Hereditary glucocorticoid resistance

S.W.J. Lamberts
Department of Medicine, University Hospital Dijkzigt, Rotterdam, The Netherlands.

There exists variability in the sensitivity to glucocorticoids within the normal population. These differences in glucocorticoid sensitivity are probably relatively minor, as they become only apparent when supraphysiological doses of glucocorticoids are administered as a therapy for non-endocrinological diseases. An extreme example of decreased sensitivity to glucocorticoids is hereditary (generalized) cortisol resistance (CR) in which the relative insensitivity of target tissues to the effects of glucocorticoids leads to clinical signs and symptoms. CR was first described by Vingerhoeds et al. [15] who reported a patient with hypercortisolism without signs and symptoms of Cushing’s disease.

The secretion of cortisol by the adrenal gland is regulated by the components of the hypothalamic-pituitary-adrenal (HPA) axis. Of principal importance in this system is the ability of glucocorticoids to exert a negative feedback action at both the hypothalamic and the pituitary level, in order to keep a perfect balance between cortisol requirement and cortisol secretion. In cases of generalized CR, patients present with increased cortisol concentrations compared with the normal population [1, 8, 9]. Keeping the structure of the HPA axis in mind, it is clear that in patients with generalized CR, the negative glucocorticoid feedback on both corticotrophin-releasing hormone (CRH) and adrenocorticotropic (ACTH) secretion is reduced as...
a consequence of diminished sensitivity to glucocorticoid [1, 8, 9]. As a result, the HPA axis is set at a higher level. CRH and ACTH secretion increase, resulting in higher serum cortisol concentrations. In this way, the body tries to achieve a balance between cortisol requirement and cortisol secretion. The increased cortisol concentrations appear to compensate adequately for the reduced sensitivity. However, patients suffering from CR do not show signs or symptoms of cortisol excess, because they simply need these increased serum cortisol concentrations. The sensitivity to glucocorticoids is diminished at the hypothalamic and the pituitary level, but also at the peripheral target tissue level. The clinical symptoms seen in patients with CR are therefore not due to glucocorticoid excess, but secondary to the activation of the HPA axis, which results in an increased production of ACTH, resulting in the stimulation of mineralocorticoid and androgen secretion. So in case of CR, increased ACTH secretion leads to a secondary adrenal overproduction of hormones with mineralocorticoid activity, such as deoxycorticosterone, and with androgen activity such as androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulphate.

CR is a rare disease, which has been described in only about 30 patients and (a)symptomatic family members since the first description in 1976 by Vingerhoeds et al. [15]. The clinical presentation of the patients is variable. Generally, symptoms of glucocorticoid deficiency were not seen in the patients, indicating that they all could compensate adequately for their glucocorticoid insensitivity by activation of the HPA axis. Moreover, most of the “patients” evaluated in the context of family studies (i.e. affected family members) were asymptomatic despite increased peripheral glucocorticoid levels. As a result of the increased secretion of steroids with mineralocorticoid activity, patients may present with hypertension or even hypokalaemic alkalosis [7, 9, 15]. In women with CR, the secondary overproduction of adrenal androgens was reported to result in acne, hirsutism, male pattern baldness (geheimratsecken), and manifestations affecting the reproductive system such as oligomenorrhoea and infertility [8, 9]. Malchoff et al. [11] described a seriously affected boy, who presented with precocious puberty resulting from adrenal androgen overproduction.

Biochemically, the disease is characterized by increased concentrations of plasma cortisol and increased 24 h urinary free cortisol excretion, a normal circadian pattern of ACTH and cortisol secretion and resistance to adrenal suppression by dexamethasone (DEX), without signs or symptoms of Cushing’s syndrome. It is known that in cases of Cushing’s syndrome, resistance to adrenal suppression by DEX exists as well. Therefore, clinical evaluation for signs and symptoms of Cushing’s syndrome is of crucial diagnostic importance once hypercortisolism has been biochemically established. Nevertheless, in most patients with hypercortisolism due to Cushing’s syndrome, the circadian pattern of cortisol secretion is lost, which therefore is another important differential diagnostic parameter. Because of effects of glucocorticoids are exerted by the GR, the next step in the diagnostic evaluation of patients with hypercortisolism without Cushing’s syndrome is the evaluation of the GR in these patients. Ligand binding capacity and affinity are important determinants of glucocorticoid sensitivity. GR characteristics can be adequately determined in whole mononuclear leucocytes [9]. Furthermore, in vitro determining the glucocorticoid induced inhibition of [3H]thymidine incorporation in activated mononuclear leucocytes give an impression of glucocorticoid sensitivity [9].

As early as 1980, it was suggested that CR could be the result of a defect in the intracellular cascade of events from the entrance of glucocorticoids into the cell to their final effect on cellular function. Even before the structure of the GR was known, studies performed in patients with CR showed alterations in receptor number of ligand binding affinity of the GR. Following cloning of the GR gene and subsequent studies of the genomic structure, PCR amplification and sequencing of genomic DNA have been possible. This strategy has revealed point mutations and/or microdeletions in the GR gene in patients with familial CR [4, 6, 7, 11]. Circulating peripheral mononuclear leucocytes from affected members from a family had only 50 % of the normal number of receptors [9]. The proband of these kindred, a 26-year-old woman, presented with hirsutism, male pattern baldness and menstrual irregularities. She had no hypertension and normal serum potassium concentrations. It appeared that the patient had marked elevated levels of adrenal androgens, explaining the clinical symptoms. Furthermore, she had greatly elevated plasma cortisol levels, which were insufficiently suppressed in a 1 mg overnight DEX suppression test. The father and two of the brothers of the patient had elevated basal plasma cortisol levels, which were insufficiently suppressed by 1 mg overnight DEX suppression, but they were clinically unaffected with normal blood pressure and normal serum potassium levels. In contrast to the proband of this family, the father and affected brothers had normal androgen concentration. This was to be expected because of the much higher gonadal androgen production in males compared with females. Therefore, the overproduction of adrenal androgens gives rise to signs of hyperandrogenism in females only, and is mostly asymptomatic in males [9]. Karl et al. [6] identified a 4 base pair deletion at the 3’ boundary of exon and intron 6. Mutations or deletions involving the first two nucleotides of
an intron disrupt normal splicing generating aberrant mRNA variants. These variants are probably more susceptible to nuclease digestion, which precludes the production of mature mRNA, and may therefore exclude the expression of the encoded protein. This could explain why the receptor number on peripheral mononuclear leukocytes was reduced by 50 % in the affected members of this family. Vingerhoeds et al. [15] described CR for the first time in a father (and an asymptomatic son) who presented with severe hypertension and hypokalaemia. In the biochemical analysis, it appeared that the ligand affinity of GR in peripheral mononuclear leukocytes of this patient was decreased by a factor of three. Molecular analysis revealed a homozygous (father) and heterozygous (son) aspartic acid to valine change at codon 641 of the GR [4]. An amino acid change such as this in the hormone binding domain of the GR might explain the lowered ligand affinity of the receptor in these patients [2]. Malchoff et al. [10] described a young boy who presented with isosexual precocity, caused by increased adrenal androgen production associated with CR. Biochemical analysis showed a two-fold higher dissociation constant for the GR in peripheral mononuclear leukocytes of the boy. Molecular analysis of the GR gene revealed a homozygous valine to isoleucine change at codon 729 of the GR protein [11]. Recently the mutation in the GR gene of a young male presenting with hypertension, hypokalaemia and infertility has been elucidated. Analysis of the GR gene of this patient disclosed a novel point mutation (T to A) in exon 5 at cDNA position 1808 [7]. This base change predicted the substitution of the neutral and hydrophobic amino acid isoleucine by the neutral and polar asparagine at codon 559 in the proximal region of the hormone binding domain. By sequence the cDNA transcripts of the patient’s lymphoblasts and fibroblasts, the expression of both wild type and mutant receptor in the patient’s tissues was confirmed. It became apparent that this mutant receptor had a dominant negative effect on the wild-type receptors.

Recently four new molecularly characterized cases of GC were described: one female with hyperandrogenism with an mutation in codon 747 (Ile-747 Met) [16], one female with hypokalemia and virilization with a Val 571 Ala mutation [12], as well as two female patients with hyperandrogenism with a Arg 477. His mutation (the first with a mutation in the DNA-binding domain), and one with a fly 679 Ser mutation [14]. The molecular basis of the only patient ever described with cortisol hypersensitivity has not yet been elucidated [5].

Recently, we described 5 patients with biochemical and clinical CR [3]. All patients showed a diurnal rhythm of serum cortisol concentrations, albeit at a high level, an insufficient suppression of serum cortisol concentration in reaction to 1 mg of dexamethasone (DEX), variable degrees of androgen overproduction, in the absence of clinical signs and symptoms of Cushing’s syndrome. Three of the four female patients presented with complaints of androgen overproduction, two of them in combination with fatigue. The other female patient had severe steroid resistant asthma. The only male patient and his son were asymptomatic. In 4 patients, we investigated receptor protein characteristics on mononuclear leukocytes in a whole cell DEX binding assay, and studied the ability of DEX to inhibit mitogen induced cell proliferation in mononuclear leukocytes in vitro. In all patients investigated, we found alterations in receptor number of ligand affinity and/or the ability of DEX to inhibit mitogen induced cell proliferation. In order to investigate the molecular defects leading to the clinical and biochemical pictures in these patients, we screened the GR gene using PCR/SSCP/sequency analysis. No GR gene alterations were found in these patients. In conclusion, the five patients described had clinical and biochemical evidence of CR but no abnormalities were demonstrated in the GR gene. Probably, as yet undefined alterations somewhere in the cascade of events starting with ligand binding to the GR protein, and finally resulting in the regulation of the expression of GC responsive genes, or post-receptor defects or interactions with other nuclear factors from the pathophysiological basis of CR in these patients [3].

Finally, New et al. [13] described a 14-yr-old native American girl from the Iroquois Nation was referred as a potential patient with the syndrome of apparent mineralocorticoid excess. Instead, her evaluation revealed resistance to glucocorticoids, mineralocorticoids, and androgens, but no resistance to vitamin D or thyroid hormones. She lacked Cushingoid features despite significantly high cortisol levels. Menstruation was regular, and there was no clinical evidence of masculinization despite high serum androgen levels in the male range. The patient’s sister had similar clinical features. Partial resistance to exogenous glucocorticoid and mineralocorticoid administration was well demonstrated in both patients. It was proposed that these patients represent the first cases of partial resistance to multiple steroids, possibly due to a coactivator defect [3].

**CONCLUSIONS**

In cases of the extremes of the spectrum of glucocorticoid sensitivity, these differences in sensitivity may lead to clinical syndromes such as cortisol resistance or the cortisol hyperactivity syndrome. Ever since the first description 1976 [15], only about 30 patients and (asymptomatic) family members with CR have been described.
To date, the molecular basis for CR has been described in eight patients and affected family members. In seven cases, mutations in the hormone binding domain of the GR gene were responsible for the clinical manifestation of CR, while in one individual a mutation in the DNA-binding domain was observed. Reports of a significant prevalence of possible abnormalities in the GR in patients attending the endocrine clinic for hypokalaemia, hypertension, acne, hirsutism and menstrual disorders prompted many investigators to carry out a thorough investigation with respect to GR protein and GR gene in such patients [3,17].

REFERENCES