BIOCHEMICAL LETTERS

Untreated type 1 diabetes does not impair adenosine-mediated inhibition of lipolysis in human adipocytes

It has been suggested that the inhibitory arm of the regulation of adenylate cyclase, mediated by the guanine-nucleotide-binding protein G_i from receptors on the plasma membrane, is defective in diabetic experimental animals (Gawler *et al.*, 1987; Bushfield *et al.*, 1990; Strassheim *et al.*, 1990). We have measured the inhibition of isoproterenol-stimulated lipolysis by N^6 -(phenylisopropyl)adenosine in adipocytes prepared from untreated newly diagnosed type 1 human diabetics and show here that it is not impaired as compared to control subjects.

In adipocytes, the release of fatty acids and glycerol from intracellular triacylglycerols is accomplished by the enzyme hormone-sensitive lipase that is activated by cyclic AMP-dependent phosphorylation. Adenylate cyclase, and therefore lipolysis, is under dual regulation. β -Adrenergic receptors stimulate the cyclase via the guanine nucleotide binding stimulatory protein G_s . Different analogous inhibitory G_i proteins mediate inhibitory impulses from adenosine and α -adrenergic receptors on the plasma membrane to adenylate cyclase, thus inhibiting cyclic AMP accumulation and lipolysis. The inhibitory arm of this dual regulatory system has been reported to be altered in hypothyroidism (Ohisalo & Stouffer, 1979), obesity (Ohisalo *et al.*, 1986), by ageing and by corticosteroids. A decrease in this inhibitory system in diabetes would be concordant with the stimulated lipolysis observed.

Indeed, it has been suggested recently that diabetes causes selective changes in the functioning of the guanine nucleotide binding inhibitory G-protein (G_i) in hepatocytes and in adipocytes in animal models of diabetes (Gawler *et al.*, 1987; Bushfield *et al.*, 1990; Strassheim *et al.*, 1990). This has been suggested to remove the tonic GTP-mediated inhibitory function of this G-protein on adenylate cyclase. To test the hypothesis that G_i -mediated inhibition of lipolysis is impaired in diabetes, we obtained adipose tissue samples from two untreated type 1 diabetics (ages 44 and 25 years; body mass indices 20.2 and 19.5 kg/m²) who had just been hospitalized to start insulin treatment. They had lost 13 and 8 kg and both had marked polyuria. The blood glucose values were 14 and 20 mM (normal 3.5–5.5) and both had high glycosylated haemoglobin values (14.8 and 13.3%; normal range 5–7%). Glucagon-stimulated proinsulin C-peptide levels were 0.38 and 0.05 nM (normal range 1.1–2.3). Samples were taken from lower abdominal adipose tissue under local anaesthesia with 1% lidocaine. Small adipose tissue pieces were excised from a small incision. The tissue pieces were placed in iso-osmotic saline for transportation to the laboratory. Insulin treatment was started immediately after taking the samples. Both patients and controls gave an informed consent to taking the samples. Approval of the ethical committee of the III Department of Medicine of Helsinki University Central Hospital was also obtained.

Fat cells were isolated by a modification of the method of

Rodbell (1964) in the presence of collagenase (0.5 mg/ml) under constant shaking at 2 Hz at 37 °C in a buffer containing 125 mM-NaCl, 5 mM-KCl, 1 mM-CaCl₂, 2.5 mM-MgCl₂, 1 mM-KH₂PO₄, 4 mM-glucose, 2% bovine serum albumin and 25 mM-Tris at pH 7.4. After 60 min, the cells were filtered through a nylon cloth and washed three times with the same buffer without collagenase. The isolated cells were incubated for 120 min in the presence of 2 μ M-DL-isoproterenol and adenosine deaminase (1 μ g/ml) as described previously (Stoneham *et al.*, 1988). Adenosine deaminase was dialysed against iso-osmotic NaCl immediately before use. Different concentrations of N^6 -(phenylisopropyl)-adenosine were added as shown in Fig. 1. The incubations were stopped by boiling and the glycerol released was measured as described previously (Kather *et al.*, 1984).

Adenosine deaminase (type VIII from calf intestine), N^6 -(phenylisopropyl)adenosine, fatty acid free bovine fraction V albumin, collagenase (type II from *Clostridium histolyticum*) and isoproterenol·HCl were purchased from Sigma Chemical Co.

The response of isoproterenol-stimulated lipolysis to different concentrations of N^6 -(phenylisopropyl)adenosine in fat cells from the two untreated type 1 diabetics is shown in Fig. 1. The mean dose-response curve for ten lean control subjects (BMI 21.5 $\pm$ 0.9 kg/m²; mean $\pm$ S.E.M.) done similarly (Stoneham *et al.*, 1988) is shown for comparison. It is evident that the inhibition was not impaired in adipocytes isolated from the diabetic patients. This is in accordance with our previous report (Ohisalo *et al.*,

Fig. 1. Inhibition of lipolysis by N^6 -(phenylisopropyl)adenosine in fat cells from untreated type 1 diabetic patients

Samples were taken and fat cells were prepared as described in the text. The cells were incubated with 2 μ M-DL-isoproterenol and adenosine deaminase (1 μ g/ml) and different concentrations of N^6 -(phenylisopropyl)adenosine (PIA) as shown. The maximal stimulated rates of glycerol release were 1.1 and 1.7 nmol·h⁻¹·(μ l of cells)⁻¹ in cells prepared from the diabetics as compared to 1.1 $\pm$ 0.2 nmol·h⁻¹·(μ l of cells)⁻¹ (mean $\pm$ S.E.M.) in the controls. ●, mean values of ten lean control subjects; □, diabetics.

1989), showing that G_i levels are also normal. Strassheim *et al.* (1990) observed a severe resistance of adenylate cyclase to inhibition by 5'-guanylylimidodiphosphate in plasma membranes prepared from adipocytes of diabetic rats. Normal inhibition of cyclase via the adenosine receptor but attenuated inhibition by a GTP analogue would suggest the presence of discrete pools of G_i that would be regulated separately and differently. Our results, of course, do not completely rule out the possibility that some hypothetical pool of G_i other than that connected with the adenosine receptor is dysfunctional, though they show that inhibition of adenylate cyclase via the adenosine receptor and G_i is not impaired in adipocytes prepared from untreated type 1 diabetics.

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Functioning of the inhibitory regulation of adenylate cyclase in diabetic states: a reply

We were rather intrigued that Ohisalo and co-workers [1] felt able to conclude from their studies, which examined N^6 -(phenylisopropyl)adenosine (PIA)-mediated inhibition of lipolysis in intact adipocytes, that the functioning of the inhibitory guanine nucleotide regulatory protein G_i was normal in type-1 diabetic subjects. Stranger still that they thought their study on human adipocytes was at odds with our studies, done on streptozotocin-induced diabetes [2] in rats, which was entitled 'Diabetes abolishes the GTP-dependent but not the receptor-dependent inhibitory function of the guanine nucleotide-binding regulatory protein G_i '. Indeed, there we found that the induction of diabetes had no obvious effect upon the ability of saturating PIA concentrations to mediate the inhibition of adenylate cyclase and thus we would not expect to see any diabetes-induced alterations in PIA-mediated inhibition of lipolysis unless events downstream of the membrane signalling apparatus occurred. Rather we found that a rather subtle effect ensued concerning G_i functioning in streptozotocin-induced diabetes, namely that the tonic inhibitory effect that physiological concentrations of GTP exert upon adenylate cyclase was lost: an effect which can only be gauged by determining adenylate cyclase activity in isolated

Fig. 1. Phosphorylation of G_i -2 causes loss of GTP-elicited but not receptor-mediated inhibition of adenylate cyclase

It is suggested that the phosphorylation of the α -subunit of G_i -2 leads to the loss of the tonic GTP-elicited inhibition of adenylate cyclase that is likely to occur under physiological conditions due to the high concentrations of GTP present intracellularly. Inhibition of adenylate cyclase is considered to still occur through this system, presumably because coupling of an inhibitory receptor to G_i -2 has an overpowering effect upon the conformation of this G-protein and hence its functioning.

membranes and not by measuring lipolysis. Indeed, both ourselves [2,3] and others [4–6] have noted alterations in the GTP-elicited inhibition of adenylate cyclase in streptozotocin-induced diabetes as well as in a number of animal models of insulin resistance associated with either diabetes or obesity [see e.g. 7,8]. We have demonstrated that it is the phosphorylation of the α subunit of G_i -2, by protein kinase C, which provides the molecular mechanism leading to the loss of GTP-induced inhibition of adenylate cyclase [3] and have suggested that why receptor-mediated inhibition still ensues is that because receptor coupling to G_i -2 causes such an overpowering conformational change in G_i -2 that it overcomes any attenuating effect of phosphorylation (Fig. 1). In diabetes (and insulin-resistant states) then, the active phosphorylation/dephosphorylation cycle which modulates the functioning of G_i -2 [9] may be altered, either by enhanced protein kinase C action or attenuated phosphatase activity, leading to the loss of GTP-elicited inhibition of adenylate cyclase. Thus detection demands analysis of GTP-mediated inhibition of adenylate cyclase in isolated membranes and not receptor-mediated downstream actions such as the effect of PIA on lipolysis in intact adipocytes.

It is, however, interesting to consider the study of Ohisalo and co-workers [1] alongside the recent work of Green & Johnson [4] who, whilst confirming our identification [2] of the loss of guanine nucleotide-mediated inhibition of adenylate cyclase in the adipocytes of streptozotocin-induced diabetic rats, actually noted a reduced sensitivity in the ability of PIA to inhibit lipolysis, as detected by a rightward shift in the dose-effect curve for PIA. Indeed, a similar attenuated responsiveness for the action of PIA on adipocyte lipolysis in streptozotocin-diabetic rats has also been noted independently by Saggerson and co-workers [5,10]. These consistent data, performed after statistical analyses on adipocyte preparations from a number of diabetic and normal animals, may imply a difference in the functioning or action of inhibitory adenosine receptors in diabetic human beings and rats or even that streptozotocin-induced diabetes does not mirror the human condition. However, before such conjecture, it