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PI-3-Kinase Inhibitor Wortmannin Blocks the Insulin-like Effects of Growth Hormone in Isolated Rat Adipocytes

Martin Ridderstråle* and Hans Tornqvist

Departments of Paediatrics and *Medical and Physiological Chemistry,
University of Lund, Sweden

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The effect of wortmannin, a selective phosphatidylinositol 3-kinase inhibitor, on the insulin-like effects of growth hormone in isolated adipocytes from rat was investigated. Wortmannin inhibited both the lipogenic and the antilipolytic effects (IC_{50} ≈20 nM) with no effect on [^{125}I]-growth hormone binding to the adipocytes. These data suggest that phosphatidylinositol 3-kinase might play an important role in the insulin-like actions of growth hormone.

Growth hormone (GH) exerts acute insulin-like effects, including antilipolysis and increased lipogenesis, in adipocytes that have been incubated in absence of hormones for three hours (1-3). The detailed molecular signaling mechanisms for these actions are not known although involvement of tyrosine kinases (4 and M. Ridderstråle *et al* unpublished data), proteinkinase C (PKC) (5) and phospholipase C (6), have been suggested.

Phosphatidylinositol 3-kinase (PI-3-kinase), consisting of an 85-kDa regulatory- and 110-kDa catalytic subunit (7-10), is a dual specificity (11), lipid- and serine kinase, which is activated in response to several growth factors (reviewed in 12). The physiologically important product of PI-3-kinase is thought to be phosphatidylinositol (3,4,5)-triphosphate (PIP_3) (13). PIP_3 has been shown to stimulate the activity of PKC ζ *in vitro* (14) which would indicate a possible role as second messenger. It has been shown that insulin stimulates the activity of PI-3-kinase in adipocytes (15-16) and recently that the metabolic actions of insulin, GLUT4 translocation (17) as well as increase in hexose uptake and antilipolysis (18), can be blocked by a selective PI-3-kinase inhibitor, wortmannin (19).

Abbreviations: PI-3-kinase, phosphatidylinositol 3-kinase; GH, growth hormone; PKC, protein kinase C; PIP_3, phosphatidylinositol (3,4,5)-triphosphate; PCV, packed cell volume, KRH, modified Krebs-Ringer medium with HEPES; BSA, bovine serum albumin; DMSO, dimethyl sulphoxide; hGH, human growth hormone; NA, noradrenaline.

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Based on these observations we used wortmannin to examine whether PI-3-kinase has a role in GH action. Wortmannin completely inhibited GH stimulated lipogenesis and antilipolysis without compromising GH binding to the adipocytes, suggesting a role for PI-3-kinase in GH signalling leading to its insulin-like effects in adipocytes.

MATERIALS AND METHODS

Cell preparation: Adipocytes were essentially prepared according to Rodbell (20) with modifications (21) from 36 day old Sprague-Dawley rats (ALAB, Sweden) fasted over night prior to the experiments. The cells were kept as a 5% (v/v) suspension in a modified Krebs-Ringer medium (KRH) at pH 7.5 containing 24 mM HEPES, 119 mM NaCl, 4.95 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4$, 2 mM glucose, 200 nM adenosine, 1% (w/v) bovine serum albumin (BSA) at 37°C on a reciprocal shaker for three hours to make them responsive to GH stimulation of insulin-like effects (22). Phenylisopropyladenosine (100 nM) and adenosine deaminase (0.5 I.U./mL) were routinely added followed by wortmannin (Sigma) or vehicle (DMSO) for 10 minutes before hormone stimulation. Recombinant hGH and human insulin were generously supplied by Novo Nordisk, Denmark.

Lipolysis: 1 mL aliquots of a 2% (v/v) cell suspension (KRH, 3.5% BSA) were added to vials containing the appropriate hormones. Lipolysis, stimulated by noradrenaline (NA) (Sigma), was measured as proportional to glycerol released to the medium during a 30 minute incubation and determined as described (23) and expressed as nmol glycerol/mL packed cell volume (PCV)/min.

Lipogenesis: 1 mL aliquots in triplicates of a 2% cell suspension (KRH, 3.5% BSA, 0.55 mM glucose) were added to vials containing the appropriate hormones and 0.11 μCi/mL D-(3-^{3}H)glucose (Amersham, UK) and assayed for the incorporation of ^{3}H from ^{3}H-glucose into adipocyte triglycerides during a 30 minute incubation (24). Results were expressed as % of the insulin (1 nM) effect in absence of inhibitor.

^{125}I-hGH-binding: ^{125}I-hGH (45 pM, 63 μCi/μg, Novo Nordisk, Denmark) was added to 1 mL aliquots in triplicates of a 5% (v/v) cell suspension (KRH, 3.5% BSA) in the presence (non-specific binding) or absence (specific binding) of 4.6 μM unlabelled hGH at different concentrations of the inhibitor. Incubations were terminated after 60 minutes and the cells separated from the media (250 μL aliquots equal to 12.5 μL PCV) by rapid centrifugation through dinonylphtalate. The tubes were frozen and then cut through the oil and cell-bound radioactivity counted in a Beckman γ-counter.

Students t-test for paired observations was used for statistical evaluations.

RESULTS AND DISCUSSION

To investigate the effect of wortmannin on the insulin-like effect of GH in adipocytes from rat, GH stimulated lipogenesis and antilipolysis was measured. In absence of wortmannin GH stimulated lipogenesis 2.8 ±0.3-fold (mean ±S.E., n=4, $p < 0.01$). This effect was completely blocked by wortmannin at 100 nM ($IC_{50} \approx 20$ nM) (Fig. 1A). The IC_{50} value for inhibition of GH action was essentially the same as that reported for wortmannin on insulin stimulation of hexose transport, the first step in lipogenesis, in adipocytes (18). As a comparison wortmannin completely inhibited insulin stimulated lipogenesis, 6.1 ±0.8-fold (mean ±S.E, n=4) increase in absence of inhibitor, with an IC_{50} of ≈100 nM.

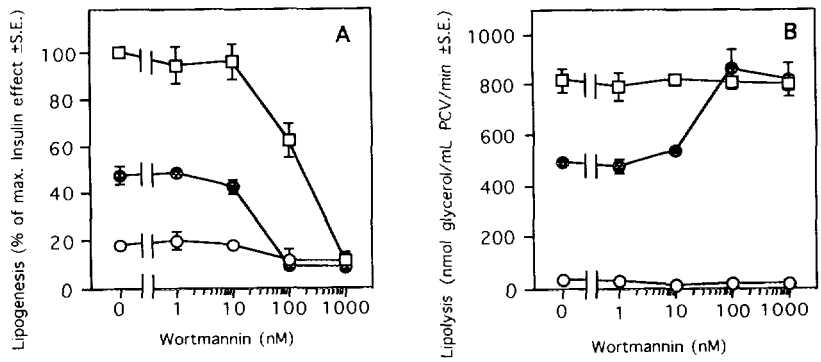

Figure 1. Effect of wortmannin on the insulin-like effects of GH. Adipocytes, incubated for 3 hours in absence of hormones, were incubated in presence of vehicle (DMSO) or wortmannin at the indicated concentrations for 10 min before being subject to hormonal stimulation: (A) Lipogenesis: basal (O), hGH at 23 nM (●) or insulin at 1 nM (□). Results are expressed as % of the effect caused by insulin at 1 nM in absence of inhibitor. Each point represents mean values ±S.E. from four seperate experiments, triplicate incubations for each condition. (B) Lipolysis: basal (O), NA at 100 nM alone (□) or NA at 100 nM together with hGH at 23 nM (●). Each point represents mean values ±S.E. from three seperate experiments analyzed in triplicate for each condition.

The antilipolytic effect of GH was measured as the ability to counteract lipolysis induced by NA at 100 nM (815 ±47 nmol glycerol/mL PCV/min. (mean ±S.E., n=3)). GH (23 nM) inhibited lipolysis by 40 ±5 % to 493 ±6 nmol glycerol/mL PCV/min. ($p<0.001$). Wortmannin completely inhibited this antilipolytic effect with an IC_{50} of ≈20 nM (Fig. 1B), similar to that obtained for lipogenesis (cf. Fig. 1A). Wortmannin had no effect on NA stimulated or basal lipolysis (28 ±11 nmol glycerol/mL PCV/min.) indicating that the inhibitory effect was not on cAMP-dependent protein kinase A or due to cell toxicity.

Wortmannin inhibits PI-3-kinase as a result of direct association with the 110-kDa catalytic subunit at low concentration (25). Other kinases such as the myosin light chain kinase (26) and phosphatidylinostol 4-kinase (19) have been reported to be inhibited by wortmannin at higher concentrations than those used in the present investigation. It does not inhibit cyclic nucleotide dependent protein kinases, calmodulin-dependent protein kinase II or PKC (26) nor the insulin stimulated tyrosine autophosphorylation of the insulin receptor 95-kDa β-subunit (17-18). Therefore inhibition of kinases other than PI-3-kinase seems an unlikely explanation for our results. In addition wortmannin did not significantly alter total ^{125}I-hGH binding to the adipocytes (Fig.2).

In conclusion our data establish wortmannin, a selective PI-3-kinase inhibitor, as an inhibitor of the insulin-like effects of GH in adipocytes. Although we do not show direct stimulation of PI-3-kinase activity in response to GH our data suggest a role for PI-3-kinase in GH action. The detailed mechanism for the antilipolytic action of GH is presently under investigation in our laboratory.

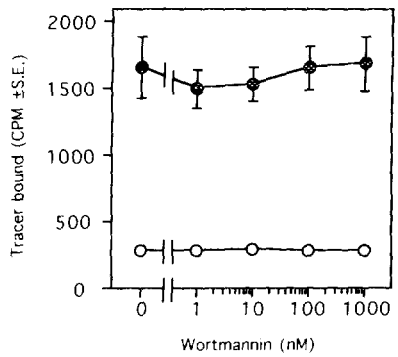

Figure 2. Effect of wortmannin on total ^{125}I-hGH binding. Adipocytes were incubated for 3 hours in absence of hormones before addition of hormone: ^{125}I-hGH at 45 pM alone (specific binding, ●) or with 4.6 μM unlabelled hGH (non-specific binding, O) in the presence of wortmannin at the indicated concentrations. Each point represents mean values ±S.E. from three seperate experiments analyzed in triplicate for each condition.

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