EUROSTERONE MEETING

Glucocorticoid and mineralocorticoid resistance/hypersensitivity syndromes

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Abstract

Glucocorticoids and mineralocorticoids regulate diverse functions important to maintain central nervous system, cardiovascular, metabolic, and immune homeostasis. The actions of these hormones are mediated by their specific intracellular receptors: the glucocorticoid (GR) and mineralocorticoid (MR) receptors. Pathologic conditions associated with changes of tissue sensitivity to these hormones have been described. The syndrome of familial glucocorticoid resistance is characterized by hypercortisolism without Cushing’s syndrome stigmata. The molecular defects of four kindreds and one sporadic case have been elucidated as inactivating mutations in the ligand-binding domain of GR. Two cases developed glucocorticoid resistance at the heterozygous state. In these patients, mutant receptors possessed transdominant negative activity upon the wild type receptor. Insensitivity to mineralocorticoids (which may also be caused by loss of function mutations of the MR gene) was found in one sporadic case and four autosomal dominant cases of Pseudohypoaldosteronism type 1. These included two frameshift mutations and a premature termination codon in exon 2, leading to gene products lacking the entire DNA- and ligand-binding domains, and a single base-pair deletion in the intron-5 splice donor site. Tissue hypersensitivity to glucocorticoids was recently hypothesized in patients with Human Immunodeficiency Virus (HIV) type-1 infection via the accessory proteins Vpr and Tat which enhance GR transactivation. Since HIV-1 long terminal repeat (LTR) and glucocorticoid-responsive promoters use the same set of coactivators, these proteins may stimulate HIV-1-LTR and glucocorticoid-inducible genes concurrently. The former may directly stimulate viral proliferation, while the latter may indirectly enhance viral propagation by suppressing the host immune system through glucocorticoid-mediated mechanisms.


Introduction

Two adrenal corticosteroids, the glucocorticoid cortisol and the mineralocorticoid aldosterone, exert profound influences on many physiologic functions by virtue of their diverse roles in growth, development and maintenance of homeostasis (Munck et al. 1984, Clark et al. 1992). Their actions are mediated by intracellular receptor proteins, the glucocorticoid (GR) and mineralocorticoid (MR) receptors, which function as hormone-activated transcription factors, that regulate the expression of, respectively, the glucocorticoid and mineralocorticoid target genes (Beato & Sanchez-Pacheco 1996).

The GR is ubiquitously expressed in almost all human tissues and organs. The presence of glucocorticoids is crucial for the integrity of central nervous system (CNS) function and for maintenance of cardiovascular, metabolic and immune homeostasis (Chrousos 1995). Increased glucocorticoid secretion during stress alters CNS function, assists with adjustments in energy expenditures and modulates the inflammatory/immune response (Chrousos 1995). Since glucocorticoids possess a broad array of life-sustaining functions, only partial or incomplete glucocorticoid resistance has been reported so far, suggesting that complete inability of glucocorticoids to exert their effects on their target tissues are incompatible with human life (Chrousos et al. 1993). Over ten kindreds and individual patients suffering from congenital glucocorticoid resistance have been described to date and the molecular mechanisms of their resistance have been analyzed in some of them (Arai & Chrousos 1995, Chrousos 1995, de Castro & Chrousos 1997).
In addition to the generalized glucocorticoid resistance described above, tissue-specific reduction in sensitivity to glucocorticoids may be found in several pathologic conditions (Chrousos et al. 1993, Chrousos 1995, Leung et al. 1997). Thus, rheumatoid arthritis, systemic lupus erythematosus and a subtype of bronchial asthma have been associated with tissue-specific reduction of sensitivity to glucocorticoids, while patients with acquired immunodeficiency syndrome (AIDS) possibly develop glucocorticoid hypersensitivity (Kino et al. 1999).

The MR mediates the sodium-retaining effects of aldosterone in the kidney, salivary glands, sweat glands and colon (Jorgensen 1986). In addition, the MR located in the CNS – also called GR type I – appears to have a role in the regulation of the stress response and the feedback control of the hypothalamic–pituitary–adrenal (HPA) axis (de Kloet et al. 1990). Recently, inactivating mutations in the MR were shown to cause pseudohypoaldosteronism type 1 (PHA1), i.e. mineralocorticoid resistance (Geller et al. 1998). This disease, however, is overwhelmingly due to loss-of-function mutations in the subunits of the amiloride-sensitive sodium channel (ASSC), which represent a post MR step in the signaling cascade of aldosterone in its target tissues (Chang et al. 1996, Strautnieks et al. 1996).

Structure and actions of the GR and MR

The GR and the MR are members of the steroid/sterol/thyroid/retinoid/orphan receptor superfamily of nuclear transactivating factors, with over 150 members currently cloned and characterized across species (Mangelsdorf et al. 1995). Together with the progesterone and androgen receptors, GR and MR form the steroid receptor subfamily. Steroid receptors display a modular structure comprised of five to six regions (A–F), with the N-terminal A/B region harboring an autonomous activation function (activation function 1), and the C and E regions corresponding to the DNA- and ligand-binding domains (Fig. 1). The GR and MR consist of 777 and 984 amino acids respectively, and have almost identical DNA binding domains (94%) and very similar ligand-binding domains (57%), but divergent N-terminal A/B regions (<15%) (Arai & Chrousos 1995). The GR and MR in their unliganded state are located primarily in the cytoplasm, as part of hetero-oligomeric complexes containing heat shock proteins 90, 70 and 50, and, possibly, other proteins (Beato & Sanchez-Pacheco 1996).

After binding to their respective ligand, the GR and MR undergo conformational changes, dissociate from the heat shock proteins, homodimerize and translocate into the

Figure 1 Location of the known mutations of the (A) GR and (B) MR in their genomic structures.
nucleus, where they interact with hormone-responsive elements and/or other transcription factors in the promoter regions of target genes (Bamberger et al. 1995). Both the GR and MR bind to and modulate transcription driven by the glucocorticoid-responsive element (GRE)-containing murine mammary tumor virus (MMTV) promoter. No specific mineralocorticoid-responsive elements have been characterized in the regulatory regions of genes physiologically regulated by aldosterone as yet (Rupprecht et al. 1993). Active endogenous GREs, on the other hand, are present in the promoter regions of many glucocorticoid-responsive genes. The GR as a dimer/monomer also modulates the transcription rates of non-GRE-containing genes regulated by other transcription factors, such as AP-1 (Schule et al. 1993). Protein–protein interactions with these factors.

The human GR cDNA was isolated by expression cloning in 1985 (Hollenberg et al. 1985). The cDNA for the human MR was subsequently isolated by low-stringency hybridization, using the human GR cDNA as a probe in 1987 (Arriza et al. 1987). The genes of the MR and GR consist of 9 exons each; their loci are on chromosomes 4 and 5 respectively (Fig. 1). For the GR, there are two 3' splicing variants, GRα and β, from alternative use of a different terminal exon 9α or 9β. GRα is the classic GR which binds with glucocorticoids and transactivates or transrepresses glucocorticoid-responsive promoters. On the other hand, GRβ does not bind glucocorticoids and functions as a weak dominant negative inhibitor of GRα on GRE-containing, glucocorticoid-responsive promoters (Bamberger et al. 1995). For the MR, alternative promoters of the MR gene have been reported regulating production of the same final receptor protein; the functional significance of this is not clear (Zennaro et al. 1995).

MR has a high affinity for both aldosterone and cortisol, and the circulating levels of cortisol are over 100 times higher than those of aldosterone. The MR of the distal convoluted tubule and possibly other mineralocorticoid target tissues are protected from the actions of cortisol by expression of 11β-hydroxysteroid dehydrogenase type 2, which converts cortisol into the inactive cortisone (Funder 1997).

Hormone resistance caused by mutations in the GR and MR genes

GR mutations

The syndrome of familiar glucocorticoid resistance, first described in 1976, is a disorder characterized by hypercortisolism without Cushingoid features (Vingerhoeds et al. 1976, Chrousos et al. 1982). Since then, over ten kindreds and sporadic cases have been reported (Iida et al. 1985, Lamberts et al. 1986, 1992, Nawata et al. 1987, Vecsei et al. 1989, Hurley et al. 1991, Karl et al. 1993, 1996, Malchoff et al. 1993). Abnormalities of the GR number, affinity for glucocorticoids, stability and translocation into the nucleus have been described. The molecular defects of four kindreds and one sporadic case have been elucidated so far (Fig. 1 and Table 1). The proposition of the original kindred was found by Hurley et al. (1991) to be a homozygote for a single non-conservative point mutation, replacing aspartic acid with valine at amino acid

Table 1 Mutations of the GR causing glucocorticoid resistance

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Mutation position</th>
<th>Localization</th>
<th>Biochemical phenotype</th>
<th>Genotype/transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hurley et al.</td>
<td>1991</td>
<td>2054 (A→T)</td>
<td>E</td>
<td>Affinity ↓</td>
<td>Homozygote/autosomal recessive</td>
</tr>
<tr>
<td>Karl et al.</td>
<td>1993</td>
<td>Δ4 at the 3' boundary of exon and intron 6</td>
<td>E</td>
<td>Number ↓ Inactivation of the affected allele</td>
<td>Heterozygote/autosomal dominant</td>
</tr>
<tr>
<td>Malchoff et al.</td>
<td>1993</td>
<td>2317 (G→A)</td>
<td>E</td>
<td>Affinity ↓ Transactivation ↓</td>
<td>Heterozygote/autosomal dominant</td>
</tr>
<tr>
<td>Karl et al.</td>
<td>1996</td>
<td>1808 (T→A)</td>
<td>D</td>
<td>Number ↓ Transactivation ↓ Dominant negative activity on wild-type</td>
<td>Heterozygote/sporadic</td>
</tr>
<tr>
<td>Vottero et al.</td>
<td>1999</td>
<td>2373 (T→G)</td>
<td>E</td>
<td>Affinity↓ Transactivation↓ Dominant negative activity on wild-type</td>
<td>Heterozygote/autosomal dominant</td>
</tr>
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Localization represents location of mutations in modular structure of the receptor (see Fig. 1).
641 in the hormone-binding domain of the GR; this mutation reduced binding affinity for dexamethasone by threefold and caused loss of transactivation activity on the MMTV promoter. The proposita of the second family had 4-base deletion at the 3'-boundary of exon 6, removing a donor splice site. This resulted in complete ablation of one of the GR alleles in affected members of the family (Karl et al. 1993). The propositus of the third kindred had a single homozygotic point mutation at amino acid 529 (valine to isoleucine) in the hormone-binding domain, which reduced both the affinity and transactivation activity of the GR (Malchoff et al. 1993). There was also an interesting sporadic case of a man with a de novo, germ-line, heterozygotic GR mutation at amino acid 559 (isoleucin to asparagine) in the hormone-binding domain, at the hinge region, proximal to the DNA-binding domain. This mutant GR bound no ligand but exerted dominant negative activity upon the wild-type receptor and was associated with later development of Cushing's disease due to an adrenocorticotropic (ACTH)-secreting pituitary adenoma (Karl et al. 1996). Recently, study of a fifth case/kindred with glucocorticoid resistance and a heterozygotic GR mutation in the ligand-binding domain (amino acid 747, replacing isoleucine with methionine) was determined; the mutant receptor had reduced affinity for dexamethasone and decreased transactivation activity; interestingly, it also had dominant negative activity upon the wild-type receptor (A Vottero, H Combe, T Kino, P Lecomte & GP Chrousos, unpublished data).

A complex negative feedback system that regulates glucocorticoid homeostasis exists in the human CNS. Glucocorticoids exert negative feedback effects on both hypothalamic corticotropic-releasing hormone (CRH) and arginine vasopressin (AVP) secretion and inhibit pituitary ACTH secretion itself. In addition, glucocorticoids influence the activity of suprarehthalamic centers that control the activity of CRH and AVP neurons (Chrousos 1995). This complex regulatory system is activated in patients with loss-of-function GR mutations, resulting in compensatory increases in ACTH and cortisol secretion (Fig. 2). The patients retain the circadian rhythm and responsiveness of cortisol to stress and are resistant to single or multiple doses of dexamethasone. Although adequate compensation is apparently achieved by elevated cortisol concentrations in the great majority of the patients described, excess ACTH secretion also results in increased production of adrenal steroids with mineralocorticoid activity and enhanced secretion of adrenal androgens. The former, together with cortisol, is responsible for causing symptoms and signs of mineralocorticoid excess, such as hypertension and/or hypokalemic alkalosis, whereas the latter cause varying manifestations of hyperandrogenism, such as acne, hirsutism, male pattern baldness, menstrual irregularities and infertility in women. Precocious puberty has been seen in a child due to early and excessive prepubertal adrenal androgen secretion. In the male, oligospermia and infertility have been observed, possibly as a result of disturbances in follicle-stimulating hormone regulation caused by excessive adrenal androgens. However, the spectrum of clinical manifestations in patients with GR mutations is quite broad, as a large number of subjects are asymptomatic and show only biochemical changes.

Patients are treated with high doses of mineralocorticoid-sparing synthetic glucocorticoids. The goal is to suppress the increased levels of ACTH, which cause overproduction of mineralocorticoids and androgens (Fig. 2). As all cases described thus far have had partial inactivation of GR activity, synthetic potent glucocorticoids with minimal intrinsic mineralocorticoid activity, such as dexamethasone, is a rational approach. These steroids achieve activation of the mutated GR in homozygous cases or of the wild-type receptor in heterozygous cases that is sufficient to suppress the compensatory increases of ACTH, and hence the adrenal mineralocorticoids and androgens causing the clinical manifestations of the condition. The patients should be treated with high, individualized doses of oral dexamethasone, which would be pharmacologic for the normal population (1–3 mg/day). Dexamethasone indeed suppresses ACTH and therefore endogenous cortisol, deoxycorticosterone (DOC), corticosterone and adrenal androgen secretion, correcting the mineralocorticoid and androgen excess states of these patients.

**MR mutations**

The mechanism by which aldosterone stimulates sodium transport in its target tissues may involve the synthesis of a protein associated with the function of the ASSC. The latter is located in the apical membrane of epithelial cells of the renal distal convoluted tubule, and in the plasma membranes of cells in other tissues involved with salt conservation (Canessa et al. 1993, 1994). The phenotype of patients with loss-of-function mutations of the MR mimics that of patients with defects in the subunits of the ASSC (Geller et al. 1998), who, however, represent the bulk of patients with PHA1.

Cheek & Perry (1958) first reported PHA1 in an infant with severe salt-wasting syndrome; PHA1 was subsequently reported in more than 70 patients (Speiser et al. 1986). This syndrome usually presents in infancy with urinary salt wasting and failure to thrive. The levels of plasma renin activity and aldosterone concentrations are markedly elevated. Approximately one fifth of these cases are familial (Arai & Chrousos 1995). All patients have renal tubular unresponsiveness to aldosterone, whereas some have multiple mineralocorticoid target tissue involvement, including the sweat and salivary glands and the colonic epithelium (Oberfield et al. 1977, Savage et al. 1982, Armanini et al. 1985, Caufriez et al. 1986).
In kindreds with PHA1, both an autosomal dominant and recessive form of genetic transmission have been observed. The autosomal recessive form was associated with severe disease, with manifestations persisting into adulthood. We and others have failed to find pathologic mutations in the MR gene in our sporadic and familial cases with autosomal recessive PHA1, and concluded that, most likely, this condition was due to a defect in a post-MR step of aldosterone action (Zennaro et al. 1994, Arai et al. 1995). Indeed, in 1996, PHA1 was found to be caused by loss-of-function mutations in genes encoding subunits of the ASSC (Chang et al. 1996, Strautnieks et al. 1996). However, recently, Geller et al. (1998) identified heterozygotic MR gene loss-of-function mutations in one sporadic case and four autosomal dominant cases of PHA1 (Fig. 1B and Table 2). These included two frameshift mutations, each deleting a single base pair in exon 2; the resultant frame shifts led into a gene product lacking the entire DNA- and hormone-binding domains, as well as a dimerization motif. Two families had an identical mutation, introducing a premature termination codon in exon 2 at position 537. One case showed a single base pair deletion in the intron-5 splice donor site. We recently suggested that double heterozygocity between MR and ASSC subunit gene mutations might also result in PHA1 (Arai et al. 1999).

Patients with MR mutations present with dehydration, low to normal levels of serum sodium, hyperkalemia, acidosis, hyperreninemia, and ‘paradoxically’ elevated plasma and urinary aldosterone levels, especially in infancy. A mild presentation and improvement with age are usually seen in patients with MR mutations when compared with patients carrying mutations in the ASSC subunits, who also present with a similar early phenotype.

Figure 2 Pathophysiologic mechanism of glucocorticoid resistance induced by loss-of-function GR mutations. The elaborate negative feedback mechanism responsible for maintenance of glucocorticoid homeostasis compensates for tissue insensitivity to glucocorticoids by resetting the HPA axis at a higher level. Thus, CRH/AVP, ACTH and cortisol secretion are increased. The compensatory increase in ACTH production augments the secretion of cortisol and glucocorticoid precursors with mineralocorticoid activity (B, cortisosterone), as well as the secretion of several adrenal androgens, including Δ4-androstenedione, which has considerable androgen activity.
but have a more severe presentation and course (Geller et al. 1998). The improvement observed in PHA1 of either etiology with age is consistent with reduced dependence of the patients on aldosterone action as they grow older (Rosler 1984). Patients with PHA1 require supplemental sodium chloride, which usually corrects their hyponatremia and hyperkalemia, improves their symptoms and enhances their growth. After infancy, the disorder typically abates enough to permit reduction or discontinuation of sodium chloride supplements, but the condition may recur during periods of dietary salt restriction (Funder et al. 1990). Treatment with high doses of the synthetic mineralocorticoid fludrocortisone and/or the 11β-hydroxysteroid dehydrogenase type 2 inhibitor carbenoxolone was tried with normalization of a PHA1 patient’s serum electrolyte concentrations, a better growth and an improved sense of well-being (Arai & Chrousos 1995). These therapeutic methods could also be tried in patients with PHA1 carrying mutations of the MR that only partially affect its ability to function.

### Possible glucocorticoid hypersensitivity in AIDS

**Human immunodeficiency virus-1 (HIV-1) infection as a glucocorticoid hypersensitive state**

AIDS patients have several manifestations compatible with tissue hypersensitivity to glucocorticoids. First of all, they develop reduction of innate and T helper 1-directed cellular immunity. Thus, secretion of interleukin (IL)-2, IL-12 and interferon-γ, which direct cellular immunity, are suppressed in AIDS patients, while the secretion of IL-4 is increased (Norbiato et al. 1997). These changes can be induced by exogenously administered glucocorticoids and are seen in hypercortisolemic patients with endogenous Cushing’s syndrome. Secondly, hippocampal atrophy seen in hypercortisolemic states has also been reported in pediatric AIDS patients (Oberfield et al. 1994). Thirdly, AIDS patients frequently present with muscle wasting and myopathy as well as dyslipidemia and visceral obesity-related insulin resistance, also seen in hypercortisolemic states (Simpson & Bender 1988, Hadigan et al. 1999, Kotler et al. 1999, Yanovski et al. 1999, Dube 2000). Therefore, it is possible that some unknown factor(s) might modulate tissue sensitivity to glucocorticoids in AIDS patients.

The apparent glucocorticoid hypersensitivity of certain tissues in AIDS patients appears to be restricted to the immune system, the brain, the musculoskeletal system and the fat and liver, while the HPA axis remains intact, suggesting that appropriate negative feedback sensitivity to glucocorticoids is preserved (Yanovski et al. 1999). To explore the possibility of glucocorticoid hypersensitivity in AIDS, we recently focused on one of the HIV-1 accessory proteins, 96 amino acid virion-associated protein (Vpr) with multiple functions (Emerman 1996, Pavlakis 1996). Vpr is known to enhance the replication of HIV-1 virus in lymphocyte- and monocyte-derived cell lines (Ayyavoo et al. 1997). It also behaves as a transcriptional activator of several viral promoters and as an enhancer of HIV-1 long terminal repeat promoter activated by Tat (Sawaya et al. 2000). Vpr also causes host cell arrest in the G2/M phase of the cell cycle, and induces terminal differentiation in some cell lines (Levy et al. 1993, He et al. 1995). Furthermore, Vpr increases the translocation of the HIV-1 pre-integration complex into the nucleus, and promotes efficient infection of non-dividing macrophages (Vodicka et al. 1998, Foucquier & Malim 1999).

Since Vpr circulates at detectable levels in HIV-1-infected individuals and is able to penetrate the cell membrane, its effects may be extended to cells not infected by HIV-1 (Levy et al. 1994, Henklein et al. 2000). We found that this small HIV-1 protein increases tissue sensitivity to glucocorticoids by functioning as a coactivator of the GR on its responsive promoters (Kino et al. 1999). Indeed, Vpr contains a nuclear receptor signature...
motif LXXLL at amino acids 64–68. This motif is used by host nuclear receptor coactivators to bind nuclear receptors (McKenna et al. 1999). Similarly, through this motif, Vpr directly binds to the GR and co-operatively enhances GR activity on its responsive promoters along with host nuclear receptor coactivators SRC1a and p300/CBP (Kino et al. 1999). Vpr directly binds to p300 at its C terminal, at amino acids 2045–2191, functioning as a potentiator of the nuclear receptor coactivator system (T Kino, A Gragerov, JB Kopp, GN Pavlakis & GP Chrousos, unpublished data). In Fig. 3, we present the interactions of Vpr with p300 and propose a simplified model of its action on a glucocorticoid-responsive promoter.

We also found that Tat, another key HIV-1 accessory protein, moderately potentiates GR activity, possibly by helping the accumulation of the pTEFβ complex on glucocorticoid-responsive promoters (T Kino, A Gragerov, JB Kopp, GN Pavlakis & GP Chrousos, unpublished data). Since Tat also readily penetrates cell membranes (Fawell et al. 1994), it is possible that, similarly to Vpr, it modulates tissue sensitivity to glucocorticoids irrespective of infection of the cells by HIV-1 (Fig. 3).

Therefore, through its encoding proteins, Vpr and Tat, HIV-1 may facilitate the transcription of genes encoding its own proteins by directly stimulating viral proliferation. On the other hand, by inducing hypersensitivity to glucocorticoids, these proteins contribute to the proliferation of the virus indirectly by suppressing the host immune system. Extensive further clinical and basic investigations are crucial to examine the clinical importance of glucocorticoid hypersensitivity and to develop novel effective therapeutic approaches in AIDS.

References


Beato M & Sanchez-Pacheco A 1996 Interaction of steroid hormone receptors with the transcription initiation complex. Endocrine Reviews 17 587–609.


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