

Lipolysis Induced by Alloxan in Rat Adipocytes is Not Inhibited by Insulin

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Summary

Isolated rat adipocytes were incubated with adrenaline, adrenaline plus insulin, alloxan or alloxan plus insulin. Glycerol release was taken as a measure of lipolysis. It was observed that alloxan in the concentration of 3, 10 and 20 mmol/l intensifies lipolysis in adipocytes in the absence of adrenaline. Insulin (10^{-6} mol/l) treatment of cells did not inhibit lipolysis caused by this compound, but significantly restricted lipolysis induced by adrenaline (10^{-6} mol/l). It was also shown that alloxan in the concentration of 3 and 10 mmol/l intensified lipolysis stimulated by adrenaline (10^{-6} mol/l). Addition of 20 mmol/l of alloxan strongly inhibited glycerol release in the presence of adrenaline. The results presented here clearly indicate that the action of alloxan concerns cells of the white adipose tissue.

Key words

Adipocytes • Alloxan • Lipolysis • Adrenaline • Insulin • Rat

Introduction

One of the methods of inducing experimental diabetes is the administration of alloxan to animals (Dunn *et al.* 1943). This substance is also widely used in studies on the physiology of pancreatic B cells and its mode of action in these cells is quite well known. It is commonly accepted that it is a complex process involving free radicals which cause cell damage (Zhang *et al.* 1992). Alloxan exhibits a relatively high selectivity in relation to insulin secreting cells, although its cytotoxic activity may affect many other types of cells leading to damage of some internal organs (Boquist *et al.* 1983, Chen *et al.* 1994, Szkudelski *et al.* 1998). Therefore, changes observed in animals suffering from

alloxan diabetes may be caused not only by impairment of pancreatic B cells, but may also result from a direct action of alloxan on cells of other tissues. No information is available concerning the influence of this compound on the cell metabolism of white adipose tissue. This problem requires elucidation because alloxan injected to rats causes, after a very short time, a rapid loss of this tissue (non-published observations). These changes may occur despite simultaneous elevation of insulin concentrations in the blood which under normal conditions exerts an inhibitory influence on lipolysis. Therefore, it seems important to determine if alloxan influences the cell metabolism of white adipose tissue.

The aim of the present study was to determine the direct effect of alloxan on non-stimulated and

adrenaline-stimulated lipolysis in isolated adipocytes of rats. Furthermore, tests were carried out to check if insulin has any influence on the action of alloxan in these cells.

Methods

Adipocytes were obtained from 15 male Wistar rats of 150 ± 5 g body weight. Before the experiment, the animals were kept under standard conditions with free access to water and were fed a complete diet *ad libitum*. The rats were decapitated, samples of epididymal adipose tissue were pooled and the cells were isolated according to Rodbell (1963) with some modifications. Adipocytes isolation was carried out for 90 min at 37°C , in a Krebs-Ringer buffer of 7.4 pH, which 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 rinsed several times with the same buffer but without collagenase and filtered through a nylon mesh. The obtained adipocytes were diluted with the Krebs-Ringer buffer so that the number of cells in the

incubation mixture was always $10^6/\text{ml}$. Adipocytes were either incubated in the buffer alone, in the buffer with alloxan, with alloxan plus adrenaline or alloxan plus insulin. Furthermore, cells were also incubated with adrenaline, with insulin or with both these hormones simultaneously. Each treatment was repeated six times. Alloxan was added in concentrations of 3, 10 or 20 mmol/l, while insulin and adrenaline were present in the concentration of 10^{-6} mol/l. Insulin and alloxan were added to the medium 10 min before adrenaline administration, whereas alloxan was added 10 min after insulin administration. After 90 min of incubation, cells were aspirated and the intensity of lipolysis was inferred from the quantity of glycerol released from cells to the incubation medium. The concentration of glycerol was determined by means of the Boehringer Mannheim UV-test. Insulin (Actrapid, porcine monocomponent) was from Novo Nordisk. Adrenaline and all other reagents were obtained from Sigma.

The obtained results were statistically verified by means of one-way analysis of variance.

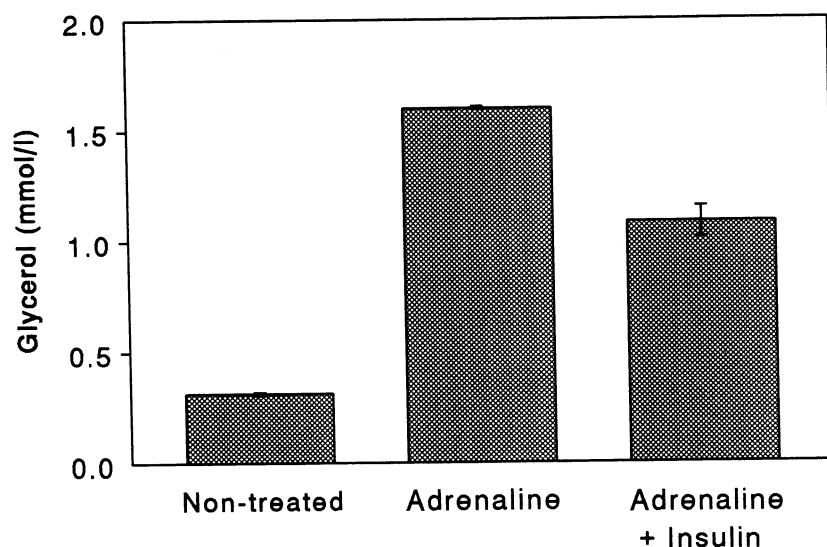


Fig. 1. Inhibitory effect of insulin (10^{-6} mol/l) on adrenaline (10^{-6} mol/l)-stimulated lipolysis (expressed as glycerol concentration in the incubation medium) in isolated rat adipocytes. Values are means \pm S.E.M., $n=6$. The differences were statistically significant between all groups ($p < 0.01$).

Results

The treatment of adipocytes with adrenaline at the concentration of 10^{-6} mol/l increased the glycerol concentration in the incubation medium by 409 % as compared to the cells which had not been treated by this hormone (Fig. 1).

On the other hand, 10^{-6} mol/l concentration of insulin had an inhibitory effect on adrenaline-stimulated

lipolysis; a 32 % decrease of glycerol concentrations in the incubation medium was observed in comparison with its amount in the medium containing cells treated with adrenaline only (Fig. 1).

In comparison with the controls, 3, 10 and 20 mmol/l concentrations of alloxan increased the glycerol content by 27 %, 51 % and 85 %, respectively (Fig. 2). The addition of insulin 10 min before treatment of the cells with alloxan did not cause significant changes in the glycerol content (Fig. 3).

The concentration of glycerol in the incubation medium containing cells treated with 3 and 10 mmol/l of alloxan and then subjected to the influence of adrenaline increased by 19 % and 21 %, respectively, in comparison with the glycerol content in the medium in which adipocytes had been exposed to adrenaline alone (see

Figs 1 and 4). When alloxan was added in a concentration of 20 mmol/l, the glycerol concentration was lowered by 47 % in comparison to the treatment in which cells had been treated with adrenaline only (Fig. 4).

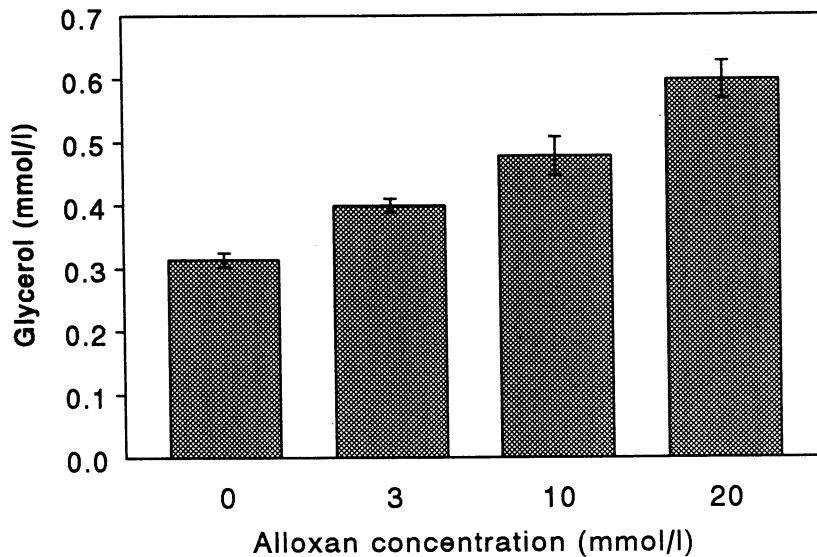
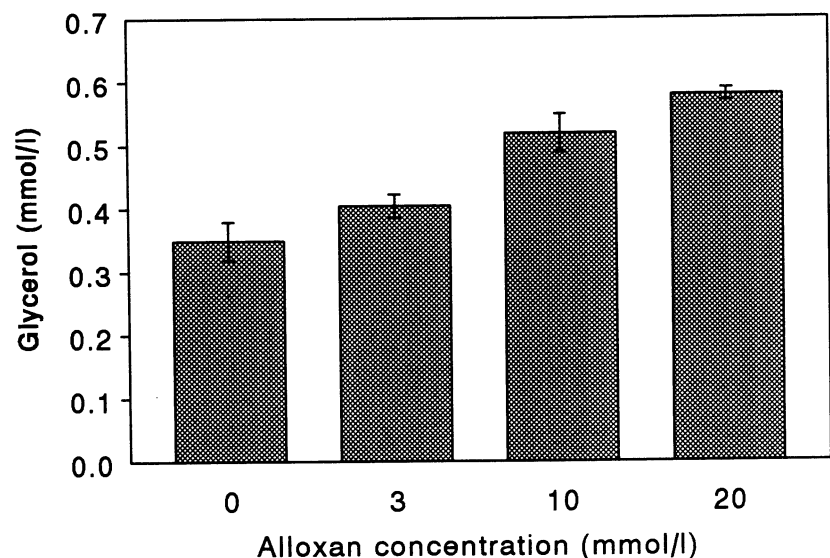


Fig. 2. Alloxan-induced lipolysis (expressed as glycerol concentration in the incubation medium) in isolated rat adipocytes. Values are means \pm S.E.M., $n=6$. The differences were statistically significant between all groups ($p<0.01$).

Fig. 3. Effect of insulin (10^{-6} mol/l) on alloxan-induced lipolysis (expressed as glycerol concentration in the incubation medium) in isolated rat adipocytes. Values are means \pm S.E.M., $n=6$. The differences were statistically significant between all groups ($p<0.01$).



Discussion

It is quite clear from these experiments that alloxan present in the incubation medium in the

concentrations of 3, 10 and 20 mmol/l led to significant lipolysis in adipocytes. The intensity of this process depended on the alloxan concentration (Fig. 2).

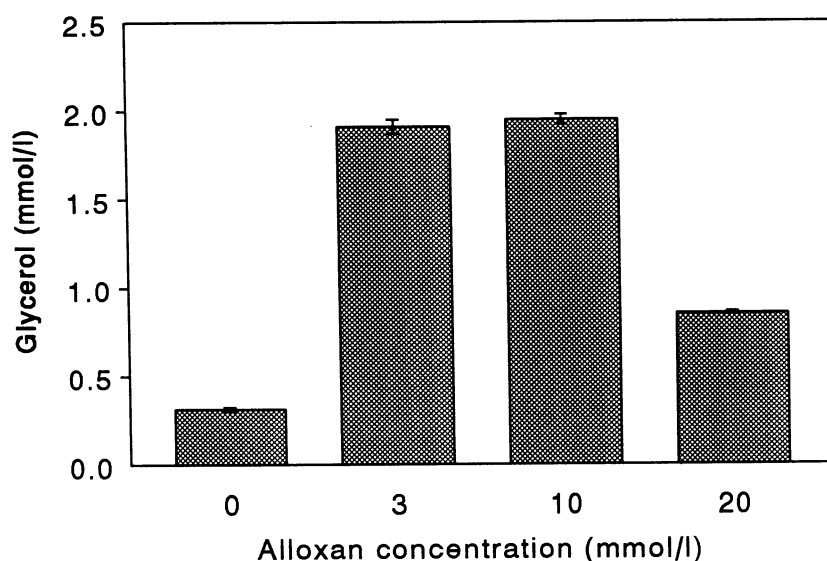


Fig. 4. Effect of alloxan on lipolysis (expressed as glycerol concentration in the incubation medium) in isolated rat adipocytes in the presence of adrenaline (10^{-6} mol/l). Values are means \pm S.E.M., $n=6$. The differences were statistically significant between all groups ($p<0.01$), except the comparison between concentrations of alloxan 3 and 10 mmol/l.

During perfusion of the pancreas with a solution containing alloxan in a concentration of 4.1 mmol/l, it was observed a rapid activation of B cells resulted in an initially high release of insulin into the medium which then led to an inability of these cells to respond even to high glucose concentrations. This lack of a response was presumably an effect of permanent impairment of B cells by alloxan (Kliber *et al.* 1996). However, the enhanced lipolysis induced by alloxan cannot be attributed exclusively to a permanent damage of adipocytes. It was observed that cells treated with this substance in concentrations of 3 and 10 mmol/l were still able to respond to adrenaline; further intensification of lipolysis was recorded under the influence of this hormone. It was not until cells were treated with 20 mmol/l of alloxan that adrenaline was found to have no lipolytic activity (Fig. 4). The inhibition of adrenaline-induced lipolysis only at very high concentrations of alloxan indicates that the resistance of adipocytes to the cytotoxic action of this substance is relatively high. Furthermore, it is obvious that changes caused by alloxan in the cells of white adipose tissue are the result of the action of this substance within a few minutes from its addition to the incubation mixture because alloxan decomposes rapidly under these conditions (Lenzen and Munday 1991).

It is quite possible that the alloxan-dependent enhancement of lipolysis may, to a certain degree, be attributed to its capability to form free radicals. Tsujita *et al.* (1995) showed that active hormone-sensitive lipase is present in adipocytes even in the absence of adrenaline

and there is some system for inhibiting the lipolytic activity of this enzyme. Thus, not only the addition of adrenaline but also the inactivation of this inhibitory system results in lipolysis (Tsujita *et al.* 1995). It is possible that lipolysis caused by alloxan may still persist even after decomposition of this drug and may occur as a result of inactivation of this inhibitory system.

In our experiments, insulin was not found to inhibit alloxan-stimulated lipolysis in adipocytes (Fig. 3), although this hormone clearly restricted decomposition of triglycerides stimulated by adrenaline under the same conditions. This indicates that the mechanism of the lipolytic action may be different for adrenaline and alloxan. It is well known that insulin is a strong anti-lipolytic factor which inhibits lipid mobilization by stimulating phosphorylation of cAMP phosphodiesterase. Insulin activates this enzyme and reduces the cAMP content in the cell (Degerman *et al.* 1990, Eriksson *et al.* 1995). In this way, insulin inhibits lipolysis stimulated by various substances increasing the content of cAMP. On the other hand, it was observed in other experiments that the anti-lipolytic activity of insulin is neutralized completely by inhibiting one of the isoforms of cAMP phosphodiesterase, i.e. cGMP-inhibited phosphodiesterase (Wesslan *et al.* 1993). It seems very probable that alloxan caused such changes in cells which prevented insulin to activate cAMP phosphodiesterase. Boylan *et al.* (1992) observed increased activity of tyrosine phosphatases in the liver of rats treated with alloxan. These enzymes catalyze

dephosphorylation of the insulin receptor and the increase of their activity may lead to insulin resistance (Boylan *et al.* 1992). Tyrosine phosphatases were also found in adipocytes (Kablouï *et al.* 1995). It is highly probable that alloxan increased the activity of these enzymes and this, in turn, led to the absence of the inhibitory effect of insulin on lipolysis caused by this compound.

The results obtained in this study clearly indicate that the action of alloxan also concerns cells of the white adipose tissue. This action enhances lipolysis

and restricts the effects of insulin. On the basis of these results, it appears that the increased discharge of insulin caused by alloxan administration in *in vivo* conditions need not necessarily constitute a factor inhibiting lipolysis in adipocytes. This could partially explain the rapid loss of fat tissue in rats observed after the treatment with alloxan. It can thus be concluded that the studies concerning lipid metabolism in alloxan diabetes should be interpreted with great caution, because alloxan itself can directly influence adipose tissue metabolism.

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Reprint requests

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