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Glucose as a Lipolytic Agent: Studies on Isolated Rat Adipocytes

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Summary

In order to elucidate the direct effect of glucose on lipolysis in isolated rat adipocytes, cells were incubated in a buffer with different concentrations of this sugar: 2, 8 or 16 mmol/l. The increase in glucose concentration from 2 mmol/l to 8 or 16 mmol/l enhanced basal lipolysis by 30% and 47 %, respectively. Epinephrine-induced lipolysis (1 μmol/l) was also increased by 31 % and 32 %, when glucose concentration was increased from 2 mmol/l to 8 or 16 mmol/l, respectively. The rise in lipolysis caused by glucose was restricted by H-89 (an inhibitor of protein kinase A, 30 μmol/l), but insulin (1 nmol/l) had no inhibitory action. The augmentation of lipolysis by glucose did not require its metabolism (as demonstrated using 2-deoxyglucose) and was due to the action of this sugar on the final steps of the lipolytic cascade, particularly on protein kinase A. However, short-term exposure of adipocytes to higher glucose concentrations did not restrict the inhibitory action of insulin on lipolysis induced by epinephrine.

Key words
Adipocytes • Glucose • Lipolysis

Introduction

The cells of white fat tissue, called adipocytes, play an important role in lipid synthesis, release and storage in the organism. Triglycerides, which are the main form of lipids stored in adipocytes, are synthesized in these cells from glucose or from fat taken up from the plasma. When triglycerides are broken down, glycerol and free fatty acids are released into the plasma. It is evident that processes taking place in adipocytes should be precisely regulated to avoid fat excess or deficit in the whole organism. Hormonal regulation appears to be pivotal for maintaining the balance between lipid synthesis and breakdown in these cells. Under physiological conditions, epinephrine and glucagon augments lipolysis, whereas insulin has antilipolytic action and increases lipogenesis. It has repeatedly been documented that several compounds absorbed from the digestive tract can modify the hormonal response of adipocytes (for review see Kandulska and Szkudelski 1998). Glucose is such a substance but the exact mechanism of its action in these cells is not clear. It is commonly believed that *in vivo* the increase in glycemia causes a rise in blood insulin and this hormone restricts lipolysis. On the other hand, continuous hyperglycemia enhances basal and stimulated lipolysis in isolated adipocytes. Simultaneous deterioration of the ability of insulin to restrict lipolysis was observed (Hager *et al.* 1991). Recent investigations demonstrated that failure of this antilipolytic action of insulin in adipocytes may lead

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to hyperglycemia and to type 2 diabetes mellitus (Rebrin *et al.* 1995). In the light of these new findings, the elucidation of glucose action in adipocytes could certainly contribute to a better understanding the causes of some metabolic disturbances.

The purpose of this work was to elucidate the direct effect of glucose on lipolysis in isolated rat adipocytes. The ability of insulin to inhibit lipolysis in these cells depending on the glucose concentration was also studied.

Material and Methods

Preparation of adipocytes. Male Wistar rats weighing 160±5 g, were fed a complete laboratory diet *ad libitum* and kept under standard conditions. Rats were killed by decapitation, their epididymal fat tissue was pooled and adipocytes were isolated according to the method described by Rodbell (1964). The tissue was rinsed with 0.85 % NaCl, cut into pieces and transferred into a plastic flask with Krebs-Ringer buffer, pH 7.4, containing 2 mmol/l glucose, 3 % bovine serum albumin (fraction V), 10 mmol/l HEPES and 2 mg/ml collagenase (from *Clostridium histolyticum*, type II). Incubation was performed for 90 min by shaking at 37 °C. After the incubation, cells were rinsed four times with a collagenase-free Krebs-Ringer buffer and then filtered through a nylon mesh. Adipocyte counts were performed using a microscope in a Bürker-Türk counting chamber.

Lipolysis in adipocytes. Adipocytes (about 10^6 cells/ml) were incubated for 90 min by shaking in plastic tubes at 37 °C with Krebs-Ringer buffer, pH 7.4, containing 3 % BSA, 10 mmol/l HEPES, and three different glucose concentrations: 2, 8 or 16 mmol/l. Furthermore, fat cell suspensions with different glucose concentrations (2, 8 or 16 mmol/l) were incubated with insulin (1 nmol/l), epinephrine (1 µmol/l], insulin and epinephrine together or with H-89 (N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesul-fonamide, 30 µmol/l). Moreover, adipocytes were incubated in the buffer containing 2 mmol/l glucose and 2-deoxyglucose added to a total sugar concentration (glucose + 2-deoxyglucose) of 8 or 16 mmol/l.

Each treatment was performed in six replications, the final volume of every incubated tube being 1 ml. The glycerol released from adipocytes, reflecting the intensity of lipolysis, was measured according to Foster and Dunn (1973). Insulin (porcine, monocomponent) was from NOVO, Nordisk, Denmark and H-89 from ICN Pharmaceuticals, Inc, USA. All other reagents were purchased from Sigma USA. The results were statistically evaluated using one-way analysis of variance and the multiple range test.

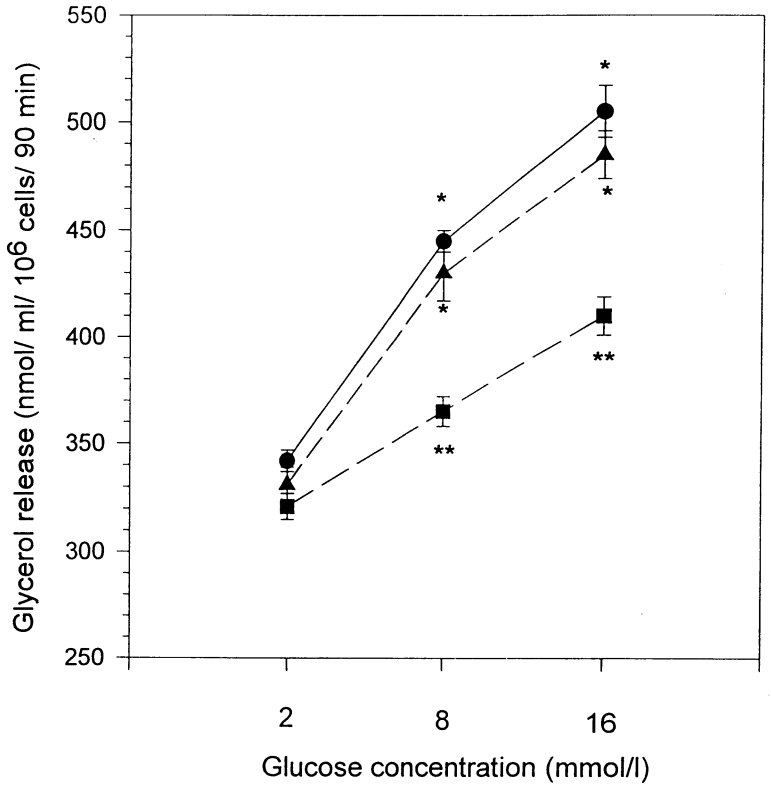

Fig. 1. The effect of glucose on basal lipolysis in isolated rat adipocytes (circles) and the inhibitory action of insulin (triangles, 1 nmol/l) or H-89 (squares, 30 µmol/l). Values are means ± S.E.M, n=6. The significant differences ($p<0.01$) between incubations with 2 mmol/l glucose and its higher concentrations (*) or between glucose alone and glucose with H-89 (**).

Results

The incubation of adipocytes in the medium with glucose at the concentration of 8 and 16 mmol/l increased basal lipolysis by 30 % and 47 %, respectively compared to lipolysis in the medium containing 2 mmol/l glucose (Fig. 1). The increase of lipolysis was not restricted by insulin, but H-89 had a significant inhibitory action (Fig. 1).

2-deoxyglucose strongly enhanced basal lipolysis by 81 % and 175 % when added to the incubation medium at the concentration of 6 mmol/l and 14 mmol/l, respectively (Fig. 2).

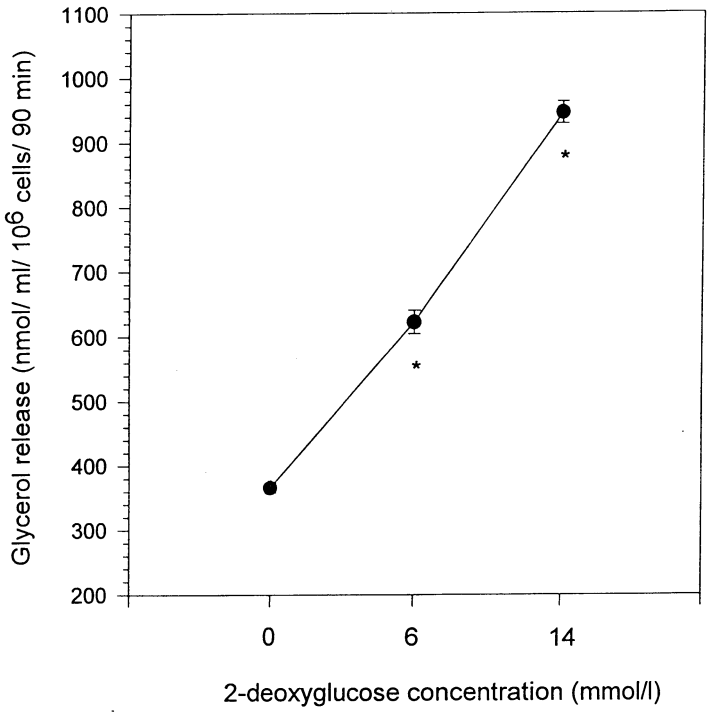

Fig. 2. *The effect of 2-deoxyglucose on basal lipolysis in isolated rat adipocytes. Values are means ± S.E.M, n=6. The significant differences (p<0.01) between incubations with 2 mmol/l glucose and 2-deoxyglucose (*).*

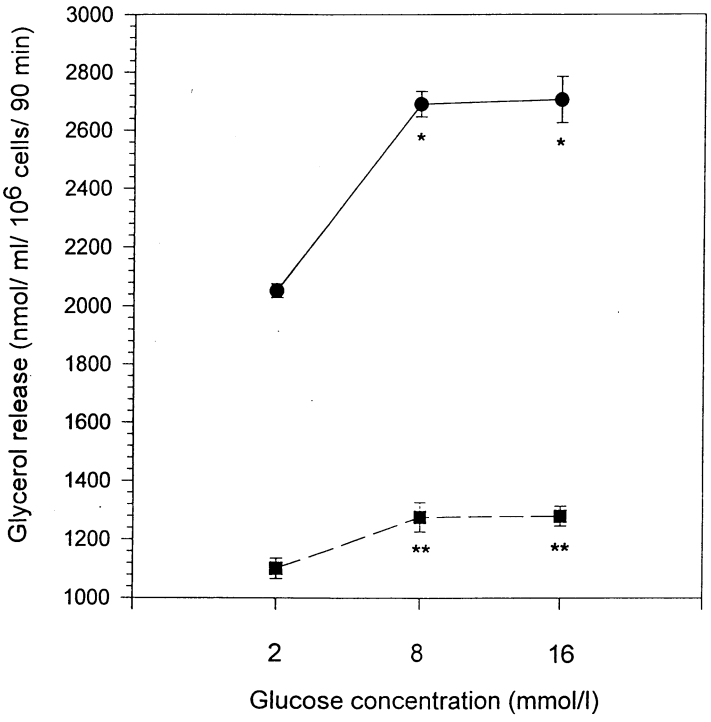

Fig. 3. *The effect of glucose on epinephrine-induced (1 μmol/l) lipolysis in isolated rat adipocytes (circles) and the inhibitory action of insulin (squares, 1 nmol/l). Values are means ± S.E.M, n=6. The significant differences (p<0.01) between incubations with 2 mmol/l glucose and its higher concentrations (*) or between epinephrine and epinephrine with insulin (**).*

The intensity of epinephrine-induced lipolysis was also elevated, when the glucose concentration was raised from 2 mmol/l to 8 mmol/l (lipolysis rose by 31 %) or to 16 mmol/l (increase by 32 %, Fig. 3). The restriction of epinephrine-induced lipolysis by insulin was not affected in the presence of higher glucose concentrations (Fig. 3).

Discussion

It was demonstrated in our experiments that basal and epinephrine-stimulated lipolysis in isolated rat adipocytes was augmented as a result of elevated glucose concentrations in the incubation medium (Figs 1 and 3). Similar results were observed previously by Naito and Okada (1975), but these authors did not suggest any explanation of this phenomenon. Recent experiments performed by Raclot *et al.* (1998) demonstrated that glucose modulated hormone-sensitive lipase (HSL) gene expression in isolated adipocytes. The incubation of cells in the medium without glucose decreased HSL mRNA with a simultaneous drop in the total activity of this enzyme. The addition of glucose had an opposite effect. These effects were, however, observed after 24-32 h of adipocytes incubation in the buffer with different glucose concentrations. In our experiment, glucose already enhanced lipolysis after 90 min. During this period its action *via* changes in gene expression may be ruled out and another mechanism of short-time duration must apparently be involved in this action. To elucidate this mechanism, we tested whether the basal lipolysis augmented by glucose was inhibited by insulin. This hormone was added at the concentration commonly used in this type of experiments (Kandulska *et al.* 1999) which inhibits the lipolysis stimulated by several physiological and pharmacological factors. Incubation of adipocytes with insulin was, however, without any inhibitory effect on the glucose-induced rise in basal lipolysis (Fig. 1). In adipocytes insulin induces phosphorylation of cAMP phospho-diesterase and activates this enzyme causing a reduction of the cAMP content (Degerman *et al.* 1990, Eriksson *et al.* 1995). This effect allows insulin to restrain the lipolysis stimulated by several agents increasing the amount of cAMP in the cells. Insulin is, however, unable to inhibit basal lipolysis (Morimoto *et al.* 1998). Thus, the lack of insulin effects on lipolysis augmented by glucose suggested that this sugar acts on the final steps of the lipolytic cascade, i.e. on protein kinase A or hormone-sensitive lipase. This assumption was additionally supported by the finding that the increase of lipolysis in the presence of higher glucose concentrations was significantly abolished by H-89, a potent and selective inhibitor of protein kinase A (Fig. 1). The inhibitory action of H-89 demonstrated that the main target for glucose is protein kinase A. Thus the activation of this enzyme plays a pivotal role in the lipolysis evoked by this sugar.

Fruhbeck *et al.* (1998) demonstrated that adipocyte-derived hormone – leptin induces lipolysis. Leptin secretion is potentiated by glucose transport and metabolism in adipocytes (Mueller *et al.* 1998). We supposed that this hormone could be involved in the increased lipolysis evoked by higher glucose concentrations. This hypothesis was verified by incubation of adipocytes with 2-deoxyglucose, a non-metabolisable glucose analogue, which was found to inhibit leptin secretion (Mueller *et al.* 1998). It was observed that 2-deoxyglucose also stimulated lipolysis (Fig. 2). The effect of this sugar was even greater than that of glucose, probably due to accumulation of 2-deoxyglucose in adipocytes (Foley and Gliemann 1981). These results suggest that leptin is not involved in glucose-induced lipolysis in adipocytes. Moreover, they demonstrate that glucose augments lipolysis *per se* and that its metabolism is not required to reveal this activity.

In the experiments performed by Hager *et al.* (1991), 72-hour glucose infusion to normal rats caused a significant rise in basal and isoproterenol-stimulated lipolysis in isolated fat cells. Inhibition of this process by insulin was blunted. A continuous glucose load was, however, accompanied by hyperinsulinemia and impairment of insulin action (Hager *et al.* 1991). In our experiment, the suppression of epinephrine-induced lipolysis by insulin was not abolished as a result of elevated glucose concentration in the incubation medium (Fig. 3). Thus, short-time exposure of adipocytes to higher glucose concentrations (8 and 16 mmol/l vs. 2 mmol/l) without simultaneous treatment with high insulin concentrations, does not restrict the antilipolytic activity of insulin in these cells. Using isolated human adipocytes, Arner *et al.* (1983) even observed an increment in the maximum effect of this hormone as a result of increased glucose concentration in the incubation medium. However, when adipocytes were incubated with 2, 8 or 16 mmol/l glucose, insulin did not restrict epinephrine-induced lipolysis exactly to the same level; the intensity of this process was still enhanced by glucose in higher concentrations (Fig. 3).

The results presented in this report clearly indicate that glucose directly stimulates lipolysis in

isolated rat adipocytes. It was also demonstrated that this stimulation does not require glucose metabolism. It is important that its lipolytic activity is not abolished by insulin, which is the main antilipolytic factor *in vivo*.

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