Adipocyte plasma-membrane G_i and G_s in insulinopenic diabetic patients

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Changes in the amounts and functions of G-proteins have been reported in diabetic rats. We determined the G_i/G_s ratio of adipocyte plasma membranes in six insulinopenic diabetic patients and matched controls by immunoblotting with antisera against synthetic peptides corresponding to regions of G-proteins. No consistent changes in the G_i/G_s ratio were observed. It is still possible that the functional status of the G-proteins may be altered in diabetes.

INTRODUCTION

Diabetes is accompanied by a multitude of metabolic and endocrine aberrations [1]. Some of these are simply due to a defective 'primary' insulin effect on glucose transport and lipid metabolism. However, alterations in the response of tissues to hormones, including insulin itself, have remained unexplained despite a massive research effort. Gawler *et al.* [2] reported decreased amounts and activity of the inhibitory guanine-nucleotide-binding protein G_i in plasma membranes of hepatocytes prepared from rats made diabetic by streptozotocin. While the present manuscript was in preparation, Lynch *et al.* [3] reported that the level of G_s is increased in hepatocytes of such diabetic rats, but in that study there was no change in G_i .

G_i and G_s are plasma-membrane proteins that mediate inhibitory and stimulatory impulses, respectively, from hormone receptors on the plasma membrane to adenylate cyclase and other systems that generate second messengers [4]. Both G_i and G_s consist of three subunits, α , β and γ . The α subunits ($G_{i\alpha}$, $G_{s\alpha}$), different in each individual G-protein, are the active subunits. There is evidence that the relative amounts of $G_{i\alpha}$ and $G_{s\alpha}$ can be regulated in rat adipocytes by thyroid hormones [5,6], which could explain the metabolic aberrations in hypothyroidism [7,8]. Also, it has been reported that the concentration and activity of G_i are dependent on insulin in rat hepatocytes [2]. Streptozotocin-induced diabetes has been reported to lead to an almost complete disappearance of hepatocyte G_i [2] or an increase in G_s [3], which could explain many of the metabolic aberrations in diabetes, such as increased hepatic output of glucose [2,3,9]. A similar change in G_i or G_s in adipocytes might be expected to lead to increased lipolysis.

In trying to explain the metabolic aberrations in human diabetes, it would be very important to assay G_i and G_s in plasma membranes prepared from the liver and adipose tissue of insulinopenic diabetic patients and corresponding controls. Liver samples from such patients are difficult to obtain, whereas adipose tissue can be sampled with less potential complications. The purpose of the present

study was to examine if insulin regulates the relative amounts of G_i and G_s in human adipose tissue.

MATERIALS AND METHODS

Patients

All the diabetic patients were admitted to hospital for the immediate institution of insulin therapy. They all had polyuria, high blood glucose levels, high haemoglobin A_1 concentrations and low C-peptide levels even 6 min after intravenous injection of glucagon. Patients 1D, 3D, 9D and 11D were on oral medication. Patients 9D and 11D had had a pancreatectomy because of haemorrhagic pancreatitis. The samples were taken in the morning before the first dose of insulin. The control patients (other than patient 10C; see below) were healthy volunteers who were selected to match the diabetic patients by age, sex and relative body weight. Details of the tissue donors are displayed in Table 1.

Written informed consent was obtained from all subjects, and the project was approved by the ethical committee of the III Department of Medicine, Helsinki University Central Hospital.

Samples

The fat samples were taken by open surgical biopsy from the lower abdomen under local anaesthesia by lidocaine, except for patient 10C, whose sample was taken from the same region under general anaesthesia during elective bowel resection done because of Crohn's disease. The samples were placed in iso-osmotic saline solution for transportation to the laboratory.

Preparation of adipocytes and plasma membranes

The tissue samples were cut into small pieces with a razor blade. Fat-cells were isolated by a modification of the method of Rodbell [10], by using 0.5 mg of collagenase/ml in 125 mM-NaCl/5 mM-KCl/1 mM-CaCl₂/2.5 mM-MgCl₂/1 mM-KH₂PO₄/25 mM-Tris/4 mM-glucose/2% (w/v) bovine serum albumin at pH 7.4. After 60 min under constant shaking at 2 Hz at 37 °C,

Abbreviations used: G_i , guanine-nucleotide-binding inhibitory protein; G_s , the corresponding stimulatory protein.

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Table 1. Details of the tissue donors

Abbreviations: D, diabetic; C, control; BMI, body mass index [weight (kg) divided by the square of height (m)]; N.D., not determined. $X = G_i(d) \times G_s(d)^{-1} \times G_i(c)^{-1} \times G_s(c)$, calculated by using the signal from the major band of $G_s\alpha$.

Patient	Sex	Age (years)	BMI (kg/m ²)	Duration of disease	Glucagon-stimulated C-peptide (μ g/l) (normal range 3.5–6.8)	Haemoglobin A ₁ (%) (normal range 5.0–7.0)	X
1D	F	43	21.2	7 years	1.68	9.8 }	1.48
2C	F	31	23.1	–	N.D.	N.D. }	
3D	M	34	20.0	Newly diagnosed	0.71	14.1 }	
4C	M	37	22.9	–	N.D.	N.D. }	0.47
5D	M	27	25.9	8 months	2.31	10.4 }	
6C	M	31	24.7	–	N.D.	N.D. }	
7D	M	33	26.4	Newly diagnosed	0.69	10.0 }	0.80
8C	M	25	24.0	–	N.D.	N.D. }	
9D	M	51	20.0	11 years	1.09	13.0 }	
10C	M	46	23.5	–	N.D.	N.D. }	1.04
11D	M	30	22.3	16 months	0.52	12.6 }	
12C	M	26	20.2	–	N.D.	N.D. }	

the cells were washed three times with the same buffer without collagenase. The cells were then homogenized by a Potter/Elvehjem Teflon/glass homogenizer in 2 vol. of 10 mM-EDTA/0.25 M-sucrose, pH 7.4, and crude plasma membranes were isolated by centrifugation as described elsewhere [11].

Determination of the G_i/G_s ratio

Relative amounts of G_i and G_s in the plasma membranes were assessed after SDS/polyacrylamide-gel electrophoresis and electroblotting on to nitrocellulose paper as described elsewhere [6,12]. The samples from a diabetic patient and the corresponding control were run in parallel. $G_i\alpha$ was detected by using antiserum SG-1, which was raised in a rabbit against a conjugate of keyhole-limpet haemocyanin (KLH) and the C-terminal decapeptide of the α subunit of transducin (KENLKDCGLF). This antiserum has been shown to recognize forms of G_i in human adipocytes [12]. $G_s\alpha$ was detected in an analogous way by using antiserum CS-1, which was raised in a rabbit against a conjugate of KLH and the C-terminal decapeptide of G_s (RMHLRQYELL) [12,13]. The second antibody was goat anti-rabbit IgG coupled to horseradish peroxidase, and the final reaction was detected by *o*-dianisidine [6]. Quantification was performed by scanning a photographic positive using a Helena Flur-Vis Autoscanner.

Protein determinations

Total protein was assayed by the method of Lowry *et al.* [14], with bovine serum albumin as the standard.

Reagents

Collagenase (type II from *Clostridium histolyticum*) and bovine serum albumin (fraction V) were purchased from Sigma, St. Louis, MO, U.S.A. Goat anti-rabbit IgG coupled to horseradish peroxidase was obtained from Bio-Rad. Prestained molecular-mass standards were purchased from Bio-Rad. Their apparent molecular masses were determined by comparison with unstained Bio-Rad molecular-mass standards.

Fig. 1. Detection of $G_s\alpha$ and $G_i\alpha$ in crude adipocyte plasma membranes

(a) Crude human adipocyte plasma-membrane protein (50 μ g) was run on a 10 %-polyacrylamide gel in the presence of SDS. The proteins were transferred electrophoretically to nitrocellulose paper and stained by antiserum CS-1 as described in the text. (b) The blot described above was restained with antiserum SG-1. Positions of molecular-mass (kDa) standards are shown to the left. Abbreviation: d.f., dye front.

RESULTS AND DISCUSSION

Fig. 1(a) displays an immunoblot of human adipocyte plasma-membrane proteins separated by SDS/polyacrylamide-gel electrophoresis and stained with antiserum CS-1, which detected two bands (42 kDa and 45 kDa) that are two forms of $G_s\alpha$ subunit. After being photographed, the same blot was stained with antiserum SG-1 (Fig. 1b). This antibody stained a single band at approx. 40 kDa. We have shown previously [12] that this protein is the major pertussis-toxin substrate in human adipocyte plasma membranes, and therefore it is the α subunit of G_i . In further experiments, a mixture of the two antisera was used to detect both $G_i\alpha$ and the two forms of $G_s\alpha$ in a single blot.

Fig. 2 shows the staining by a mixture of CS-1 and SG-1 of different amounts of crude human adipocyte plasma membranes up to an amount of 50 μ g. All the three bands were stained linearly up to 25 μ g of protein. In subsequent experiments, less than 15 μ g of protein was used.

Fig. 3 shows the staining pattern of crude adipocyte plasma-membrane proteins of a diabetic patient (7D) and a corresponding control (8C). Six such pairs were

run in different experiments. After scanning a photographic positive of the blots, the G_i/G_s ratio was calculated for each patient. Since the pairs were assayed separately and the assay gives only relative values, the results were transformed mathematically so that the G_i/G_s ratio of the control patient was arbitrarily assigned the value of 1, and the comparable value of the G_i/G_s ratio of the diabetic was calculated by using the formula

$$X = \frac{G_i(d)/G_s(d)}{G_i(c)/G_s(c)}$$

where (d) refers to the diabetic patient and (c) to the corresponding control. The units of G_i and G_s are arbitrary, but this has no effect on the final absolute value because the terms cancel each other. The values of X averaged 0.97; in three pairs the value was below and in three others over 1. There was no correlation between the value of X and the glucagon-stimulated C-peptide level of the diabetic patient. This shows clearly that the insulin-deficient diabetic patients did not have consistent changes in their G_s/G_i ratio.

It has been reported that the amount and activity of G_i in the liver plasma membranes of diabetic rats is decreased [2]. A very recent paper [3] reported an increase in the amount of G_s while the amount of G_i remained unchanged. The present results with adipocyte plasma membranes from diabetic human patients and matched

Fig. 2. Linearity of the staining by antisera CS-1 and SG-1

Different amounts of crude human adipocyte plasma membranes were run on a 10%-polyacrylamide gel in the presence of SDS, electroblotted and stained with a mixture of antisera CS-1 and SG-1 as described in the text. The resulting bands were quantified by scanning a photographic positive.

Fig. 3. Detection of $G_i\alpha$ and $G_s\alpha$ in crude adipocyte membranes of an insulinopenic diabetic patient and a matched control

The experiment was done as described in the text. The patient numbers refer to Table 1. The numbers to the left show the molecular masses of the standards in kDa.

control subjects are different from both of those two observations. Several explanations are possible. The reported change in G_i or G_s may be specific to the liver. Secondly, the change may be species-specific. Furthermore, the diabetic rats studied by Gawler *et al.* [2] and Lynch *et al.* [3] had extreme insulin deficiency for 7 days, during which time they had already lost most of their adipose tissue. Therefore, as compared with our patients, their disease was at a more advanced stage. It is possible that, if the diabetic patients would have been without insulin treatment for a longer period of time, changes in the G_i/G_s ratio would finally have been observed.

The present results exclude changes in the G_i/G_s ratio as a simple explanation of metabolic aberrations in fat-cells prepared from diabetic human patients with a considerable insulin deficiency. However, the possibility remains that the functional status of the G proteins in human adipocytes is aberrant in diabetes. Indeed, it has been shown recently that the activation of protein kinase C in rat hepatocytes can lead to phosphorylation and concomitant inactivation of G_i [15]. A similar mechanism could be operational in human adipocytes, and this mechanism could be regulated by insulin and altered in insulin deficiency.

We are much obliged to the diabetic patients and our other volunteers for participating in this study. The financial support from the Academy of Finland and the Wellcome Trust is gratefully acknowledged.

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Received 7 July 1989/13 September 1989; accepted 22 September 1989