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Genistein directly inhibits GLUT4-mediated glucose uptake in 3T3-L1 adipocytes☆

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Abstract

The isoflavone-derivative genistein is commonly applied as an inhibitor of tyrosine kinases. In this report we analyze the effect of genistein on insulin-stimulated glucose uptake in 3T3-L1 adipocytes. In these cells insulin-induced glucose uptake is primarily mediated by the GLUT4 glucose transporter. We observed that pre-treatment with genistein did not affect insulin-induced tyrosine kinase activity of the insulin receptor or activation of protein kinase B. On the other hand, genistein acted as a direct inhibitor of insulin-induced glucose uptake in 3T3-L1 adipocytes with an IC_{50} of 20 μM. We conclude that apart from acting as a general tyrosine kinase inhibitor, genistein also affects the function of other proteins such as the GLUT4 transporter. These data suggest that caution must be applied when interpreting data on the involvement of tyrosine kinase activity in glucose uptake in 3T3-L1 adipocytes.

Keywords: GLUT1; Insulin; Walker B domain; Homology

A key feature of muscle cells and adipocytes is the induction of glucose uptake in response to stimulation with insulin. In these cells the activated insulin receptor tyrosine kinase initiates a signaling cascade inducing translocation of the GLUT4 glucose transporter from an intracellular vesicular storage compartment towards the plasma-membrane [1–3]. Aside from insulin, also stress-inducing stimuli such as osmotic shock and arsenite increase glucose uptake in adipocytes by inducing GLUT4 translocation. Although these stimuli do not activate the insulin receptor, a tyrosine kinase is involved [4,5]. Thus, apparently tyrosine kinase activity is essential for all stimuli projecting towards GLUT4 translocation.

The naturally occurring isoflavone derivative genistein is commonly applied as a tyrosine kinase inhibitor [6]. As such it has been used in reports illustrating the involvement of tyrosine kinase activity in glucose uptake [7]. However, as we describe in this brief communication, genistein is a potent and direct inhibitor of GLUT4-mediated glucose uptake itself. Hence, although the involvement of tyrosine kinase activity in GLUT4-mediated glucose uptake is undisputed, genistein is ill suited as an inhibitor in investigating the role of tyrosine kinases in glucose transport.

Materials and methods

Materials. Dulbecco's modified Eagle's medium was purchased from Life Technologies; fetal calf serum was from Braunschweig, Amsterdam (Lot No. A01127-318); dexamethasone, 1-methyl-3-isobutylxanthine (IBMX), bovine insulin, and 2-deoxy-D-glucose were

☆ *Abbreviations:* IC_{50}, inhibitory concentration 50%; 2-DOG, 2-deoxyglucose; AGC, kinase superfamily defined by protein kinases A, C, and G; PKB, protein kinase B; EGF, epidermal growth factor; ATP, adenosine-tri-phosphate.

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obtained from Sigma–Aldrich. 2-Deoxy-D-[^{14}C]glucose was purchased from NEN-Dupont; genistein was obtained from Calbiochem. HRP-conjugated mouse monoclonal (anti-phosphotyrosine) pY-20 was obtained from Santa Cruz Biotechnology, rabbit polyclonal recognizing IRβ was from Transduction Laboratories, phospho-specific ser^{473} PKB was from Cell Signaling Technology, and sheep polyclonal antibodies recognizing PKB were purchased from Upstate Biotechnology.

Cell culture. 3T3-L1 fibroblasts were obtained from ATCC and differentiated to adipocytes as described previously [8]. Mature adipocytes were routinely used 7–14 days after completion of the differentiation process. Only cultures in which >95% of cells displayed adipocyte morphology were used.

Assay of 2-deoxyglucose uptake. 3T3-L1 adipocytes, grown in 12-well plates (Costar), were subjected to an assay of 2-deoxy-D-[^{14}C]glucose (2-DOG) (0.075 μCi/well) uptake as described previously [9].

Immunoprecipitation and whole cell lysate. Nine centimeter dishes of 3T3-L1 adipocytes were treated with 100 nM insulin for 5 min. Cells were lysed in a NP-40 based buffer (1 mM Na_3VO_4, 1 mM EGTA, 1 mM EDTA, 50 mM Tris–HCl. pH 7.4, 1% (w/v) NP-40, 0.5% (w/v) Na-deoxycholate, 150 mM NaCl, and 5 mM NaF in the presence of protease inhibitors (Complete, Boehringer–Mannheim)). Cells were rotated head-over-head for half an hour at 4 °C. Cell lysate was cleared from cellular debris (including the lipid droplets) by spinning at 14,000*g* for 10 min at 4 °C in a table-top centrifuge. After a pre-clear step using protA beads, 1 mg cell lysate as determined using BCA protein assay reagent (Pierce) was subjected to immunoprecipitation using 5 μg anti-IRβ rabbit polyclonal for 1.5 h at 4 °C. Immunocomplexes were harvested by incubating with ProtA beads for an additional 1.5 h at 4 °C. Beads were washed three times in lysis buffer, once in 10 mM Tris–EDTA, pH 7.4, and dissolved in sample buffer for standard immunoblot procedures. For whole cell lysate, samples were lysed in sample buffer with the β-mercaptoethanol and bromophenol-blue was added after protein concentration determination.

Statistical analysis and graph generation. Statistical analysis of the data obtained was performed with an independent-samples *t* test using SPSS 10.0. Graphs were generated using PRISM 2.01.

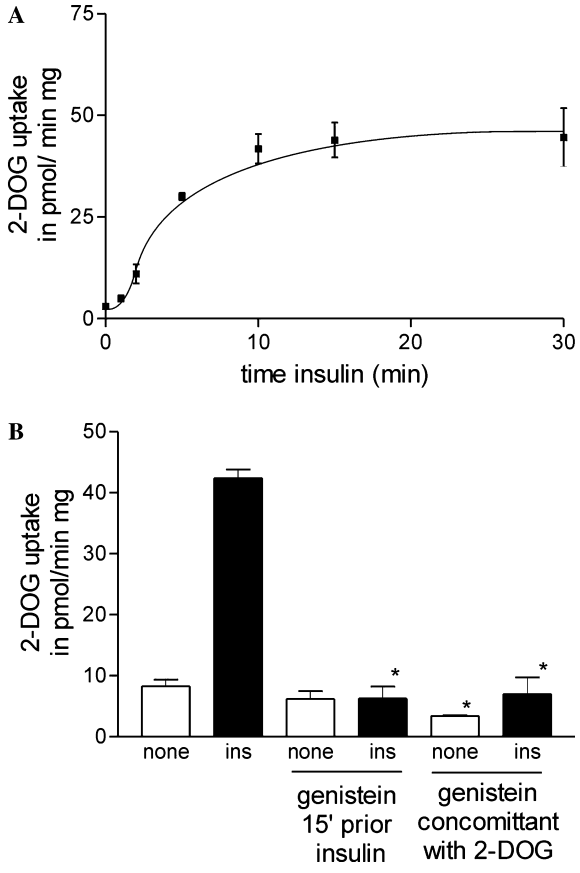

Fig. 1. Insulin-induced glucose uptake and effects of genistein. (A) 3T3-L1 adipocytes were stimulated with 100 nM insulin for the indicated times and glucose uptake was analyzed. Data shown are means ± SE of two experiments each performed in triplicate. (B) 3T3-L1 adipocytes were stimulated with 100 nM insulin for 15 min and glucose uptake was measured. Genistein treatment was as indicated, i.e., either no treatment, pre-treatment 15 min prior to the time of insulin stimulation (under the continued presence of genistein) or concomitant with the addition of radiolabel (i.e., after 15 min of uninhibited insulin-stimulation). Data shown are means ± SE of two experiments each performed in triplicate. An * indicates $p < 0.05$ compared to the corresponding uninhibited samples.

Results and discussion

Genistein inhibits glucose uptake independent of tyrosine kinase inhibition

Insulin stimulates glucose uptake in 3T3-L1 adipocytes after a brief lag-phase of roughly 1 min with a $t_{1/2}$ of 3 min (see Fig. 1A) [10]. Consequentially, after 15 min of insulin treatment, the process of GLUT4 translocation and the induction of glucose uptake is complete and does not require any further tyrosine kinase activity. When 50 μM genistein was included in the assay 15 min prior to insulin-stimulation, genistein profoundly inhibited insulin-induced glucose uptake (Fig. 1B). However, even when genistein is added concomitant with the radiolabeled 2-DOG (i.e., after 15 min of insulin-stimulation in the absence of genistein), glucose uptake is still completely reduced. These observations suggest that genistein interferes with the insulin-induced glucose uptake directly and not by inhibiting GLUT4 translocation. To further characterize this direct inhibition of GLUT4-mediated glucose uptake, 3T3-L1 adipocytes were stimulated with insulin for 15 min and subsequently genistein was added to the cells at the concentrations indicated concomitant with the addition of radiolabeled 2-DOG (Fig. 2). As can be seen in this figure, this effect of genistein has an IC_{50} of 20 μM.

When we analyzed the effects of 15 min pre-treatment with genistein on insulin-induced signaling, no effect on insulin-receptor autophosphorylation was observed (Fig. 3A). Furthermore, insulin stimulation induces activation of the AGC-kinase family member PKB [1–3]. As can be seen in Fig. 3B, the activation of PKB was also unaffected by genistein, suggesting that the insulin receptor tyrosine kinase is not sensitive to genistein. Of note, EGF receptor tyrosine phosphorylation induced by EGF was inhibited in these cells by pre-treatment with genistein (data not shown). These observations are in