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Curcumin is a direct inhibitor of glucose transport in adipocytes

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ABSTRACT

Curcumin has been reported to inhibit insulin signaling and translocation of GLUT4 to the cell surface in 3T3-L1 adipocytes. We have investigated the effect of curcumin on insulin signaling in primary rat adipocytes. Curcumin (20 μM) inhibited both basal and insulin-stimulated glucose transport (2-deoxyglucose uptake), but had no effect on insulin inhibition of lipolysis. Dose–response experiments demonstrated that curcumin (0–100 μM) inhibited basal and insulin-stimulated glucose transport, but even at the highest concentration tested did not affect lipolysis. Inhibition was equal in cells that had been pre-incubated with curcumin and in cells to which curcumin was added immediately before the glucose transport assay. Similarly, time-course experiments revealed that the inhibitory effect of curcumin was evident at the earliest time point tested (30 s). Thus it is unlikely that inhibition of insulin signaling or of translocation of GLUT4 to the cell surface is involved in the inhibitory effect of curcumin. Curcumin did not affect the stimulatory action of insulin on phosphorylation of Akt at serine 473. We conclude that curcumin is a direct inhibitor of glucose transporters in rat adipocytes.

Introduction

Recently there has been considerable interest in potential therapeutic uses of curcumin, in particular for Alzheimer's disease, multiple myeloma and colon cancer (Hatcher et al. 2008). Evidence also suggests that curcumin may be of value in treatment or prevention of type 2 diabetes (Weisberg et al. 2008). This polyphenolic compound is the major yellow-colored pigment found in the spice, turmeric. Curcumin has been used in traditional Indian medicine for centuries, and has numerous pharmacological activities, including potent anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic actions (Hatcher et al. 2008). Curcumin has been reported to inhibit peptide hormone and growth factor signaling pathways through effects on several kinases and transcription factors, including I-κB kinase and NF-κB (Singh and Aggarwal 1995). Despite these many activities, curcumin appears to be remarkably non-toxic, and human subjects have been treated with as much as 12 g curcumin with no apparent adverse effects (Lao et al. 2006).

Several reports have suggested that curcumin might be of value in treatment of diabetes. Curcumin lowered blood glucose in streptozotocin-treated rats (Mahesh et al. 2005), and has been reported to markedly improve blood glucose and insulin sensitivity in mouse models of diabetes (Weisberg et al. 2008). The authors of this latter study concluded that the improvement was likely related to the anti-inflammatory actions of curcumin. Curcumin has also been shown to inhibit glucose production in mouse hepatocytes (Fujiwara et al. 2008), and this action may also contribute to its antidiabetic activity. Finally, a recent report has demonstrated that curcumin prevents the lipolytic effects of TNFα and catecholamines in 3T3-L1 adipocytes (Xie et al. 2012), which if true *in vivo* would be expected to decrease circulating FFA and improve insulin sensitivity.

Curcumin has been reported to prevent the stimulatory effect of insulin on glucose transport in 3T3-L1 adipocytes (Ikonomov et al. 2002). This inhibitory activity was thought to be due to prevention of insulin-induced translocation to the cell surface of the insulin-sensitive GLUT4 glucose transporters. Moreover, it was reported that curcumin inhibits activation of PIKfyve, an enzyme thought to be involved in insulin signaling (Ikonomov et al. 2002). Here we report that in primary rat adipocytes, curcumin inhibits glucose transport directly, rather than through inhibition of insulin signaling. Indeed, insulin-induced phosphorylation of Akt and inhibition of lipolysis are not affected by curcumin in these cells. Furthermore, the inhibition of glucose transport is essentially immediate and hence unlikely to involve inhibition of insulin signaling or translocation of the GLUT4 glucose transporters to the cell surface. The possible relevance of the findings to diabetes is discussed.

Materials and methods

Materials

Bovine serum albumin (BSA) was from Intergen (Purchase, NY); colleganase, type 2 was from Worthington (Freehold, NJ);

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2-deoxy-[^{3}H]glucose was from Amersham (Piscataway, NJ). Curcumin and all other reagents were from Sigma (St. Louis, MO) unless otherwise noted.

Animals

Male Sprague-Dawley rats were used for all experiments. Animals (180–240 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN). They were maintained on a 12-h light–12-h dark cycle and fed PROLAB RMH 1000 (PMI Nutrition Int'l Inc., Brentwood, MO) and tap water *ad libitum*.

Adipocyte isolation

Animals were killed by CO_2 asphyxiation. Animal protocols were approved by Bassett Healthcare's Institutional Animal Care and Use Committee. Adipocytes were isolated from epididymal fat pads as previously described (Green et al. 1990, 1992). Digestion was carried out at 37 °C with constant shaking (140 cycles/min) for 45 min. Cells were filtered through nylon mesh (1 mm) and washed three times and suspended in 137 mM NaCl, 5 mM KCl, 4.2 mM $NaHCO_3$, 1.3 mM $CaC1_2$, 0.5 mM KH_2PO_4, 0.5 mM $MgCl_2$, 0.5 mM $MgSO_4$, 20 mM Hepes (pH 7.4), plus 1% bovine serum albumin (BSA).

Glucose transport assay

Uptake of 2-deoxy[^{3}H]glucose was used as an index of the rate of glucose transport as previously described (Green 1986).

Lipolysis assay

Lipolysis was measured by following glycerol release, as previously described, using a kit from Amresco (Solon, OH) for determination of glycerol concentration (Gasic and Green 1995).

Akt phosphorylation

Adipocytes were washed into the same buffer as described for the glucose transport assay, except that the final concentration of BSA was 0.1%. Cells were incubated with insulin and/or curcumin as described in the figure legends for 10 min, then the reaction was terminated by addition of Laemmli sample buffer (Laemmli 1970) and the samples were heated at 95 °C for 5 min. The samples were then resolved by SDS-PAGE (10% gels), transferred to nitrocellulose and the blots were probed with a polyclonal rabbit antibody to phospho-Akt (Ser473). Parallel blots were probed with an antibody to total Akt (both Akt antibodies were from Cell Signaling, Danvers, MA). Blots were developed with a second antibody (goat anti-rabbit HRP) and chemiluminescent detection (Amersham ECL+).

Results

Curcumin has been reported to be a potent inhibitor of PIKfyve, an enzyme believed to be important in signaling the metabolic effects of insulin. PIKfyve is believed to be "upstream" of Akt, and hence involved both in stimulation of glucose transport and inhibition of lipolysis by insulin. This would predict that curcumin would block both the stimulatory effect of insulin on glucose transport, and the inhibitory effect on lipolysis. Fig. 1 shows the effect of insulin on glucose transport and lipolysis in cells pre-incubated with or without curcumin (20 μM). As expected, insulin produced a marked increase in the rate of glucose transport (measured as 2-deoxyglucose uptake) and a pronounced inhibition of lipolysis. Preincubation with curcumin resulted in a decrease in the rate of glucose transport at all insulin concentrations (Fig. 1a) but did not affect the antilipolytic effect of insulin (Fig. 1b). This finding suggests that curcumin preferentially inhibits the effect of insulin on glucose transport over the effect on lipolysis.

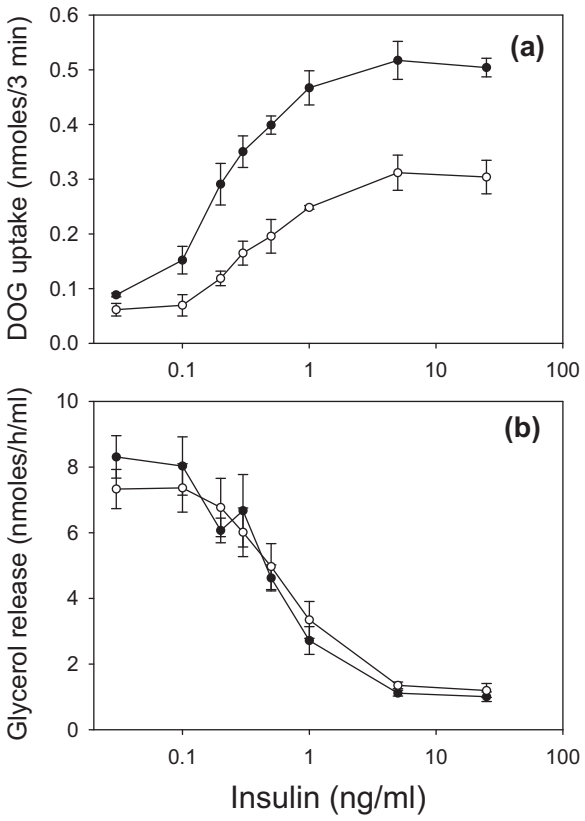

Fig. 1. Effect of curcumin on insulin action. Panel (a), glucose transport. Adipocytes were incubated without (●–●) or with (○–○) curcumin at 20 μM, plus insulin as indicated, for 45 min. Glucose transport was measured as uptake of 2-deoxy [^{3}H]glucose as described in *Methods* section. Panel (b), lipolysis. Adipocytes were incubated with adenosine deaminase without (●–●) or with (○–○) curcumin at 20 μM, plus insulin as indicated, for 1 h. Glycerol release was measured as described in *Methods* section. Data are means ± S.D. (n = 3).

Fig. 2 illustrates the dose–response relationship for effects of curcumin on glucose transport and lipolysis. Curcumin, 0–100 μM, caused a dose-related inhibition of both basal and insulin-stimulated glucose transport (Fig. 2a). In Fig. 2b the glucose transport data are normalized to the rate in the absence of curcumin. This analysis demonstrates that percent inhibition by curcumin is equal for basal and insulin-stimulated glucose transport. This observation suggests that curcumin inhibits glucose transport *per se*, rather than inhibiting the insulin signaling cascade. As before, curcumin had little or no effect on lipolysis, nor on the ability of insulin to inhibit lipolysis (Fig. 2c).

To determine whether preincubation is necessary for the inhibitory effect of curcumin, glucose transport was measured in adipocytes that had been preincubated with various concentrations of curcumin for 1 h, and in cells to which curcumin was added immediately before the assay (Fig. 3). The inhibitory effect of curcumin was similar in the two groups of cells, suggesting that the effect of curcumin is very rapid or immediate.

To investigate further the rate of onset of the inhibitory effect of curcumin on glucose transport, time-course experiments were performed. Uptake of 2-deoxyglucose was determined as a function of time both before and after addition of 100 μM curcumin (Fig. 4). In this experiment, cells were incubated with insulin for 45 min to increase the rate of glucose transport, before addition of