Streptozotocin diabetes results in increased responsiveness of adipocyte lipolysis to glucagon

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Adipocytes from streptozotocin-diabetic rats are ~50-times more sensitive to the lipolytic action of glucagon. This change is only perceived in the presence of a small quantity of adenosine deaminase which itself has little effect on basal lipolysis. Insulin treatment restores glucagon sensitivity to normal.

Adenosine deaminase *Adipocyte* *Diabetes* *Glucagon* *Lipolysis* *Streptozotocin*

1. INTRODUCTION

Adipocytes from 24 h-fasted rats are more sensitive (by a factor of ~10) to the lipolytic action of glucagon compared with cells from fed animals [1]. Adenylate cyclase in adipocyte plasma membranes is $\geq$100-times more sensitive to stimulation by glucagon after fasting [2]. It is not yet established whether these changes are due to altered glucagon–receptor interaction or result from changes in coupling between adenylate cyclase and the adipocyte glucagon receptor.

In streptozotocin-diabetes, both adipocyte lipolysis and adenylate cyclase in fat-cell ghosts show a 3-fold increase in sensitivity to adrenaline [3]. The sensitivity of adenylate cyclase to corticotropin was increased 10-fold in adipocyte ghosts from streptozotocin-diabetic rats [3]. Streptozotocin- or alloxan-induced diabetes in rats [4,5] and insulin-dependent diabetes in humans [6–8] is generally associated with hyperglucagonaemia. By analogy with the 'down regulation' of insulin receptors frequently observed with elevated insulin levels [4,9,10], one might expect tissue responsiveness to glucagon to be diminished in diabetes. However, in two human conditions exhibiting hyperglucagonaemia (untreated insulin-dependent diabetes and in the case of uremic patients) the subjects are more sensitive to the hyperglycaemic action of infused glucagon [11,12]. Although in the diabetic patients this could simply reflect the loss of the restraining action of insulin, this cannot be so in the uremic patients since plasma insulin was normal, or even elevated [12]. In the streptozotocin-diabetic rat or the partially nephrectomized rat (a uremic animal model) the resultant hyperglucagonaemia is accompanied by increased rather than decreased binding of ^{125}I-labelled glucagon to liver plasma membranes and by an increased stimulation of hepatic adenylate cyclase by glucagon [4,13].

Here, we have investigated the effect of streptozotocin diabetes upon the lipolytic response of rat adipocytes to glucagon and find it to be substantially increased. Insulin treatment of diabetic animals reversed this change.

2. MATERIALS AND METHODS

Male Sprague–Dawley rats bred in the animal colony at University College London were used throughout. Starting body weights are shown in table 1. Rats were made diabetic by subcutaneous injection on day 1 of streptozotocin (100 mg/kg) dissolved in 0.2 ml 50 mM citrate buffer (pH 4.0) containing 0.15 M NaCl. Control animals were similarly injected with saline–citrate alone. Control animals were adjudged as being suitable for

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Table 1

Body weights and plasma glucose concentrations of experimental animals

Treatment of animals		Day 1	Day 3	Day 5
Control (30)	Body wt (g)	159 ± 1	170 ± 1	–
	Plasma glucose (mM)	–	11.8 ± 1.2	–
Diabetic (31)	Body wt (g)	160 ± 1	151 ± 2	–
	Plasma glucose (mM)	–	33.0 ± 1.4	–
Diabetic, insulin-treated (on day 3) (25)	Body wt (g)	164 ± 1	151 ± 2	177 ± 1
	Plasma glucose (mM)	–	–	8.0 ± 0.9

Animals were treated as described in the text. The values are means ± SEM. The number of animals in each group is shown in parentheses

the experiment if their growth over the following two days was normal (~5 g/day). On day 3, animals were selected as diabetic if showing a strongly positive 'Clinistix' test for urinary glucose (>0.5% glucose, w/v). These animals lost weight and showed marked hyperglycaemia (table 1). Diabetic rats received bovine insulin subcutaneously on days 3 and 4 at ~10:00 h (20 units/kg of protamine zinc beef insulin from Weddel Pharmaceuticals, London). Insulin-treated diabetic animals regained weight and showed normal glycaemia (table 1). Blood was collected into heparinized vessels immediately after decapitation and centrifuged to obtain plasma for enzymatic measurement of glucose [14].

Chemicals were obtained as in [1]. Adenosine deaminase was dialysed to remove ammonium sulphate and standardized spectrophotometrically on the morning of the experiment, as in [1]. One milliunit of adenosine deaminase deaminates 1 nmol adenosine/min at 25°C.

Adipocytes were isolated after disaggregation of epididymal adipose tissues with collagenase [15] and were then incubated in 4 ml lots of Krebs-Henseleit saline medium [16] containing defatted albumin (40 mg/ml) and 5 mM glucose as in [1]. Generally cells equivalent to 1/5 of those obtained from one rat were added to each flask. After incubation, the flask contents were deproteinized with $HClO_4$ and glycerol measured [17]. Adipocyte DNA was measured as in [18].

3. RESULTS AND DISCUSSION

Glucagon is not generally assigned a significant lipolytic role in mammalian adipose tissue [19]. In part, this is because, under the conditions generally used in vitro, high, unphysiological concentrations of the hormone are necessary to elicit a response which, even at its fullest, is considerably smaller than that seen with maximally effective concentrations of other lipolytic agents. Incubated adipocytes release small quantities of adenosine which, through interaction with 'R_i'-type adenosine receptors [20] inhibits adenylate cyclase and attenuates the effects of lipolytic hormones [21–24]. The lipolytic effect of glucagon is particularly susceptible to the attenuating effect of adenosine. Removal of adenosine from cell incubations with adenosine deaminase increases the lipolytic efficacy of glucagon to a level comparable with that of noradrenaline or corticotropin [1,25] and also reveals the substantial increase in sensitivity to glucagon seen in the fasted state [1].

Fig.1a shows that addition of glucagon alone at up to 30 nM only elicited a weak lipolytic response under the 3 experimental conditions. Fig.1b shows that inclusion of a small amount of adenosine deaminase (8 munits/ml) in the incubations of cells had only a slight effect on basal lipolysis. Inclusion of adenosine deaminase, however, facilitated the lipolytic action of higher concentrations of glucagon in cells from control animals or from insulin-treated diabetic rats (fig.1b). This facilitation of glucagon-stimulated lipolysis was substantially greater with the cells from diabetic

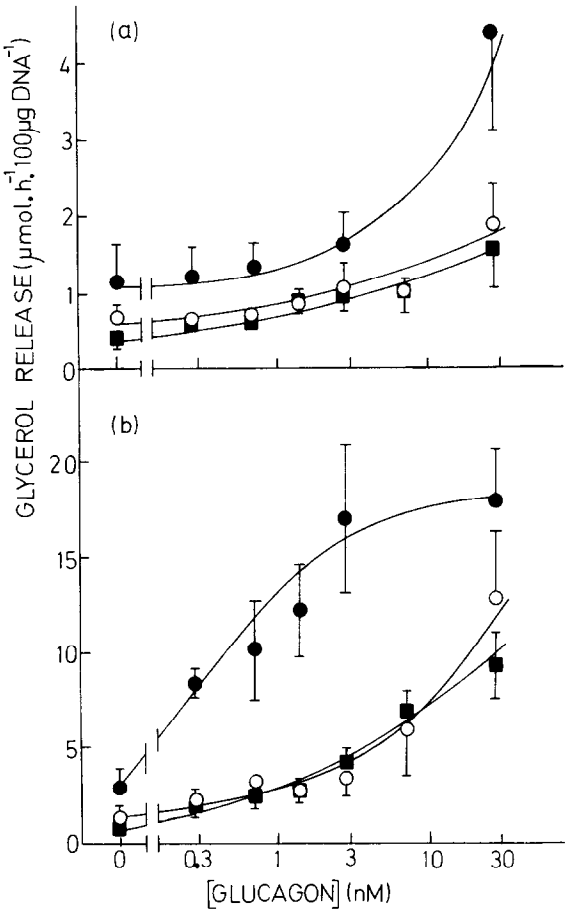

Fig.1. Dose–response curves of glucagon-stimulated lipolysis. Adipocytes were incubated for 1 h without (a) or with (b) adenosine deaminase (8 m units/ml). The values are means ± SEM. In the presence of adenosine deaminase diabetes resulted in significantly increased lipolysis compared with the controls at the following [glucagon]: 3×10^{-10} M and 8×10^{-10} M, $p < 0.05$; 1.5×10^{-9} M and 3×10^{-9} M, $p < 0.01$. Similar values for comparison of diabetics versus insulin-treated diabetics are: 3×10^{-10} M and 3×10^{-8} M, $p < 0.05$; 8×10^{-10} M and 3×10^{-9} M, $p < 0.02$; 1.5×10^{-9} M, $p < 0.01$. (○) Controls ($n = 5$) (●) diabetics ($n = 6$); (■) insulin-treated diabetics ($n = 4$). The mean adipocyte DNA/ml flask contents was: controls, 6.6 μg; diabetics, 5.4 μg; insulin-treated diabetics, 7.9 μg.

animals. About 4×10^{-10} M glucagon caused half-maximal stimulation of lipolysis in the diabetic state. Although a comparable value cannot be calculated directly from this experiment for the normal state, the maximum rate of glucagon-stimulated lipolysis observed in control cells with adenosine deaminase present is ~25–30 $\mu mol.h^{-1}.100\,\mu g$ DNA^{-1} [1,25]; i.e., comparable to or even greater than in the diabetic state. On this basis $\sim 2 \times 10^{-8}$ M glucagon would be necessary to elicit half-maximal response in the normal state; i.e., the enhancement in sensitivity to glucagon in the diabetic state is ~50-fold. Plasma glucagon levels in the rat have been reported over 80–2000 pg/ml; i.e., $\sim 2 \times 10^{-11}$–5×10^{-10} M [5,26,27], the top end of this range encompassing various pathological states, including diabetic ketoacidosis. It is concluded therefore, that physiological concentrations of the hormone may be capable of eliciting an appreciable lipolytic response in the diabetic condition, but not in the normal state. The responsiveness shown here is obviously a minimal estimate since the biological potency of the commercial glucagon preparation used here is unknown. It is not known at present whether this apparent adaptation of the glucagon responsiveness of tissue is due to altered interaction of glucagon with its receptor or is a post-receptor event. In the diabetic animal it will be accompanied by a fall in insulin and a rise in glucagon concentrations.

Fig.1b also shows that insulin-treatment of diabetic animals abolished the hypersensitivity to the hormone. It is not known if this may be attributed to a direct action of insulin on the tissue

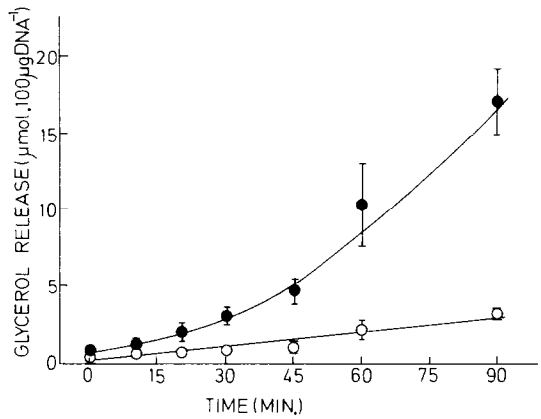

Fig.2. Time courses of glucagon-stimulated lipolysis. Adipocytes were incubated with glucagon (3×10^{-9} M) and adenosine deaminase (8 munits/ml). Time courses in the absence of glucagon were linear (not shown). The values are means ± SEM. (○) Controls ($n = 4$); (●) Diabetics ($n = 7$). The mean adipocyte DNA/ml flask contents was: controls, 4.6 μg; diabetics, 7.0 μg.

or could be secondary to the reduction in hyperglucagonaemia observed after administration of insulin to streptozotocin-diabetic rats [4]. Lipolytic hypersensitivity to adrenaline in diabetes is also reversible by insulin treatment [28].

Degradation of glucagon by incubated adipocytes may be considerable [29,30]. It was therefore considered possible that the difference in sensitivity might only be apparent and could be attributable to more rapid glucagon degradation in the normal state. If this were so, one would not expect sustained, linear, time courses of glucagon-stimulated lipolysis in response to non-saturating concentrations of the hormone in the normal state. However, this was observed with the intermediate glucagon dose of 3×10^{-9} M (fig.2). Furthermore, and surprisingly, the time course with diabetic cells actually accelerated such that the lipolytic rate over the 60–90 min interval was 9-times that seen in control cells. This is another unexplained facet of the action of glucagon in this tissue that requires consideration in further studies.

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