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Defective Stimulation of Adipocyte Adenylate Cyclase, Blunted Lipolysis, and Obesity in Pseudohypoparathyroidism 1a

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ABSTRACT. Adipocyte plasma membranes were isolated from four patients with type 1a pseudohypoparathyroidism, a disease in which deficiency of the stimulatory guanine nucleotide binding protein G_s has been reported, and from controls. Stimulation of adenylate cyclase by isoproterenol was defective, whereas inhibition of forskolin-stimulated cyclase activity by N^6-(phenylisopropyl)adenosine was normal. The patients had low serum FFA concentrations and developed obesity in childhood. These results suggest that pseudohypoparathyroidism 1a is connected with a blunted stimulatory response of adenylate cyclase, possibly because of low G_s activity, and that this blunted response may lead to decreased lipolysis and to obesity. (*Pediatr Res* 35: 594–597, 1994)

Abbreviations

G_i, inhibitory guanine nucleotide binding protein
G_s, stimulatory guanine nucleotide binding protein
PHPT, pseudohypoparathyroidism

Adoption and twin studies (1, 2) have suggested that obesity is inherited genetically and not only socially. Furthermore, it is known that although losing weight by dieting is easy, returning to the previous higher weight thereafter is almost inevitable. This suggests that there are molecular mechanisms leading to and maintaining obesity. Defective stimulation of lipolysis could be a mechanism causing or maintaining an increased adipose tissue mass. In fact, there is evidence that stimulation of lipolysis is defective in obesity both in human subjects (3–5) and in experimental animals (6, 7). Lipolysis is controlled by intracellular cAMP, whose concentrations in the fat cell are increased by hormone receptors, stimulating adenylate cyclase via the stimulatory guanine nucleotide binding protein, G_s (8). G_s is present in concentrations that are orders of magnitude higher than the receptors. A decreased amount of β-adrenergic receptors (5) or G_s (9, 10) in adipocyte plasma membranes or their altered functional status (7) could be an explanation for the development and maintenance of obesity. A decreased amount of G_s has, in fact, been reported in obesity (9, 10).

PHPT type 1a is a syndrome characterized by the clinical picture of hypoparathyroidism but elevated circulating parathormone levels, short stature, obesity, congenital hypothyroidism, Albright's hereditary osteodystrophy, brachydactyly, and mild mental retardation (11). In this dominantly inherited disease, the transmembrane signaling system is defective, and the amount of $G_s\alpha$-protein has been reported to be decreased by approximately 50% (12–14). A mutation of the start codon of protein synthesis and a concomitantly untranslated or mistranslated mRNA has been described (15).

Here we report investigations on fat metabolism in adipose tissue in PHPT type 1a in which a G_s deficiency has been reported.

MATERIALS AND METHODS

Patients. All the pseudohypoparathyroid patients described here had brachydactyly as well as other features of Albright's hereditary osteodystrophy and somewhat but not grossly retarded mental development. They all had elevated serum parathormone levels even when made normocalcemic with dihydrotachysterol, indicating parathormone resistance, and elevated TSH values before thyroid hormone replacement (Table 1). It is therefore clear that all the patients had PHPT type 1a, which has been shown to be connected with low plasma membrane G_s activity. Patients 1, 2, and 3 were siblings; their pedigree is shown in Figure 1. Their mother has osteodystrophy but has normal TSH, PTH, and calcium values. Patient 6 is the mother of patient 5; she also has a newborn daughter who has the disease and an obviously healthy son. All the patients were normocalcemic and euthyroid at the time when fat biopsies and samples for plasma FFA determinations were taken. All four patients whose adipocyte membranes were studied were obese. The exact determination of the degree of obesity is difficult because the body mass index is not useful with children and because the patients are of very short stature and stocky build. The body weights of patients 1–8 were compared with age-, sex-, and height-matched Finnish national statistics. This comparison gave an average overweight of 50 ± 13% (mean ± SEM).

Table 1. *Characteristics of the patients**

Patient	Age	Sex	PTH (ng/L) (NR 10–55)	TSH (mU/L) (NR 0.3–4)
1	15	F	307	11.6
2	12	M	352	16.1
3	6	M	336	11.0
4	13	F	134	14.1
5	2	M	466	8.9
6	30	F	427	8.2
7	28	F	95	7.0
8	3	M	115	33.6

* The PTH determinations were done at a time when the patients were normocalcemic (with or without dihydrotachysterol), and TSH values were determined earlier when the patients had not been receiving thyroid hormone replacement. NR, normal range.

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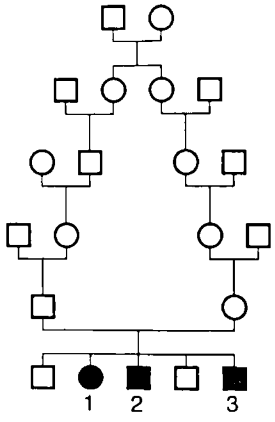

Fig. 1. The family tree of patients 1, 2, and 3.

It was not possible to obtain fat samples from age- and weight-matched controls because the patients were children. The control material used therefore was from adults. The exact characteristics will be given in the Results section, and the justification for using adult controls will be discussed below.

Isolation of cell membranes. Subcutaneous adipose tissue samples were excised from the middle abdominal region from a small incision with the subjects under local anesthesia (without adrenaline) or under general anesthesia at the beginning of routine operations such as cholecystectomies and hysterectomies. Fat cells were isolated from these samples by a modification of the method of Rodbell (16) in the presence of collagenase (0.5 mg/mL) with constant shaking at 2 Hz at 37°C in a buffer containing 125 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 2.5 mM $MgCl_2$, 1 mM KH_2PO_4, 4 mM glucose, 2% BSA, 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 cells were homogenized in 10 volumes of buffer containing 150 mM NaCl, 1 mM EDTA, 10 mM Tris at pH 7.4, 1 μM leupeptin, and 4 μM phenylmethylsulfonyl fluoride using a Potter-Elvehjem glass-Teflon homogenizer. The homogenate was centrifuged at 10 000 × *g* for 20 min and the pellet was resuspended in the homogenization buffer.

Adenylate cyclase assay. Adenylate cyclase activity of fresh adipocyte plasma membranes was assayed in a total volume of 500 μL in the presence of 150 mM NaCl, 10 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 2 mM DTT, 1 mM ATP, 100 μM papaverine, 1 mM guanosine triphosphate, BSA (1 mg/mL), 50 mM Tris-HCl, pH 7.5, and adenosine deaminase (1 μg/mL). 7-Deacetyl-6-(N-acetylglycyl)forskolin (20 μM) and different concentrations of N^6-(phenylisopropyl)adenosine or isoproterenol were added as indicated. After 20 min at 37°C, the incubations were terminated by boiling the tubes for 2 min. The boiled samples were then centrifuged and the supernatants were assayed for cAMP using a kit from Advanced Magnetics (Cambridge, MA).

FFA. Samples for serum FFA determinations were taken the morning after an overnight fast. The assay was done by using the NEFA C-kit (Wako Chemicals GmbH, Neuss, Germany) and oleic acid as the standard.

Reagents. Adenosine deaminase (type VIII from calf intestine), N^6-(phenylisopropyl)adenosine, fatty acid-free BSA, isoproterenol·HCl, and collagenase were purchased from Sigma Chemical Co. (St. Louis, MO). 7-Deacetyl-6-(N-acetylglycyl)forskolin was from Calbiochem (San Diego, CA), and the cAMP assay kit (catalog no. 6301) from Advanced Magnetics.

Ethical considerations. The study was approved by the ethical commissions of the 3rd Department of Medicine and 2nd Department of Surgery of Helsinki University Central Hospital.

RESULTS

The adenylate cyclase assay was linear with respect to protein concentration up to 70 μg per assay, corresponding to approximately 200 μL of fat cells. When we studied the PHPT patients, we used plasma membranes derived from 12–16 μL of cells per assay tube. The small size of the samples from PHPT patients prevented reliable protein assay, but the harmonic means of forskolin-stimulated cyclase activities were 26 and 39 pmol/min/μL fat cells in the control and PHPT groups, respectively. However, there was much interindividual variation in both groups. Our experience is that this is caused by variation in the yield in plasma membrane isolation.

The dose-response curve of adipocyte plasma membrane adenylate cyclase to isoproterenol of patients 1–4 compared with 12 controls is displayed in Figure 2. The body mass indices of the controls were 32.1 ± 1.7 and ages 51.6 ± 4.8 y (means ± SEM). The adenylate cyclase activities are shown on a scale of 0 to 100%, measured in the presence of 7-deacetyl-6-(N-acetylglycyl)forskolin, which stimulates the cyclase directly. It is evident that the basal unstimulated activities were similar but the membranes prepared from the type 1a pseudohypoparathyroid patients had decreased responsiveness to isoproterenol. The difference was statistically significant at $p = 0.041$ (repeated measures analysis of variance).

The inhibitory arm of cyclase regulation by N^6-(phenylisopropyl)adenosine, an adenosine A1 receptor agonist, was studied in the presence of exogenous adenosine deaminase to remove endogenous adenosine. The samples from pseudohypoparathyroid patients were the same as described above; the control material came from six subjects whose ages were 52.7 ± 4.5 y and body mass indices 26.5 ± 1.9 kg/m² (means ± SEM). Inhibition of 7-deacetyl-6-(N-acetylglycyl)forskolin–stimulated adenylate cyclase by N^6-(phenylisopropyl)adenosine was similar in both groups (Fig. 3).

Fasting serum FFA of patients 1–5 (relative body weight, 144 ± 17%; means ± SEM) were measured and compared with five age-matched control subjects (relative body weight, 124 ± 10%) selected from hospital records of 200 FFA determinations. All the control subjects were found healthy at the time of investigation. Four of the control subjects had an advanced bone age compared with calendar age, as was also the case with the

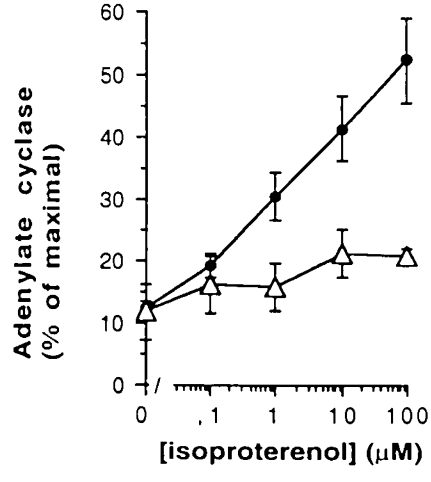

Fig. 2. Effect of isoproterenol on adenylate cyclase activity of fat cell plasma membranes prepared from patients 1–4 and controls. Fat cell plasma membranes were prepared as described in the text. The membranes were incubated with different concentrations of isoproterenol and the cAMP formed was measured by RIA. Cyclase activity is expressed on a scale from zero activity to 100% measured in the presence of 20 μM 7-deacetyl-6-(N-acetylglycyl)forskolin. *Vertical bars* indicate SEM. △, pseudohypoparathyroid; ●, controls.

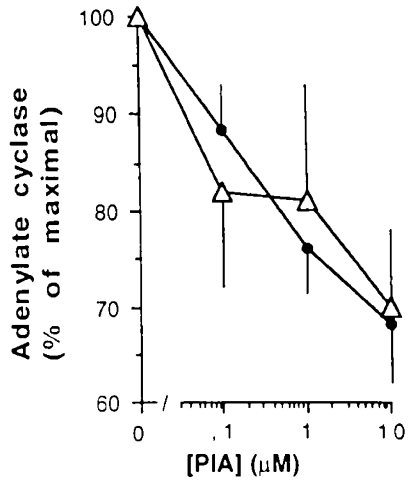

Fig. 3. Inhibition of adipocyte plasma membrane adenylate cyclase by N^6-(phenylisopropyl)adenosine (*PIA*) in pseudohypoprathyroid and control subjects. Plasma membranes were isolated as described in the text. Adenylate cyclase was stimulated by 20 μM 7-deacetyl-6-(N-acetyl-glycyl)forskolin in the presence of adenosine deaminase, and different concentrations of N^6-(phenylisopropyl)adenosine were added. Cyclase activity is expressed on a scale from zero activity to 100% measured in the presence of 20 μM 7-deacetyl-6-(N-acetyl-glycyl)forskolin. *Vertical bars* indicate SEM. △, pseudohypoparathyroid; ●, controls.

Table 2. *Serum FFA concentrations in pseudohypoparathyroid and control subjects**

	Serum FFA (μmol/L)	Age (y)
Control	735 ± 198	10.8 ± 1.3
Pseudohypoparathyroid	190 ± 54	9.6 ± 2.4

* The values are mean ± SEM of five subjects.

pseudohypoparathyroid patients. One of the control subjects had hypoglycemia in the past. Two of the control subjects were obese. Serum FFA were low in the pseudohypoparathyroid patients compared with controls (Table 2); the difference was statistically significant at $p = 0.029$. The mean value in the pseudohypoparathyroid patients is below the normal range of 0.3–1.0 mmol/L given by the manufacturer of the assay kit.

DISCUSSION

The present results suggest that the stimulation of adenylate cyclase via the β-adrenergic receptor and G_s is defective in fat cells in PHPT type 1a. The finding of low serum FFA concentrations in the patients is in accordance with this; in obese individuals, FFA concentrations are usually elevated (17). Control samples for studies of cyclase regulation could not be obtained from age-matched material. However, there is a lot of evidence suggesting that adult controls could be used. Most importantly, direct measurements have shown that the stimulatory arm of cyclase regulation is similar in infants and adults (18, 19). Secondly, small fat cells are more sensitive to stimulation by isoproterenol than large cells (Kaartinen *et al.*, unpublished manuscript); because cells are smaller in childhood, if anything the cells of children would be expected to be more and not less sensitive. Furthermore, the responses of the older two patients who already had reached puberty were similar to those of the smaller children. Finally, the inhibitory responses were similar in the patients and controls. It is also possible, of course, that there are differences in adenylate cyclase *per se*.

The finding of low FFA concentration in PHPT is in contrast with the report of Carlson *et al.* (20). A closer look at the data of Carlson *et al.*, however, shows that their patient material consisted of only two type 1a and four type 1b patients who do not have a G-protein defect. The isoproterenol-stimulated FFA concentrations, in fact, were lower in pseudohypoparathyroid patients than in controls and the SEM values shown at 15 min do not overlap. Other reasons for their different conclusion are that their samples were stored for long periods and their basal FFA levels were well above the upper limit of normal values.

G_i-mediated events in platelets have been reported to be normal in PHPT (21). The inhibition of adipocyte adenylate cyclase via the adenosine A1 receptor was normal in our patients, which is in contrast with what is usually found in obesity (4, 22). This could be caused by an effect of G_i on G_s through common βγ-subunits by the law of mass action.

We conclude that adipocyte plasma membranes isolated from patients with PHPT type 1a had defective stimulatory responses to β-adrenergic stimulation but normal inhibitory response to an adenosine analog. The patients had low serum FFA concentrations and developed obesity early in life.

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