

Food-dependent Cushing's syndrome

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ABSTRACT

It has recently been proposed that other hormones than ACTH can control cortisol production in Cushing's syndrome with bilateral adrenal hyperplasia. We present a case of food-dependent Cushing's syndrome. After a positive response of cortisol production during mixed meals, several tests identified glucose-dependent insulinotropic polypeptide (GIP) as the driving hormone responsible for the cortisol overproduction. Identification of aberrant hormone receptor expression is of importance because it may create a possibility for pharmacological treatment.

KEYWORDS

ACTH-independent macronodular adrenal hyperplasia (AIMAH), food-dependent Cushing's syndrome, glucose-dependent insulinotropic polypeptide (GIP)

INTRODUCTION

Cushing's syndrome is usually caused by excessive ACTH secretion, such as in pituitary adenomas (as in Cushing's disease) and ectopic ACTH secretion (as in small cell lung carcinomas and carcinoids); less frequently it is ACTH-independent, frequently caused by cortisol-producing adrenal adenomas or carcinomas. However, ACTH-independent bilateral adrenal hyperplasia has also been described. Adrenocortical cells then have the capacity to secrete cortisol in the absence of detectable ACTH levels. In recent years, the presence of aberrant hormone receptors on adrenal cells of patients with ACTH-independent Cushing's syndrome has been demonstrated.¹ Hormones or peptides other than ACTH control cortisol secretion in these cases, for example glucose-dependent insulinotropic

polypeptide (GIP) via binding to ectopic receptors on adrenal cells.²

We report a new case of bilateral adrenal enlargement based on food-dependent Cushing's syndrome, a less known variant of Cushing's syndrome.

CASE REPORT

A 57-year-old woman was referred to our outpatient clinic because of accidentally discovered bilateral adrenal enlargement. She complained of weight gain, abdominal discomfort, easy bruising, trembling and palpitations. No muscle weakness or psychological problems, such as depression, were reported. Physical examination revealed hypertension (160/100 mmHg), centripetal fat distribution without signs of muscle atrophy and ankle oedema. No moon face or buffalo hump were noticed. The early morning fasting plasma cortisol level could not be suppressed (323 nmol/l; reference value <50 nmol/l) by 1 mg dexamethasone administered the previous evening. The 24-hour urinary free cortisol excretion was also elevated (539 nmol/24 hours; reference values 30 to 150 nmol/24 hours). ACTH levels were below the detection limit (<10 ng/l) at all times. Further investigations showed that the patient's 24-hour urinary free cortisol excretion appeared to be lower (444 nmol/l) during a fasting day as compared with values on non-fasting days (539 nmol/l). Plasma cortisol levels increased after mixed meals and decreased during fasting. Prolonged fasting was associated with a progressive decrease in serum cortisol levels (*figure 1*). Food-dependent Cushing's syndrome was suspected. On intravenous infusion of GIP (0.6 µg/kg, while fasting) the plasma cortisol level more than doubled already 15 minutes after infusion (*figure 2*). The response of cortisol to ACTH administration (250 µg) was similar and

Figure 1. Plasma cortisol levels while fasting (■) and during normal food intake (▼)

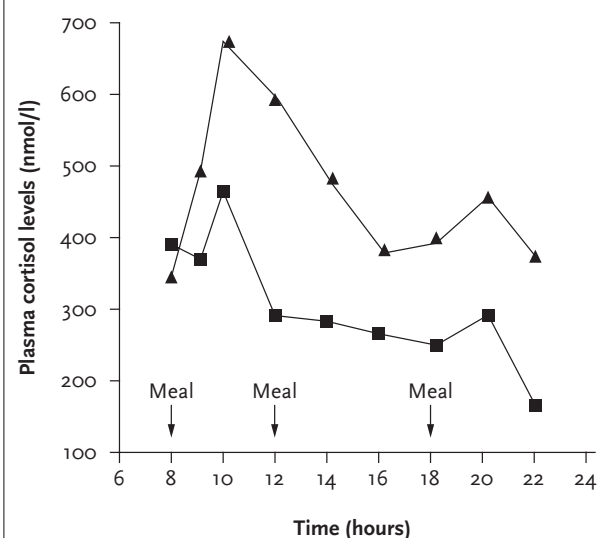


Figure 3. Plasma cortisol levels during food intake (■) and during treatment with octreotide pre-meals (▼)

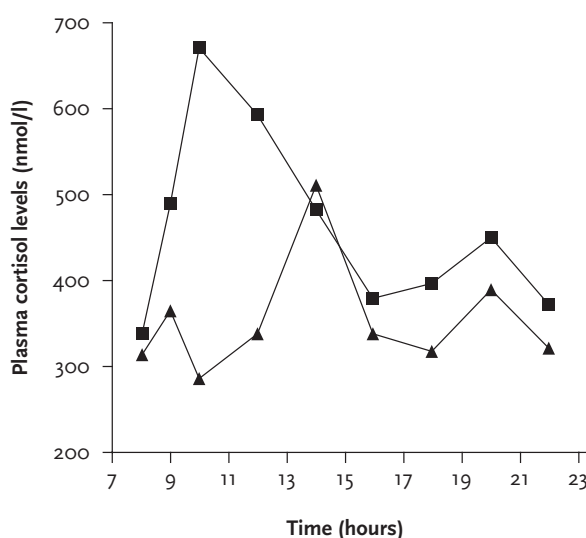
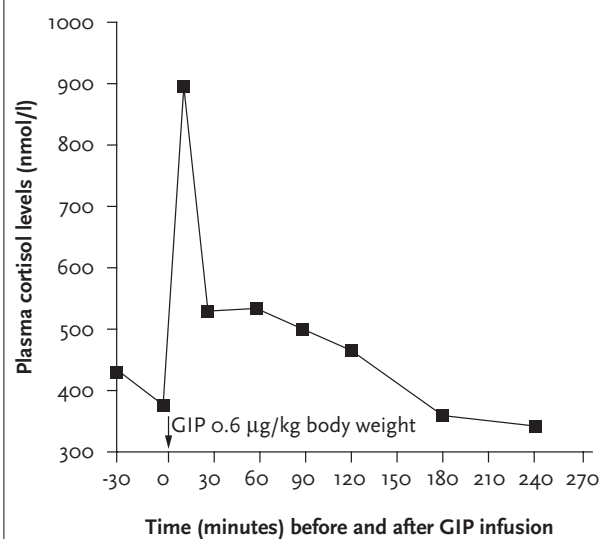


Figure 2. Effect of a single infusion of GIP (0.6 µg/kg body weight) on plasma cortisol levels



served as a reference test (data not shown). This proved food-dependent Cushing's syndrome in this patient.

A posture test was performed to screen for receptors to angiotensin II, vasopressin and catecholamines and the tests were negative.

Octreotide treatment before meals (0.1 mg subcutaneously 3 times/day) reduced plasma cortisol levels (figure 3). Urinary cortisol excretion was 414 nmol/l, lower than the other values (in the fasting or the non-fasting state).

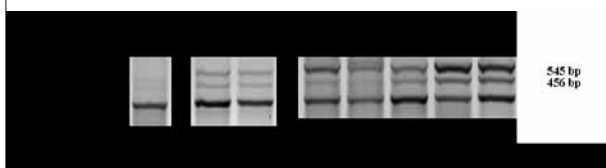
According to our experience and that of others reported in the literature,^{3,4} octreotide therapy only results in short-term suppression of the hypercortisolism. It was, therefore, decided not to treat the patient with octreotide. She subsequently underwent laparoscopic bilateral adrenalectomy. The left adrenal tissue weighed 11 grams and the right adrenal lesion weighed 120 grams. Both lesions were categorised as benign adrenocortical adenomas.

Total RNA was extracted from adrenals using Trizol (Invitrogen). Multiplex RT-PCR using QuantumRNA 18S rRNA as an endogenous control (Spencer & Christensen 1999) was performed according to recommendations by the manufacturer, as follows: the RT-PCR reaction was terminated when all samples were in the linear range of amplification. For each gene, seven RT-PCR reactions with a pool of cDNA from normal adrenals were performed using the specific primers, submitted to PCR and one tube was removed every four cycles, starting from the 12th cycle; then, all the PCR products were resolved in ethidium bromide stained 1.5% agarose gel, detected by Typhoon and quantified by ImageQuant. Cycle number was plotted against the log of the signal and a straight line was obtained for samples in linear range of amplification; the medium point was chosen. Multiplex PCR using two sets of primers, one to amplify the cDNA of interest and a second to amplify an endogenous control (18S rRNA - QuantumRNA 18S Internal Standards, Ambion Inc. Austin-Texas, USA), was performed. The product level of the gene of interest was normalised against the product from the control reaction. The PCR reactions contained 10 mM Tris-HCl (pH 8.3), 1.5 mM

MgCl₂, 50 mM KCl, 0.2 mM each of deoxy-dNTP, 20 pmol each of sense and anti-sense primers specific for the gene of interest, 2 µl of RT reaction, 2.5 U Taq DNA polymerase and 2 µl of 18S PCR Primer Pair.⁵ GIP receptor was found to be over-expressed on the adrenal tumour cells as compared with a normal adrenal or normal human pancreas (figure 4).

She gained weight in the first year postoperatively, and then lost a total of 3 kg. The hypertension resolved. The patient developed type 2 diabetes five years postoperatively, which is now well controlled with oral glucose-lowering drugs.

Figure 4. GIP receptor expression in control pancreas, control adrenal and in 5 cases of GIP-dependent adrenal AIMAH with Cushing's syndrome



Relative quantitative RT-PCR products in agarose gel, showing the two GIPR isoforms (545 and 456 bp) and the internal control r18s (324bp). Sample in lane 3 with the asterisk is the patient reported in this study.

DISCUSSION

In recent years, new aetiologies of ACTH-independent unilateral and bilateral adrenal hyperplasia have been added to the spectrum of ACTH-independent Cushing's syndrome.

It has been established that the aberrant stimulation of steroidogenesis in ACTH-independent macronodular adrenal hyperplasia (AIMAH) and in some unilateral adenomas can be driven by ectopic receptors such as those for glucose-dependent insulintropic peptide or gastric inhibitory polypeptide (GIPR), β-adrenergic receptors, vasopressin (V₂-V₃ vasopressin receptor), serotonin (5-HT₇ receptor) and probably angiotensin II receptor (AT₁R); it can also result from increased expression or altered activity of eutopic receptors such as those for vasopressin (V₁ vasopressin receptor), luteinising hormone/human chorionic gonadotropin (LH/hCGR) and serotonin (5-HT₄ receptor).¹ The molecular mechanisms leading to the abnormal expression of eutopic and ectopic receptors in the adrenal glands of patients with AIMAH and less commonly in adrenal adenomas remains not completely understood.

In our case, the driving force behind the increased adrenal cortisol production appeared to be meals, i.e. a peptide

or hormone released after meals, instead of ACTH. All healthy persons produce GIP from endocrine K cells in the small intestine (duodenum and proximal jejunum) after oral food intake.⁶ This release increases with higher fat and carbohydrate intake and decreases with protein intake. GIP has a positive effect on the release and synthesis of insulin, thus its name: glucose-dependent insulintropic polypeptide.

In patients with food-dependent Cushing's syndrome, GIP is released in physiological concentrations in the small intestine; however, because of the binding to the ectopic or overexpressed adrenal GIP receptors this results in a postprandial increase in plasma cortisol. Cortisol exerts its negative feedback on ACTH and CRH synthesis and leads to suppressed levels of plasma ACTH and thereby to low fasting cortisol levels. In some patients with GIP-dependent Cushing's syndrome, plasma cortisol levels are not as low, possibly as the result of the expression of more than one aberrant receptor. In this case report it was remarkable that in the fasting state cortisol levels also increased around the meal times (morning and evening). This could be a result of the presence of other aberrant receptors, but this has not been tested. In addition, no cortisol increase was found after lunch during feeding and fasting; this could be due to a bigger contribution of physiological morning and evening increases in cortisol than the result of the influence of GIP. The investigators did not screen for all possible receptors such as the glucagon receptor and the serotonin (5-HT₄) receptor; a posture test was performed to screen for receptors to angiotensin II, vasopressin and catecholamines; these tests were negative. The presence of other aberrant receptors could, however, have influenced the cortisol pattern in this patient.

Until recently GIP receptors (GIPR) had not been found on either normal human adrenal cells² or on adrenal cells of a non-food-dependent Cushing's syndrome patient.⁷ Baldacchino *et al.* found GIPR to be expressed in a large number of human adult and foetal tissues. Data obtained by gene array and semi-quantitative RT-PCR showed an increase in the expression of several genes implicated in GIPR expression in the adrenal adenomas or bilateral macronodular hyperplasia of patients with GIP-dependent Cushing's syndrome. They were, however, also increased in some patients with non-GIP-dependent cortisol-secreting adenomas or with ACTH-dependent Cushing's disease.⁷ Further studies are necessary to clarify the molecular mechanisms responsible for the over expression of GIPR in zona fasciculata cells.

Octreotide administration before each meal prevented meal-induced increase of plasma cortisol and GIP levels.³

However, pretreatment with octreotide only decreases the cortisol response to meals for a few weeks or months, in most other cases.^{4,8} This is possibly due to downregulation of somatostatin receptors on GIP-secreting intestinal cells. Adrenalectomy seems to be the best treatment for GIP-dependent Cushing's syndrome. As an alternative to surgery, new pharmacological therapy will require development of molecules which can block the GIP receptor efficiently.

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