Resistance to epinephrine and hypersensitivity (hyperresponsiveness) to CB1 antagonists in a patient with pseudohyoparathyroidism type Ic

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Abstract

Pseudohyoparathyroidism (PHP) covers a heterogeneous group of disorders, which have in common resistance to parathyroid hormone (PTH). However, they differ in many aspects such as site of the defect in signal transduction, clinical picture (with or without Albright’s hereditary osteodystrophy (AHO)), extension of hormone resistance, and the tissue activity of protein Gs. PHP type Ic, a rare subtype, is characterized by resistance to several hormones, the presence of AHO, and normal activity of protein Gs. We present the case of a patient with PHP type Ic. Although resistance to TSH was suggested at the age of 12 months, diagnosis was made when she presented with hypocalcemia and resistance to PTH. Resistance to GH was also detected, and partial resistance to gonadotropins became clear after puberty. We demonstrated a defective lipolytic response to epinephrine, suggesting a role of this resistance in the pathogenesis of her morbid obesity. In view of the difficulties in the management of overweight in this disorder, treatment with a cannabinoid receptor type 1 (CB1) antagonist was started, and it proved to be highly effective, lowering the patient’s body mass index from 40.5 to 33.5, which was quite impressive. We propose that an underactive melanocortin-4 receptor, which is found in certain patients with PHP, leads to upregulation of the CB1 receptor and consequently to a good response to treatment with CB1 antagonists. Another interesting finding was the GNAS mutation that was identified in this patient. A nonsense mutation resulted in a truncated Gsa that was able to stimulate adenylyl cyclase efficiently, but could not bind to receptors in a normal way.

Introduction

Pseudohyoparathyroidism (PHP) is an uncommon metabolic disorder characterized by functional hypoparathyroidism, target organ resistance to the action of parathyroid hormone (PTH), and increased secretion of PTH. In addition to functional hypoparathyroidism, many patients display a distinctive constellation of physical features, referred to as Albright’s hereditary osteodystrophy (AHO). PHP was first described by Fuller Albright et al. in 1942 when they reported three patients with hypocalcemia in whom the administration of bovine parathyroid extracts failed to promote an increase in serum calcium or a rise in phosphaturia. Moreover, these patients exhibited developmental and skeletal defects such as rounded face, short stature, obesity, brachydactyly, short and low-set nasal bridge, strabismus, and ectopic calcifications, later designated as AHO (1). AHO is a unique syndrome of developmental and skeletal defects. Mental retardation has also been reported. However, these features vary greatly between patients, even between family members, and some patients may have either few or no features of the syndrome. Pseudopseudohyoparathyroidism (PPHP) is the term used for the occurrence of AHO without hormone resistance.

PHP is subdivided into two main types based on an exogenous PTH administration test. Patients with PHP type I fail to show an appropriate increase in urinary excretion of both nephrogenous cAMP and phosphate, while patients with PHP type II, with no AHO, show normal urinary cAMP excretion but no phosphaturic response to PTH. PHP type I is a heterogeneous group of disorders that can be differentiated by the presence or absence of AHO, hormone resistance that is specific (PTH alone) or more generalized, and normal or
reduced tissue (erythrocytes) activity of G protein (Gs) which mediates the stimulation of adenylyl cyclase and the production of cAMP. Patients with type Ia have generalized deficiency in Gs\(\alpha\), which results in \(\sim 50\%\) reduction in the expression or activity of the \(\alpha\)-chain of Gs (Gs\(\alpha\)) in membranes from a wide variety of cells and tissues. This generalized deficiency in Gs\(\alpha\) may impair the ability of PTH as well as that of other hormones to activate adenylyl cyclase, thereby accounting for the multihormone resistance in these patients. AHO is also present. By contrast, patients with PHP type Ib do not have features of AHO, but have hormone resistance that is limited to PTH, although resistance to TSH has occasionally been reported, and normal levels of Gs\(\alpha\) protein in cell membranes. Patients with type Ic have resistance to several hormones and exhibit AHO, but they do not have any demonstrable deficiency in Gs\(\alpha\).

The human Gs\(\alpha\) gene (GNAS) is located on chromosome 20q13.2–13.3. Originally, this gene was defined by the 13 exons that encode Gs\(\alpha\). It is now known that GNAS is a complex gene that contains at least four alternative promoters and first exons that splice onto a common set of downstream exons (exons 2–13) to produce different proteins. The most downstream of these exons is Gs\(\alpha\) exon 1. As a result of alternative splicing of exon 3, there are two long (Gs\(\alpha\)-1 and Gs\(\alpha\)-2) and two short (Gs\(\alpha\)-3 and Gs\(\alpha\)-4) forms of Gs\(\alpha\). Using an alternative splice acceptor site for exon 4 leads to insertion of an extra serine residue in Gs\(\alpha\)-2 and Gs\(\alpha\)-4 (2). The most upstream of these promoters is NESP, which produces transcripts that encode neuroendocrine secretory protein 55 (NESP55). The next promoter, which is located downstream from NESP, is XL\(\alpha\) that produces transcripts encoding XL\(\alpha\)s, an isoform of Gs\(\alpha\) with a long amino-terminal extension, whose biological function remains to be determined. The next promoter is the alternative first exon (A/B or A1). It produces transcripts, which are presumed to be untranslated mRNAs (3). GNAS also encodes for NESP55 antisense transcript, which may have a role in the regulation of imprinted gene expression from the GNAS locus (4) (Fig. 1).

GNAS presents a tissue- and parent-specific imprinting phenomenon, which explains the variations in phenotype. Genomic imprinting is an epigenetic phenomenon, which by affecting a gene leads to expression from only one parental allele. For some of these imprinted genes, the imprinting is tissue specific, leading to biallelic expression in some tissues and monoallelic expression in others. Most imprinted genes contain specific regions in which the cytosines within CpG dinucleotides are differentially methylated, and the differential methylation of these regions is probably important for establishing imprinting (5). GNAS has a complicated imprinting pattern that leads to the expression of some gene products from the maternal allele and others from the paternal allele. All the promoters of GNAS transcripts, except the first exon of Gs\(\alpha\), are differentially methylated. NESP is methylated on the paternal allele (Fig. 1), and therefore, NESP55 is only expressed from the maternal allele. In contrast, XL\(\alpha\)s is only expressed from the paternal allele. The exon 1A promoter region is methylated on the maternal allele. Gs\(\alpha\) exon 1 is expressed biallelically in the majority of human tissues. However, it is maternally expressed in a few tissues, such as the proximal renal tubules, the thyroid, the gonads, the pituitary gland, and the hypothalamus. Both thyroid and ovary imprinting are partial (6). The GNAS imprinting within the pituitary gland is cell type specific as it affects only the somatotrophs (7). The hypothalamic imprinting of GNAS was recently proposed by Long et al. to explain the differences in body mass index (BMI) between PHP type Ia and PPHP (8).

Heterozygous loss-of-function mutations of GNAS in the active maternal allele cause PHP type Ia. These mutations lead to the absence of Gs\(\alpha\) expression in tissues, in which expression is usually monoallelic (inducing hormone resistance), and to 50% Gs\(\alpha\) expression in other tissues (haploinsufficiency), which manifests as AHO (Fig. 2).

PHP type Ib is a disorder due to deregulated imprinting of GNAS. The most consistent molecular finding is the loss of methylation of the A/B exon, which leads to silencing of the GNAS promoter on the maternal allele specifically in those tissues in which expression is normally monoallelic (9, 10). Heterozygous microdeletions in STX16, the gene encoding syntaxin 16, have been identified in patients with PHP type Ib. These microdeletions probably disrupt a cis-acting element that controls imprinting at the A/B exon (11).

We report here the case of a patient with PHP type Ic. Although she had an elevated TSH level at the age of 12 months, the diagnosis was made only at the age of 10 years when she exhibited symptoms of hypocalcemia
with clinical features of AHO and resistance to PTH. Resistance to GH was also detected, and partial resistance to gonadotropins became clear after puberty. She had morbid obesity, and we demonstrated a defective lipolytic response to epinephrine as a potential causative factor. When treatment with CB1 antagonist was initiated, our patient showed a remarkable response to this treatment, losing 16 kg within 6 months, which was quite impressive. Another interesting finding was the GNAS mutation that was identified in this patient. This nonsense mutation resulted in a truncated Gsα that could stimulate adenylyl cyclase, but could not bind to the PTH receptor normally.

**Case history**

The patient was born at term in June 1979, with a birth length of 48 cm (15th percentile) and a birth weight of 3.370 kg (50th percentile). Her clinical history began at the age of 12 months, when her pediatrician noticed a decreased growth rate and accelerated weight gain (+2 s.d.); she therefore ordered a thyroid function assessment which showed a low thyroxine (T₄) level (46 ng/l; n > 64) with moderately elevated TSH (10.8 mIU/l; n < 9). The TRH test showed an exaggerated TSH response, increasing from a basal 5 to 55 mIU/l 10 min after stimulation. Thyroid scintigraphy showed normal position of the thyroid gland. The pituitary scan was also normal, so a hypothalamic origin was suspected and the patient was treated with l-T₄ for 2 years. At the age of 7 years, thyroid function tests showed a normal free T₄ level (16 pmol/l; n: 8.5–25), but elevated TSH level (8.9 mIU/l; n: 0.5–5).

At the age of 10 years and 6 months, she was hospitalized following an episode of fainting, and at the time of hospitalization, there were many clinical signs suggestive of AHO such as short stature (133 cm, i.e. −1 s.d.), obesity (44 kg, i.e. +3.5 s.d.), a round face, and brachydactyly involving the fourth and fifth metacarpals of both hands and the fourth and fifth right metatarsals. Laboratory evaluation revealed hypocalcemia at 1.63 mmol/l (6.5 mg/dl) with low ionized calcium levels, high phosphorus levels (2.43 mmol/l, 7.5 mg/dl), magnesium levels in the low normal range (1.7 mg/dl=0.71 mmol/l), hypocaliuria, and hypophosphaturia. 25-OH vitamin D levels were normal (16 µg/dl), and the PTH level was extremely elevated at 458 ng/l (n < 55). An exogenous PTH administration test was then performed that showed neither a rise in calcium levels nor an increase in phosphaturia. The plasma cAMP concentration showed no response to PTH (10 nmol/l before PTH administration, and did not exceed 12 nmol/l after PTH administration). The same applied to urinary cAMP levels, which were at 215 µmol/mol creatinine before PTH administration, 210 µmol/mol creatinine 1 h after PTH administration, and 230 µmol/mol creatinine 4 h after PTH administration. The diagnosis of PHP type I was therefore based on the existence of AHO and PTH resistance. At that time, the TSH was 11.8 mIU/l and showed an exaggerated response to TRH (peak TSH concentrations of 87 mIU/l, and even

**Figure 2** Molecular pathogenesis of PTH resistance and AHO by inactivation and imprinting of the GNAS gene.
after 2 h, the TSH was still elevated at 31 mIU/l, thus also suggesting resistance to TSH. Measurement of Gs\(_{\alpha}\) protein activity was normal (108\%, n: 85–115\%), and PHP type I c was therefore diagnosed. Evaluation of GH secretion revealed a reduced night-time GH profile and a decreased GH response to GHRH. Treatment with GH was therefore initiated.

Genetic study conducted in 1997 revealed a heterozygous nonsense mutation in codon 391 of exon 13 of the GNAS gene (Y391X), replacing the codon (TAC), a tyrosine codon, with a stop codon (TAG), only four amino acids before the wild-type stop codon, thus resulting in a truncated protein. Genetic study of the family showed that neither her parents nor her sister carried this mutation. Genetic analysis also showed that this de novo mutation was on the maternal allele.

Spontaneous menarche occurred at 15 years of age. However, 4 years later, she still had menstrual disorders such as oligomenorrhea and anovulation. A GnRH test was performed, which showed an exaggerated response of LH to GnRH (58 and 55 IU/l 30 and 90 min after GnRH injection respectively), confirming the partial resistance to gonadotropins.

In 2004, she experienced a pulmonary embolism without any predisposing factor except oral contraceptives and obesity. Extensive evaluation for genetic thrombophilia was negative.

Considering the significant obesity in our patient (88 kg for a height of 1.51 m, BMI of 38.6, and a waist circumference of 97 cm) despite her dietetic and physical activity efforts to lose weight, we decided to investigate epinephrine resistance. We therefore performed an epinephrine-induced lipolysis test which confirmed the resistance. Non-esterified fatty acids did not reveal a significant increase in response to epinephrine infusion (0.40 mmol/l before infusion versus 0.62 mmol/l after infusion), nor did glycerol levels (0.22 mmol/l before infusion versus 0.32 mmol/l after infusion). Plasma epinephrine levels and urinary metanephrine levels were normal before the test, while plasma norepinephrine levels were low. The leptin level was high at 43.3 ng/ml (n: 0.3–15), and the adiponectin level was low at 7.3 \(\mu g/ml\) (n: 7.6–26.6). Body composition was also measured using dual energy X-ray absorptiometry, and showed that fat mass was 49% of her body weight. Treatment with 20 mg rimonabant (Acomplia) was initiated in January 2008 because she reached a maximum weight of 92.3 kg (BMI of 40.5; Fig. 3). The results achieved with treatment were impressive as she lost 16 kg in 6 months, thus returning to a weight of 76.3 kg (BMI of 33.5). The adiponectin level increased to 12.2 \(\mu g/ml\) and the leptin level fell to 35.1 ng/ml. However, this treatment unfortunately had to be stopped when the drug was withdrawn from the market in October 2008, and in August 2009, her weight again went up to 87 kg while maintaining her diet and exercise.

Discussion

AHO is a syndrome which includes several developmental and skeletal defects, and many of them were present in our patient. However, there is a wide variability between patients. For example, mental retardation was not present in our patient, whereas it is present in 50–75% of AHO patients. There is one report on 25 patients with PHP type 1, whose intelligence was assessed and Gs\(_{\alpha}\) protein activity was determined. The authors suggested that decreased Gs\(_{\alpha}\) activity and/or reduced cAMP levels are responsible for mental retardation (12). cAMP is known to be important for learning and memory, and mutation of adenyl cyclase or protein kinase A (PKA) produces learning and memory defects in animals, but the exact mechanism of mental retardation in AHO and why it occurs in only a subset of AHO patients remain to be determined. Other factors such as hypothyroidism might contribute to mental retardation in patients with AHO. However, early detection and early institution of therapy do not seem to prevent mental retardation, reinforcing the importance of Gs\(_{\alpha}\) deficiency as a primary abnormality of mental retardation in these patients.

Obesity is one of the cardinal features of AHO, and many mechanisms have been proposed to explain this association. First, \(\beta\)-adrenergic stimulation stimulates lipolysis in adipocytes by activating Gs\(_{\alpha}\) and raising intracellular cAMP levels, resulting in phosphorylation of perilipin by PKA. In the non-phosphorylated state, perilipin is localized at the surface of intracellular lipid droplets, and upon phosphorylation, perilipin moves away from the lipid droplets, allowing hormone-sensitive lipase to have access to the lipid substrate. Studies in patients with PHP type Ia have found decreased circulating free fatty acid (FFA) levels, decreased cAMP response to \(\beta\)-adrenergic stimulation in fat cell membranes (mostly \(\beta3\)-receptor responsiveness) (13), and a
reduced basal and epinephrine-stimulated glycerol production rate (14). Secondly, Gsα deficiency may promote adipocyte differentiation from fibroblasts (15). Finally, some studies have suggested that decreased activity of the sympathetic nerves may have a role in the development of obesity.

We showed in the patient discussed here that there was no lipolytic response to epinephrine in adipose tissue, with blunted FFA release and glycogen production. This is highly significant in relation to her morbid obesity since impaired lipolysis induced by catecholamines (epinephrine) has an important role in the pathogenesis of obesity (16). Basal epinephrine levels were normal, but norepinephrine levels were low, as has been observed already by Carel et al.

Interestingly, a recent study has elucidated an important role of rimonabant in sympathetic nervous system in mice. Besides inducing the expression of β3-receptor gene, rimonabant represses the expression of catechol-O-methyltransferase, a methyltransferase involved in the degradation of catecholamines (17). Taken together, these effects would accentuate the sympathetic tone. This is of special interest in those patients with PHP in whom the sympathetic tone is downregulated.

Furthermore, a role for underactivity of the hypothalamic melanocortin-4 receptor (MCR-4), a receptor bound to Gs, which mediates the central effects of leptin on the inhibition of food intake, has been proposed as a cause of obesity and hyperphagia in patients with PHP type Ia (not in those with PPHP, because of presumed paternal imprinting in the hypothalamus) (18). When it is activated by α-melanocyte-stimulating hormone, MCR-4 inhibits feeding by increasing cAMP, which is known to be a biochemical signal mediating satiety. Reduced MCR-4 signaling could lead to obesity in patients with PHP1a as mentioned before. This could be highly relevant in our patient to explain the good response to cannabinoid receptor type 1 (CB1) antagonist. CB1 is a receptor bound to Gi protein, and upon activation, it inhibits the adenylyl cyclase-mediated conversion of ATP to cAMP, thus decreasing cAMP and increasing appetite. A recent study has shown a synergistic functional interaction between the cannabinoid and melanocortin systems in regulating food intake. It also suggested that the cannabinoid receptors are located downstream from the melanocortin system (19), thus explaining the conserved response to CB1 antagonist in spite of an underactive MCR-4. Our patient lost 16 kg in 6 months, which was remarkable. It is of note that in the Rimonabant in Obesity (RIO) program, four randomized placebo-controlled, double-blind trials (RIO-Europe, RIO-Lipids, RIO-North America, and RIO-Diabetes) investigating the efficacy of rimonabant for the treatment of obesity, body weight reductions of 6.6, 6.9, 6.3, and 5.3 kg respectively were noticed after 1 year (20). Interestingly, in the RIO-Europe study, a similar effect on weight loss was recorded in patients with BMI > 40 compared with the whole study population (21). One putative mechanism to explain the impressive response observed in our patient may be that underactive MCR-4 leads to upregulated CB1 receptors, which respond favorably to treatment with antagonist. Two recent studies have supported this hypothesis: the first showed elevated endocannabinoid levels following prolonged blockade of MCR-4 (22), and the second reported that leptin receptor deficiency led to upregulation of CB1 receptor (23).

Our patient experienced a pulmonary embolism while she was taking oral contraceptives, and it is well known that any oral contraceptive increases the risk of thrombotic events. However, there is one report of a boy with PHP and prothrombotic state. His platelets displayed pronounced Gsα deficiency and were spontaneously hyperreactive, resulting in a prothrombotic state due to extremely low cAMP levels (24). We did not measure Gsα activity in our patient’s platelets to establish whether they were hyperreactive or not.

As has been analyzed and discussed already in another publication (25), the mutation identified in our patient is very interesting. First, because of its location, the carboxyl terminus (especially residues 384–394) is important for interaction with receptors, and mutations of Gsα carboxyl-terminal residues prevent or alter the specificity of G protein-receptor coupling. However, there is no role for the carboxyl terminus in adenylyl cyclase stimulation, which explains the conserved Gsα activity. Secondly, the identification of this mutation in a patient with PHP type Ic is also important, implying that certain patients with PHP type Ic could have a genetic defect within the GNAS locus, and that it may be useful to investigate GNAS mutation in these patients.

In conclusion, this interesting case shows the important role of resistance to epinephrine in the pathogenesis of obesity in PHP I patients (especially types a and c). Moreover, this case also suggests that treatment with CB1 antagonists might be very valuable in view of the abnormalities observed in these patients either in the attenuated sympathetic tone or in food intake regulation (particularly, MCR-4 underactivity).

Declaration of interest
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