Involvement of PI 3-kinase in stimulation of glucose transport and recruitment of transferrin receptors in 3T3-L1 adipocytes

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PI 3-kinase is acutely activated by insulin and it is apparent that PI 3-kinase activity is required for insulin stimulation of a range of diverse responses. These include stimulation of glucose transport [1-4], activation of ribosomal protein S6-kinase [3,5], induction of membrane ruffling [6], inhibition of lipolysis [7], activation of the MAP kinase cascade [8] and activation of glycogen synthase [PRS unpublished observation]. In our hands wortmannin inhibited insulin stimulation of glucose transport and recruitment of transferrin receptors to the plasma membrane in a dose dependent manner (IC50 = 15 nM) (data not shown). We find wortmannin inhibits PI 3-kinase activity in these cells with a similar potency (IC50 = 10 nM) (data not shown). This suggests that PI 3-kinase activity is necessary for growth factor stimulation of glucose transport and transferrin receptor recruitment in 3T3-L1 adipocytes. However, it is not clear whether activation of PI 3-kinase activity is sufficient to induce any of these endpoint responses.

To investigate this we correlated insulin, IGF-1 and PDGF-bb stimulation of PI 3-kinase with the ability of these growth factors to stimulate DNA synthesis, glucose transport and recruitment of transferrin receptors. At maximally effective concentrations (insulin 10 nM, IGF-1 37 ng/ml and PDGF-bb 20 ng/ml), these growth factors all significantly stimulated [H]methyl thymidine uptake into 3T3-L1 adipocytes (Table 1). This established that signalling mechanisms for these ligands existed in this cell type. However, at these concentrations only insulin and IGF-1 were able to stimulate 2 deoxy-glucose uptake (Table 1). Further, insulin and IGF-1 caused significant stimulation of transferrin receptor recruitment to the plasma membrane while stimulation by PDGF-bb was not significant (Table 1). Insulin potently stimulated PI 3-kinase activity in anti-phosphotyrosine immunoprecipitates from cells that had been stimulated with growth factor at 4°C to cells that had been stimulated with growth factor at 37°C for 20 minutes. PI 3-kinase activity was determined in anti-phosphotyrosine immunoprecipitates from cells that had been stimulated for 10 minutes with growth factor.

Table 1 Insulin, IGF-1 and PDGF-bb Stimulation of DNA Synthesis, Glucose Transport, Transferrin Receptor Recruitment and PI 3-kinase Activity in 3T3-L1 Adipocytes

<table>
<thead>
<tr>
<th></th>
<th>Insulin (10 nM)</th>
<th>IGF-1 (37 ng/ml)</th>
<th>PDGF-bb (20 ng/ml)</th>
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<tbody>
<tr>
<td>DNA Synthesis</td>
<td>498 ± 169</td>
<td>838 ± 301</td>
<td>1125 ± 101</td>
</tr>
<tr>
<td>Glucose Transport</td>
<td>875 ± 105</td>
<td>860 ± 80</td>
<td>169 ± 14</td>
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<tr>
<td>Transferrin Binding</td>
<td>179 ± 6</td>
<td>186 ± 18</td>
<td>107 ± 7</td>
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<tr>
<td>PI 3-kinase activity</td>
<td>1400 ± 155</td>
<td>N.D.</td>
<td>2560 ± 110</td>
</tr>
</tbody>
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References