k, and the specific PI 3-kinase inhibitor wortmannin, to investigate the involvement of PI 3-kinase and p70^6k in insulin stimulation of glucose transport and glycogen synthesis in 3T3-L1 adipocytes. We find that wortmannin inhibits PI 3-kinase activity in anti IRS-1 and anti-PI 3-kinase immunoprecipitates from 3T3-L1 adipocytes in a dose dependent manner (IC50 of ~10 nM), with inhibition being complete at 100 nM (data not shown). Wortmannin inhibited insulin stimulated glucose transport in a similar dose dependent manner in the 3T3-L1 adipocytes (IC50 = 15nM) (Fig. 1). The potency of these inhibitory effects of wortmannin agrees with previous reports [3,4].

Insulin stimulated glycogen synthase activity 2-3 fold in these cells. Wortmannin inhibited insulin stimulated activation of glycogen synthase with a similar dose dependency to its inhibition of insulin stimulated glucose transport and PI 3-kinase. Inhibition of activation was complete at 100nM wortmannin although basal activity was not affected (Fig. 2). We sought to find the downstream mechanism for the effects of PI 3-kinase on glycogen synthase activation. Activity of glycogen synthase is controlled by serine phosphorylation cascades. However, recent evidence suggests that the MAP kinase cascade is not involved in this process in the 3T3-L1 adipocyte [5]. Activation of the p70^6k is known to be independent of the MAP kinase cascade but dependent on PI 3-kinase activity [6]. We investigated the role of p70^6k in insulin stimulation of glycogen synthase using rapamycin, a specific inhibitor of growth factor activation of p70^6k. Rapamycin attenuated insulin activation of glycogen synthase in a dose dependent manner (IC50 = 0.8ng/ml) with a maximal inhibition of ~75%. Rapamycin did not inhibit insulin's stimulation of glucose transport although it inhibited insulin stimulated glucose incorporation into glycogen with a similar dose dependency to the inhibition of glycogen synthase activation. The potency of the inhibition closely matches the reported IC50 for inhibition of p70^6k [6] suggesting that rapamycin's effects on glycogen synthase and glycogen synthesis are exerted through p70^6k.

We conclude that insulin's activation of glycogen synthase in 3T3-L1 adipocytes involves a novel pathway dependent on PI 3-kinase and p70^6k activity.

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References

Figure 1. Wortmannin Inhibits Insulin Stimulated 2-deoxyglucose Uptake in 3T3-L1 Adipocytes Serum starved 3T3-L1 adipocytes were incubated in glucose free media with indicated concentration of wortmannin for 5 minutes prior to addition of 10 nM insulin for 10 minutes prior to determination of 3H-2-deoxyglucose uptake. Each point represents mean of triplicate determination ± s.d.

Figure 2. Wortmannin Inhibits Insulin Activation of Glycogen Synthase in 3T3-L1 Adipocytes Serum starved 3T3-L1 adipocytes were incubated with 100 nM wortmannin for 5 minutes prior to addition of 10 nM insulin for 30 minutes. 3H-UDPG glucose incorporation into glycogen was measured in cell lysates + or - glucose 6-phosphate and activity ratios calculated. Each point represents mean of 4 determinations ± s.d.