EUROSTERONE MEETING

Glucocorticoid and mineralocorticoid resistance/hypersensitivity syndromes

T Kino and G P Chrousos

Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA

(Requests for offprints should be addressed to G P Chrousos, Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bldg 10, Rm 9D42, 10 Center Drive MSC 1583, Bethesda, Maryland 20892–1583, USA; Email: chrousog@mail.nih.gov)

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.

Journal of Endocrinology (2001) **169**, 437–445

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).

A **B**

Figure 1 Location of the known mutations of the (A) GR and (B) MR in their genomic structures.

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 the 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

Table 1 Mutations of the GR causing glucocorticoid resistance

Author	Year	Mutation position		Localization	Biochemical phenotype	Genotype/transmission
		cDNA	Amino acid			
Hurley <i>et al.</i>	1991	2054 (A→T)	641 (D→V)	E	Affinity ↓ Transactivation ↓	Homozygote/autosomal recessive
Karl <i>et al.</i>	1993	Δ4 at the 3' boundary of exon and intron 6		E	Number ↓ Inactivation of the affected allele	Heterozygote/autosomal dominant
Malchoff <i>et al.</i>	1993	2317 (G→A)	729 (V→I)	E	Affinity ↓ Transactivation ↓	Homozygote/autosomal recessive
Karl <i>et al.</i>	1996	1808 (T→A)	559 (I→N)	D	Number ↓ Transactivation ↓ Dominant negative activity on wild-type	Heterozygote/sporadic
Vottero <i>et al.</i>	1999	2373 (T→G)	747 (I→M)	E	Affinity ↓ Transactivation ↓ Dominant negative activity on wild-type	Heterozygote/autosomal dominant

Localization represents location of mutations in modular structure of the receptor (see Fig. 1).

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.* 1990), nuclear factor-κB (Caldenhoven *et al.* 1995) and STAT5 (Stocklin *et al.* 1996), through 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 proband 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

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 proband of the third kindred had a single homozygotic point mutation at amino acid 729 (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 (isoleucine 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 adrenocorticotropin (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 transactivational 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 corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) secretion and inhibit pituitary ACTH secretion itself. In addition, glucocorticoids influence the activity of suprahypothalamic 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).

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 $\Delta 4$ -androstenedione, which has considerable androgen activity.

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 heterozygosity 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

Table 2 Mutations of the MR causing mineralocorticoid resistance*

	Mutation position		Localization	Genotype/transmission	Clinical phenotype
	cDNA	Amino acid			
Case 1	ΔG1226	409 Frameshift	A/B	Heterozygote/sporadic	PHA1
Kindred 2	ΔT 1597	533 Frameshift	A/B	Heterozygote/autosomal dominant	PHA1
Kindred 3	1831 (C→T)	537 (R→Stop)	A/B	Heterozygote/autosomal dominant	PHA1
Kindred 4	ΔA at intron5 splice donor site		E	Heterozygote/autosomal dominant	PHA1

Localization represents location of mutations in modular structure of the receptor (see Fig. 1).
*Reproduced from Geller *et al.* (1998).

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, Fouchier & 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

Figure 3 (A) Linearized Vpr and p300 molecules and their mutual interaction domains. (B) A simplified model of Vpr (VPR) and Tat actions on glucocorticoid-responsive genes. P/CAF, p300/CBP-associated factor; NR, nuclear receptor; GRE, glucocorticoid-responsive element; RNPII, RNA polymerase II; CDK, cyclin-dependent kinase.

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 pTEF β 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

- Arai K & Chrousos GP 1995 Syndromes of glucocorticoid and mineralocorticoid resistance. *Steroids* **60** 173–179.
- Arai K, Tsigos C, Suzuki Y, Listwak S, Zachman K, Zangeneh F, Rapaport R, Chanoine JP & Chrousos GP 1995 No apparent mineralocorticoid receptor defect in a series of sporadic cases of pseudohypoaldosteronism. *Journal of Clinical Endocrinology and Metabolism* **80** 814–817.
- Arai K, Zachman K, Shibasaki T & Chrousos GP 1999 Polymorphisms of amiloride-sensitive sodium channel subunits in five sporadic cases of pseudohypoaldosteronism: do they have pathologic potential? *Journal of Clinical Endocrinology and Metabolism* **84** 2434–2437.
- Armanini D, Kuhnle U, Strasser T, Dorr H, Butenandt I, Weber PC, Stockigt JR, Pearce P & Funder JW 1985 Aldosterone-receptor deficiency in pseudohypoaldosteronism. *New England Journal of Medicine* **313** 1178–1181.
- Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE & Evans RM 1987 Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* **237** 268–275.
- Ayyavoo V, Mahalingam S, Rafali Y, Kudchodkar S, Chang D, Nagashunmugam T, Williams WV & Weiner DB 1997 HIV-1

- viral protein R (Vpr) regulates viral replication and cellular proliferation in T cells and monocytoid cells *in vitro*. *Journal of Leukocyte Biology* **62** 93–99.
- Bamberger CM, Bamberger AM, de Castro M & Chrousos GP 1995 Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. *Journal of Clinical Investigation* **95** 2435–2441.
- Bamberger CM, Schulte HM & Chrousos GP 1986 Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocrine Reviews* **17** 245–261.
- Beato M & Sanchez-Pacheco A 1996 Interaction of steroid hormone receptors with the transcription initiation complex. *Endocrine Reviews* **17** 587–609.
- Caldenhoven E, Liden J, Wissink S, Van de Stolpe A, Raaijmakers J, Koenderman L, Okret S, Gustafsson JA & Van der Saag PT 1995 Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the antiinflammatory action of glucocorticoids. *Molecular Endocrinology* **9** 401–412.
- Canessa CM, Horisberger JD & Rossier BC 1993 Epithelial sodium channel related to proteins involved in neurodegeneration. *Nature* **361** 467–470.
- Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD & Rossier BC 1994 Amiloride-sensitive epithelial Na⁺ channel is made of three homologous subunits. *Nature* **367** 463–467.
- de Castro M & Chrousos GP 1997 Glucocorticoid resistance. *Current Therapy in Endocrinology and Metabolism* **6** 188–189.
- Caufriez A, Golstein J, Tadjerouni A, Bosson D, Cantraine F, Robyn C & Copinschi G 1986 Modulation of immunoreactive somatomedin-C levels by sex steroids. *Acta Endocrinologia* **112** 284–289.
- Chang SS, Grunder S, Hanukoglu A, Rosler A, Mathew PM, Hanukoglu I, Schild L, Lu Y, Shimkets RA, Nelson-Williams C, Rossier BC & Lifton RP 1996 Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nature Genetics* **12** 248–253.
- Cheek DB & Perry JW 1958 A salt wasting syndrome in infancy. *Archives of Diseases in Childhood* **22** 252–256.
- Chrousos GP 1995 The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. *New England Journal of Medicine* **332** 1351–1362.
- Chrousos GP, Vingerhoeds A, Brandon D, Eil C, Pugeat M, DeVroede M, Loriaux DL & Lipsett MB 1982 Primary cortisol resistance in man. A glucocorticoid receptor-mediated disease. *Journal of Clinical Investigation* **69** 1261–1269.
- Chrousos GP, Detera-Wadleigh SD & Karl M 1993 Syndromes of glucocorticoid resistance. *Annals of Internal Medicine* **119** 1113–1124.
- Clark JK, Schrader WT & O'Malley BW 1992 Mechanism of steroid hormones. In *Williams Textbook of Endocrinology*, pp 35–90. Eds JD Wilson & DW Foster. Philadelphia: WB Saunders Co.
- Dube MP 2000 Disorders of glucose metabolism in patients infected with human immunodeficiency virus. *Clinical Infectious Diseases* **31** 1467–1475.
- Emerman M 1996 HIV-1, Vpr and the cell cycle. *Current Biology* **6** 1096–1103.
- Fawell S, Seery J, Daikh Y, Moore C, Chen LL, Pepinsky B & Barsoum J 1994 Tat-mediated delivery of heterologous proteins into cells. *PNAS* **91** 664–668.
- Fouchier RA & Malim MH 1999 Nuclear import of human immunodeficiency virus type-1 preintegration complexes. *Advances in Virus Research* **52** 275–299.
- Funder JW 1997 Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. *Annual Review of Medicine* **48** 231–240.
- Funder JW, Pearce PT, Myles K & Roy LP 1990 Apparent mineralocorticoid excess, pseudohypoaldosteronism, and urinary electrolyte excretion: toward a redefinition of mineralocorticoid action. *FASEB Journal* **4** 3234–3238.
- Geller DS, Rodriguez-Soriano J, Vallo Boado A, Schifter S, Bayer M, Chang SS & Lifton RP 1998 Mutations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypoaldosteronism type I. *Nature Genetics* **19** 279–281.
- Hadigan C, Miller K, Corcoran C, Anderson E, Baszog N & Grinspoon S 1999 Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women. *Journal of Clinical Endocrinology and Metabolism* **84** 1932–1937.
- He J, Choe S, Walker R, Di Marzio P, Morgan DO & Landau NR 1995 Human immunodeficiency virus type 1 viral protein R (Vpr) arrests cells in the G2 phase of the cell cycle by inhibiting p34 cdc2 activity. *Journal of Virology* **69** 6705–6711.
- Henklein P, Bruns K, Sherman MP, Tessmer U, Licha K, Kopp J, de Noronha CM, Greene WC, Wray V & Schubert U 2000 Functional and structural characterization of synthetic HIV-1 vpr that transduces cells, localizes to the nucleus, and induces G2 cell cycle arrest. *Journal of Biological Chemistry* **275** 32016–32026.
- Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG & Evans RM 1985 Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* **318** 635–641.
- Hurley DM, Accili D, Stratakis CA, Karl M, Vamvakopoulos N, Rorer E, Constantine K, Taylor SI & Chrousos GP 1991 Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. *Journal of Clinical Investigation* **87** 680–686.
- Iida S, Gomi M, Moriwaki K, Itoh Y, Hirobe K, Matsuzawa Y, Katagiri S, Yonezawa T & Tarui S 1985 Primary cortisol resistance accompanied by a reduction in glucocorticoid receptors in two members of the same family. *Journal of Clinical Endocrinology and Metabolism* **60** 967–971.
- Jorgensen PL 1986 Structure, function and regulation of Na,K-ATPase in the kidney. *Kidney International* **29** 10–20.
- Karl M, Lamberts SW, Detera-Wadleigh SD, Encio IJ, Stratakis CA, Hurley DM, Accili D & Chrousos GP 1993 Familial glucocorticoid resistance caused by a splice site deletion in the human glucocorticoid receptor gene. *Journal of Clinical Endocrinology and Metabolism* **76** 683–689.
- Karl M, Lamberts SW, Koper JW, Katz DA, Huizenga NE, Kino T, Haddad BR, Hughes MR & Chrousos GP 1996 Cushing's disease preceded by generalized glucocorticoid resistance: clinical consequences of a novel, dominant-negative glucocorticoid receptor mutation. *Proceedings of the Association of American Physicians* **108** 296–307.
- Kino T, Gragerov A, Kopp JB, Stauber RH, Pavlakis GN & Chrousos GP 1999 The HIV-1 virion-associated protein vpr is a coactivator of the human glucocorticoid receptor. *Journal of Experimental Medicine* **189** 51–62.
- de Kloet ER, Reul JM & Sutanto W 1990 Corticosteroids and the brain *Journal of Steroid Biochemistry and Molecular Biology* **37** 387–394.
- Kotler DP, Rosenbaum K, Wang J & Pierson RN 1999 Studies of body composition and fat distribution in HIV-infected and control subjects. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* **20** 228–237.
- Lamberts SW, Poldermans D, Zweens M & de Jong FH 1986 Familial cortisol resistance: differential diagnostic and therapeutic aspects. *Journal of Clinical Endocrinology and Metabolism* **63** 1328–1333.
- Lamberts SW, Koper JW, Biemond P, den Holder FH & de Jong FH 1992 Cortisol receptor resistance: the variability of its clinical presentation and response to treatment. *Journal of Clinical Endocrinology and Metabolism* **74** 313–321.
- Leung DY, Hamid Q, Vottero A, Szefer SJ, Surs W, Minshall E, Chrousos GP & Klemm DJ 1997 Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. *Journal of Experimental Medicine* **186** 1567–1574.

- Levy DN, Fernandes LS, Williams WV & Weiner DB 1993 Induction of cell differentiation by human immunodeficiency virus 1 vpr. *Cell* **72** 541–550.
- Levy DN, Refaeli Y, MacGregor RR & Weiner DB 1994 Serum Vpr regulates productive infection and latency of human immunodeficiency virus type 1. *PNAS* **91** 10873–10877.
- McKenna NJ, Xu J, Nawaz Z, Tsai SY, Tsai MJ & O'Malley BW 1999 Nuclear receptor coactivators: multiple enzymes, multiple complexes, multiple functions. *Journal of Steroid Biochemistry and Molecular Biology* **69** 3–12.
- Malchoff DM, Brufsky A, Reardon G, McDermott P, Javier EC, Bergh CH, Rowe D & Malchoff CD 1993 A mutation of the glucocorticoid receptor in primary cortisol resistance. *Journal of Clinical Investigation* **91** 1918–1925.
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P *et al.* 1995 The nuclear receptor superfamily: the second decade. *Cell* **83** 835–839.
- Munck A, Guyre PM & Holbrook NJ 1984 Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Reviews* **5** 25–44.
- Nawata H, Sekiya K, Higuchi K, Kato K & Ibayashi H 1987 Decreased deoxyribonucleic acid binding of glucocorticoid-receptor complex in cultured skin fibroblasts from a patient with the glucocorticoid resistance syndrome. *Journal of Clinical Endocrinology and Metabolism* **65** 219–226.
- Norbiato G, Bevilacqua M, Vago T, Taddei A & Clerici 1997 Glucocorticoids and the immune function in the human immunodeficiency virus infection: a study in hypercortisolemic and cortisol-resistant patients. *Journal of Clinical Endocrinology and Metabolism* **82** 3260–3263.
- Oberfield SE, Levine LS, Carey RM, Bejar R & New MI 1977 Pseudohypoaldosteronism: multiple target organ unresponsiveness to mineralocorticoid hormones. *Journal of Clinical Endocrinology and Metabolism* **48** 228–234.
- Oberfield SE, Cowan L, Levine LS, George A, David R, Litt A, Rojas V & Kairam R 1994 Altered cortisol response and hippocampal atrophy in pediatric HIV disease. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* **7** 57–62.
- Pavakis GN 1996 The molecular biology of HIV-1. In *AIDS: Diagnosis, Treatment and Prevention*, pp 45–74. Eds VT DeVita, S Hellman & SA Rosenberg. Philadelphia: Lippincott Raven.
- Rosler A 1984 The natural history of salt-wasting disorders of adrenal and renal origin. *Journal of Clinical Endocrinology and Metabolism* **59** 689–700.
- Rupprecht R, Arriza JL, Spengler D, Reul JM, Evans RM, Holsboer F & Damm K 1993 Transactivation and synergistic properties of the mineralocorticoid receptor: relationship to the glucocorticoid receptor. *Molecular Endocrinology* **7** 597–603.
- Savage MO, Jefferson IG, Dillon MJ, Milla PJ, Honour JW & Grant DB 1982 Pseudohypoaldosteronism: severe salt wasting in infancy caused by generalized mineralocorticoid unresponsiveness. *Journal of Pediatrics* **101** 239–242.
- Sawaya BE, Khalili K, Gordon J, Taube R & Amini S 2000 Cooperative interaction between HIV-1 regulatory proteins tat and vpr modulates transcription of the viral genome. *Journal of Biological Chemistry* **275** 35209–35214.
- Schule R, Rangarajan P, Klierer S, Ransone LJ, Bolado J, Yang N, Verma IM & Evans RM 1990 Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* **62** 1217–1226.
- Simpson DM & Bender AN 1988 Human immunodeficiency virus-associated myopathy: analysis of 11 patients. *Annals of Neurology* **24** 79–84.
- Speiser PW, Stoner E & New MI 1986 Pseudohypoaldosteronism: a review and report of two new cases. *Advances in Experimental Medicine and Biology* **196** 173–195.
- Stocklin E, Wissler M, Gouilleux F & Groner B 1996 Functional interactions between Stat5 and the glucocorticoid receptor. *Nature* **383** 726–728.
- Strautnieks SS, Thompson RJ, Gardiner RM & Chung E 1996 A novel splice-site mutation in the gamma subunit of the epithelial sodium channel gene in three pseudohypoaldosteronism type 1 families. *Nature Genetics* **13** 248–250.
- Vecsei P, Frank K, Haack D, Heinze V, Ho AD, Honour JW, Lewicka S, Schoosch M & Ziegler R 1989 Primary glucocorticoid receptor defect with likely familial involvement. *Cancer Research* **49** 2220s–2221s.
- Vingerhoeds ACM, Thijssen JHH & Schwartz F 1976 Spontaneous hypercortisolism without Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* **43** 1128–1133.
- Vodicka MA, Koeppe DM, Silver PA & Emerman M 1998 HIV-1 Vpr interacts with the nuclear transport pathway to promote macrophage infection. *Genes and Development* **12** 175–185.
- Vottero A, Combe H, Kino T, Lecomte P & Chrousos GP 1999 *Proceedings of the 81st Meeting of the Endocrine Society*, San Diego, CA, USA.
- Yanovski JA, Miller KD, Kino T, Friedman TC, Chrousos GP, Tsigos C & Falloon J 1999 Endocrine and metabolic evaluation of human immunodeficiency virus-infected patients with evidence of protease inhibitor-associated lipodystrophy. *Journal of Clinical Endocrinology and Metabolism* **84** 1925–1931.
- Zennaro MC, Borensztein P, Jeunemaitre X, Armanini D & Soubrier F 1994 No alteration in the primary structure of the mineralocorticoid receptor in a family with pseudohypoaldosteronism. *Journal of Clinical Endocrinology and Metabolism* **79** 32–38.
- Zennaro MC, Keightley MC, Kotelevtsev Y, Conway GS, Soubrier F & Fuller PJ 1995 Human mineralocorticoid receptor genomic structure and identification of expressed isoforms. *Journal of Biological Chemistry* **270** 21016–21020.

Received 22 December 2000

Accepted 6 March 2001