Streptozotocin Induces Lipolysis in Rat Adipocytes *in Vitro*

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Summary

Streptozotocin (STZ) is used to induce experimental diabetes in animals and is also applied for the treatment of patients with insulinoma. The aim of the present work was to investigate the direct effect of STZ on lipolysis in isolated rat adipocytes. After the isolation, the cells were incubated in a Krebs-Ringer buffer of pH 7.4, at the temperature 37 °C for 90 min with different concentrations of STZ: 0.5, 1 or 2 mmol/l. STZ caused a significant rise in basal values (99 %, 199 %, and 377 %, respectively) and epinephrine-stimulated (1 µmol/l) lipolysis (15 %, 24 % and 46 %, respectively). Augmentation of basal lipolysis by STZ was neither restricted by insulin (1 nmol/l) nor by H-89 (an inhibitor of protein kinase A, 50 µmol/l). These results indicate the stimulatory influence of STZ on the action of hormone-sensitive lipase in isolated cells of white adipose tissue. The obtained outcomes suggest that in studies employing STZ, it is necessary to consider its direct effect upon lipolysis in adipocytes.

Introduction

Streptozotocin (STZ) is widely used for inducing experimental diabetes in animals. This compound is also applied as an anti-insulinoma therapeutic agent in humans (Shultz *et al.* 1990, Nesovic *et al.* 1992). When administered in diabetogenic doses, STZ exerts a cytotoxic effect on pancreatic B cells. The mechanism of its action in these cells has been intensively investigated (reviewed by Szkudelski 2001). STZ is taken up by B cells *via* the glucose transporter GLUT2 and causes alkylation of DNA (Delaney *et al.* 1995) and reduction of ATP and NAD^+ content (Nukatsuka *et al.* 1990, Heller *et al.* 1994).

One of the intensively examined aspects of diabetes concerns lipid metabolism. In these investigations, the STZ-induced animal model of diabetes is commonly used. It was previously observed that STZ-induced diabetic rats develop significant disturbances in metabolism of cells of white adipose tissue. These changes are mainly due to the insulin deficiency and include a rise in the cAMP content (Chiappe de Cingolani 1986) and an increase in the activity of hormone-sensitive lipase (HSL) in adipocytes (Sztalryd *et al.* 1995). As a consequence, rats manifest augmented lipolysis leading to diabetic ketoacidosis. However, there are no literature data demonstrating whether STZ itself has any influence on metabolism of white adipose tissue cells. The elucidation of this problem seems to be important since some investigations are performed shortly after STZ treatment (Woods *et al.* 1981).

The aim of this experiment was to elucidate the direct effect of STZ upon lipolysis in isolated rat

adipocytes i.e. that not caused by hormonal and metabolic changes.

Methods

Preparation of adipocytes

Adipocytes were obtained from male Wistar rats of 160 ± 5 g body weight and kept in standard conditions. Cells isolation was performed according to the Rodbell method (1964) with minor modifications (Szkudelska *et al.* 2000). Experiments were carried out in accordance with the ethical rules approved by the University of Agriculture in Poznan. Rats were slaughtered by decapitation and samples of epididymal adipose tissue were pooled. Isolation was carried out for 90 min in a Krebs-Ringer buffer at pH 7.4 and temperature 37 °C. The buffer contained 3 mmol/l glucose, 3 % bovine serum albumin (fraction V), 10 mmol/l HEPES and 2 mg/ml collagenase (from Clostridium histolyticum, type II). After isolation, the cells were washed several times with the same buffer but without collagenase and filtered through nylon mesh. Adipocyte counts were performed using a microscope with a Bürker-Türk counting chamber.

Lipolysis in adipocytes

Adipocytes (10^6 cells/ml) were incubated at 37 °C for 90 min in the Krebs-Ringer buffer. Cells were incubated in the buffer alone, with STZ (0.5, 1 or 2 mmol/l), STZ (0.5, 1 or 2 mmol/l) and epinephrine (1 μmol/l), STZ (1 mmol/l) and insulin (1 nmol/l) or

insulin and epinephrine. Additionally, treatments in which adipocytes were incubated with STZ (1 mmol/l) and H-89 (N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesul-fonamide, 50 μmol/l), as well as epinephrine and H-89 were performed. Each treatment was performed six times. After the incubation, adipocytes were aspirated and the intensity of lipolysis was ascertained as the amount of glycerol released from cells into the incubation medium. The concentration of glycerol was determined using Boehringer Mannheim test. Insulin (Actrapid, porcine monocomponent) was from Novo, Nordisk, H-89 was from ICN Pharmaceuticals and all other reagents were purchased from Sigma. The obtained results were statistically verified employing one-way analysis of variance and multiple range test.

Results

STZ added to the incubation medium in the concentration of 0.5, 1 or 2 mmol/l significantly augmented basal lipolysis in isolated rat adipocytes. The glycerol concentration was 474, 722 and 1149 nmol/ml/10^6 cells/90 min, respectively vs. 241 nmol/ml when adipocytes were incubated without STZ (Fig. 1). Epinephrine-induced lipolysis was also augmented in the presence of STZ. The mean concentration of glycerol in the incubation medium was 1670 nmol/ml/10^6 cells/90
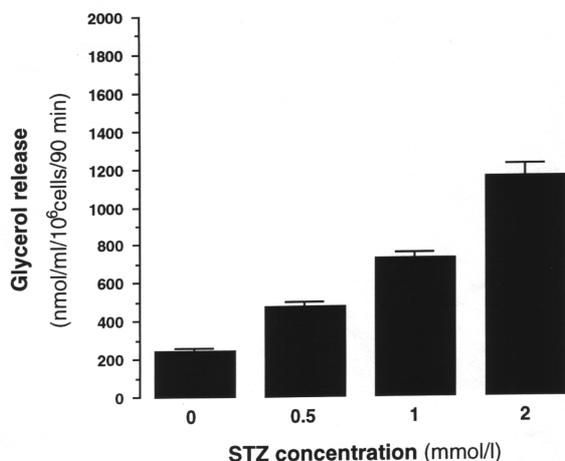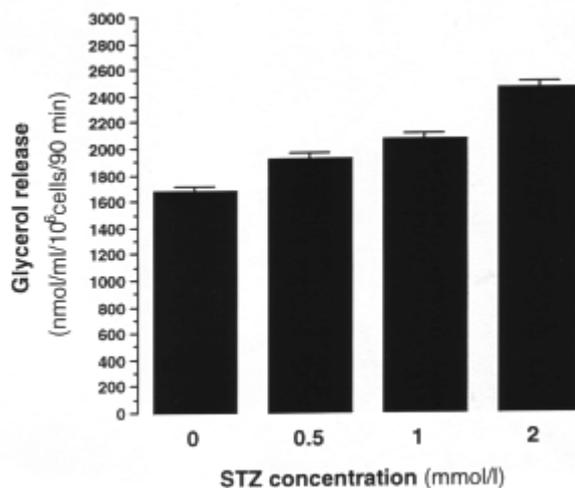
Fig. 1. *Streptozotocin-induced lipolysis in isolated rat adipocytes. Values are means ± S.E.M of six repetitions. The differences are statistically significant between all groups (p≤0.01); STZ – streptozotocin*

Fig. 2. *Streptozotocin-induced lipolysis in isolated rat adipocytes in the presence of epinephrine (1 μmol/l). Values are means ± S.E.M of six repetitions. The differences are statistically significant between all groups (p≤0.01); STZ – streptozotocin*

min when adipocytes were treated only with epinephrine and rose to 1920, 2060 and 2441 nmol/ml after treatment with epinephrine and 0.5, 1 or 2 mmol/l of STZ (Fig. 2). Insulin considerably reduced triglycerides breakdown stimulated by epinephrine. The concentration of glycerol in the incubation medium was 1670 nmol/ml/10^6 cells/90 min when cells were incubated with epinephrine alone and dropped to 1255 nmol/ml as a result of insulin addition. However, insulin was not found to have any inhibitory effect on STZ-induced glycerol release (Fig. 3). Lipolysis stimulated by epinephrine was almost completely abolished in the presence of H-89. The glycerol content was 1670 nmol/ml/10^6 cells/90 min when cells were incubated with epinephrine alone vs. 387 nmol/ml after H-89 treatment. H-89 exerted negligible effect on STZ-induced lipolysis (722 nmol/ml of glycerol/10^6 cells/90 min vs. 658 nmol/ml after incubation with H-89; Fig. 3).
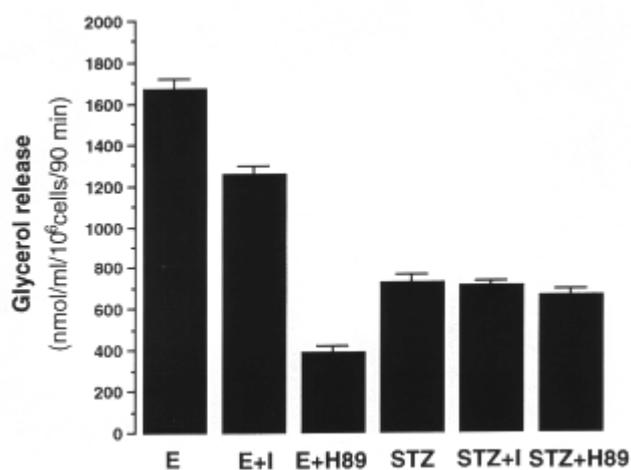

Fig. 3. *Effect of insulin and H-89 on epinephrine- and streptozotocin-induced lipolysis in isolated rat adipocytes. Values are means ± S.E.M of six repetitions. The differences are statistically significant between all groups (p≤0.01), except between STZ and STZ+I and STZ+H89; E - epinephrine (1 μmol/l), I - insulin (1 nmol/l), STZ - streptozotocin (1 mmol/l), H-89 (50 μmol/l)*

Discussion

It was demonstrated in the present report, that STZ significantly induced basal (Fig. 1) as well as epinephrine-stimulated (Fig. 2) lipolysis in isolated rat adipocytes. This effect appeared even at the concentration of 0.5 mmol/l and, at the higher concentration of STZ in

the incubation medium, further augmentation of glycerol release was noted. It is noteworthy that the stimulatory action of STZ on lipolysis was similar in the absolute values in the absence and presence of epinephrine. This observation may suggest that the STZ has an additive effect on lipolysis. Similar lipolytic properties were previously reported for another diabetogenic agent, alloxan (Kandulska *et al.* 1999).

The ability of STZ to stimulate lipolysis in the absence of epinephrine indicates that the action of this compound does not involve changes in the adrenergic receptors but suggests the STZ influence on further steps of the lipolytic cascade, i.e. on adenylate cyclase, cAMP content, protein kinase A (PKA) or HSL. This assumption is additionally supported by lack of the inhibitory effect of insulin on lipolysis caused by STZ, whereas this hormone at the same concentration clearly restricted glycerol release from cells incubated with epinephrine (Fig. 3, Szkudelski and Szkudelska 2000). It is known that insulin is a strong anti-lipolytic factor exerting its effect *via* phosphorylation of cGMP-inhibited cAMP phosphodiesterase. Activation of this enzyme reduces cAMP in the cell (Degerman *et al.* 1990, Eriksson *et al.* 1995). In this way, insulin inhibits lipolysis stimulated by various compounds acting through an increase of the cAMP content. The lack of the inhibitory effect of insulin on STZ-induced lipolysis suggests that STZ increases this process acting downstream from the adenylate cyclase in the lipolytic cascade, i.e. on PKA or HSL.

To verify this hypothesis, H-89, a specific and potent inhibitor of PKA, was used. As expected, H-89 strongly restricted the epinephrine-induced lipid mobilization from isolated fat cells. The inhibition of PKA by this compound was, however, almost completely ineffective in abolishing the stimulatory influence of STZ on triglycerides breakdown (Fig. 3). This finding indicates an effect of STZ on HSL action.

STZ-augmented lipolysis may by caused either by its direct effect on HSL or is the result of STZ-induced changes under some circumstances in the cells influencing the action of this enzyme. Okuda *et al.* (1994) and Tsujita *et al.* (1995) demonstrated that non-treated adipocytes possess lipolytic activity. This activity is, however, inhibited in intact cells and appears either after stimulation by lipolytic agents or as a result of destruction of some cellular structures. Recent experiments also demonstrated that the phosphoprotein perilipin is located at the lipid droplet surface and under basal conditions, when it is not phosphorylated by PKA,

provides a barrier against HSL action. Stimulation of lipolysis is associated with changes in localization of both perilipin and HSL (Clifford *et al.* 2000). The exact mechanism whereby STZ augments lipolysis is difficult to ascertain. Since STZ is unstable, it can be expected that STZ-induced lipolysis results from changes in adipocytes initiated within a few minutes after its addition to the incubation medium. It is known that this diabetogenic agent liberates nitric oxide (NO) and increases the activity of guanylyl cyclase and formation of cGMP (Turk *et al.* 1993). cGMP can inhibit cAMP phosphodiesterase and augment lipolysis. However, the failure of the inhibitory effect of H-89 on STZ-induced basal lipolysis demonstrated in our experiment indicates that this is not the case and that the lipolytic action of STZ is cGMP-independent. Since STZ possesses alkylating properties and is able to generate reactive

oxygen species and NO, alkylation of some cellular structures or their impairment by free radicals or by NO may be responsible for STZ-induced lipolysis.

In conclusion, we can corroborate that adipocytes are susceptible to the direct action of STZ. This susceptibility is exhibited by intensification of lipolysis *via* STZ action at the level of HSL. This action is not accompanied by permanent impairment of the cells since STZ-treated adipocytes are still able to respond to epinephrine. It was also found that insulin fails to restrict lipolysis after STZ treatment. The obtained results suggest that using STZ, the direct, early effect of this compound upon lipolysis in adipocytes should be taken into account. This effect can be expected in animals examined within a short time after STZ treatment and when STZ in used as an anti-insulinoma therapeutic agent.

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