Glucocorticoid and mineralocorticoid receptors and associated diseases

Tomoshige Kino¹ and George P. Chrousos

Pediatric and Reproductive Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-1583, U.S.A.

Abstract

Adrenal corticosteroids, ie. glucocorticoids and mineralocorticoids, play important physiological roles in humans. Their actions are mediated by intracellular receptor molecules, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), which function as hormone-dependent transcription factors. Ligand-activated receptors modulate the transcription rates of responsive genes by interacting with responsive elements in the promoters of these genes or by influencing the activities of other transcription factors, via protein–protein interactions. Natural inactivating mutations of the GR or MR genes have been reported in humans with significant clinical phenotypes. The former causes sporadic or familial glucocorticoid resistance characterized by generalized partial insensitivity of tissues to glucocorticoids and subsequent activation of the hypothalamic/pituitary/adrenal axis with resultant hyperandrogenism in children and women and/or mineralocorticoid excess symptoms in both sexes. The latter develop pseudohypoaldosteronism type 1, i.e. hypotension and hyperkalaemic acidosis, as a result of reduced aldosterone actions in the kidney. An activating mutation in the MR gene causing early-onset, periodic hypertension was reported recently. The biological relevance of the GR and MR receptors was also addressed in mice

¹To whom correspondence should be addressed (e-mail kinot@mail.nih.gov).
whose GR or MR genes were inactivated or modified by gene targeting. The results were generally confirmatory of the concepts obtained by the human studies. Similarly, natural, compensated glucocorticoid and/or mineralocorticoid ‘resistance’ were described in several mammalian species, including non-human primates and rodents. Here we discuss the actions of GR and MR and the molecular defects of naturally occurring mutations in these receptors with associated pathophysiological changes.

**Introduction**

Two adrenal corticosteroids, the glucocorticoid cortisol and the mineralocorticoid aldosterone, exert profound influences on many physiological functions by virtue of their diverse roles in growth, development and maintenance of cardiovascular, metabolic and immune homoeostasis. Their actions are mediated by intracellular receptor proteins, the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), which function as hormone-activated transcription factors that regulate the expression of glucocorticoid and mineralocorticoid target genes, respectively [1].

The GR is 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 homoeostasis. Increased glucocorticoid secretion during stress alters CNS function, assists with adjustments in energy expenditures and modulates the inflammatory/immune response. Since glucocorticoids possess a broad array of life-sustaining functions, only partial or incomplete glucocorticoid resistance — a state demonstrating reduced sensitivity/responsiveness to glucocorticoids — has been reported so far, suggesting that the complete inability of glucocorticoids to exert their effects on their target tissues is incompatible with human life. 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 analysed in some of them [2].

The MR mediates the sodium-retaining effects of aldosterone in the kidney, salivary glands, sweat glands and colon. In addition, the MR located in the CNS – also called corticosteroid type I receptor – appears to have a role in the regulation of the stress response and the feedback control of the hypothalamic/pituitary/adrenal axis (HPA axis) [2]. 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 MRs of the kidney 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. Recently, inactivating mutations in the MR were shown to cause pseudohypoaldosteronism type 1 (PHA1), i.e. mineralocorticoid resistance [2a]. This disease, however, is
mostly due to loss-of-function mutations in the subunits of the amiloride-sensitive sodium channel (ASSC), which represent a post-MR step in the signalling cascade of aldosterone in its target tissues.

**Structure and actions of GRs and MRs**

The GR and MR are members of the steroid/sterol/thyroid/retinoid/orphan receptor superfamily of nuclear transcription factors, with over 150 members currently cloned and characterized across species. Together with the progesterone, oestrogen and androgen receptors, GR and MR form the steroid receptor subfamily. Steroid receptors display a modular structure comprised of five or six regions (A–F), with the N-terminal A/B region harbouring an autonomous activation function (activation function 1) that catalyses transcriptional activity of the receptor by contacting and accumulating cofactors and basal transcriptional components, and the C and E regions corresponding to the DNA-binding domain (DBD) and ligand-binding domain (LBD) [2]. The human GR cDNA was isolated by expression cloning in 1985 [2b]. The genes of the GR consist of nine exons; its locus is on chromosome 5 (Figure 1A, a). It encodes two 3' splice variants, GRα and GRβ, produced by alternative use of different terminal exons, 9α and 9β. The GRα encodes a 777-amino-acid protein, while the GRβ contains 742 amino acids. The first 727 amino acids from the N-terminus are identical in both isoforms. GRα possesses an additional 50 amino acids, while the GRβ encodes an additional 15 non-homologous amino acids in their C-terminus. GRα is the classic GR that binds to glucocorticoids and transactivates or transrepresses glucocorticoid-responsive promoters. On the other hand, human GRβ does not bind glucocorticoids and its physiological and pathological roles are not well known (Figure 1B) [1,2]. The cDNA for the human MR was isolated by low-stringency hybridization, using the human GR cDNA as a probe, in 1987 [2c]. The genes of the MR also consist of nine exons and its locus is on chromosome 4 (Figure 1A, b). Alternative 5' promoters of the MR gene have been reported to regulate production of the same final receptor protein; the functional significance of this is not clear.

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. After binding to its agonist ligand through their C-terminal LBD, the receptors undergo conformational changes, dissociate from the heat-shock proteins, dimerize and translocate into the nucleus through the nuclear pore via an active process. There, the ligand-activated GR and MR through their DBDs directly interact with DNA sequences in the promoter regions of target genes called glucocorticoid response elements (GREs). Both the GR and MR bind to and modulate transcription driven, for example, by the GRE-containing murine mammary tumour virus promoter [1]. Active endogenous GREs are present in the
Figure 1. Genomic, complementary DNAs and protein structures of the human GR and MR.

(A) The human GR gene consists of ten exons. Exon 1 is an untranslated region, exon 2 codes for the immunogenic domain (A/B), exons 3 and 4 for the DBD (C), and exons 5–9 for the hinge region (D) and the LBD (E). The GR gene contains two terminal exons 9 (exons 9α and 9β), alternatively spliced to produce the classic GRα and the non-ligand-binding GRβ.

(B) Functional domains of the human GR. The C-terminal grey-coloured domains in GRα and GRβ show the portions that are specific to each splice variant. HR, hinge region; NL 1 and 2, nuclear-localization signals 1 and 2; AF-1 and -2, activation functions 1 and 2; ATG, start codon; TGA and TAA, stop codons.

© 2004 The Biochemical Society
promoter regions of many glucocorticoid-responsive genes, whereas no specific mineralocorticoid-response elements have been characterized in the regulatory regions of genes physiologically regulated by aldosterone as yet. The GR as a dimer/monomer also modulates the transcription rates of non-GRE-containing genes regulated by other transcription factors, such as activator protein 1, nuclear factor κB and signal transducer and activator of transcription 5 (STAT5), through protein–protein interactions with these factors.

The promoter-bound GR and MR stimulate the transcription rates of responsive genes by facilitating the formation of the transcription-initiation complex, including the RNA polymerase II and its ancillary factors. In addition to these molecules, GR and MR, via their two transactivation domains attract several proteins and protein complexes, so-called co-activators, that help transmit the glucocorticoid complex signal to the transcription-initiation complex as well as contain intrinsic histone acetyltransferase activity, through which they loosen the chromatin structure and facilitate access and/or binding of transcription machinery components to DNA [3]. They include the homologous p300 and cAMP-response element-binding protein (CREB)-binding protein (CBP), the p160 steroid receptor co-activators and the p300/CBP-associated factor (pCAF). The p300/CBP co-activators may serve as macromolecular docking 'platforms' for transcription factors from several signal transduction cascades, including, in addition to nuclear receptors, CREB, activator protein 1, nuclear factor κB, p53, Ras-dependent growth factor and STATs. pCAF, originally reported as a human homologue of yeast Gcn5 that interacts with p300/CBP, is also a broad co-activator with histone acetyltransferase activity. Steroid receptors preferentially interact with the p160 family of co-activators: steroid receptor co-activator-1 (SRC-1), transcription intermediate factor-II (TIF-II) or GR-interacting protein-1 (GRIP-1), also called SRC-2, the p300/CBP/co-integrator-associated protein (p/CIP), activator of thyroid receptor (ACTR) or receptor-associated co-activator-3 (RAC3), also called SRC-3.

The p300/CBP and p160 family co-activators contain one or more copies of the co-activator signature motif sequence Leu-Xaa-Xaa-Leu-Leu (LXXLL), also called nuclear-receptor-binding box (NRB), through which they directly bind the GR and MR [3]. It appears that p160 co-activators are first attracted to the DNA-bound steroid receptors, where they help accumulate p300/CBP and pCAF to the promoter region through their mutual interactions, indicating that p160 proteins play a central role in the transactivation by steroid receptors.

In addition to the co-activator molecules, there are several proteins whose function is to retain the steroid receptors in the repressed, inactive state. These molecules, called co-repressors, may have deacetylase activity themselves or may attract other molecules with deacetylase activity, through which they tighten chromatin structure and prevent transcription machinery components from binding to DNA [3]. Co-repressors also employ a sequence close to the LXXLL motif to bind to nuclear receptors. The distinction between co-activators and co-repressors is not absolute, as there are now several examples of co-
activators acting as co-repressors, and vice versa, depending on cell or tissue type, or state of cell activation [4].

The recent development of GR- and MR-knockout mice has provided new insights into the biological activities of these receptors. Mice harbouring complete inactivation of the GR gene died at birth from severe respiratory distress syndrome due to a deficit of lung surfactant [5]. In these mice, transcription of genes encoding gluconeogenic enzymes in the liver was decreased, proliferation of erythroid progenitors was impaired and the HPA axis was strongly up-regulated, indicating that GR plays an important role in the regulation of these activities. In contrast with GR-knockout mice, GR-knockin mice, with a mutated GR defective in GRE-mediated transactivation but intact in protein–protein interaction with other transcription factors, survived and procreated [5]. These animals demonstrated defects in the induction of gluconeogenic enzymes and proliferation of erythroid progenitors, while most of the immune-suppressive effects of glucocorticoids were preserved. Regarding their HPA axis, suppression of corticotropin-releasing hormone (CRH) synthesis by glucocorticoids was maintained, whereas pro-opiomelanocortin expression was up-regulated.

By using the Cre-LoxP-mediated recombination reaction, mice harbouring a brain-specific GR-knockout were also developed [6]. These mice demonstrated severe impairment of their HPA axis, resulting in increased corticosterone levels due to a loss of its negative-feedback action on the secretion of CRH and corticotropin (adrenocorticotrophic hormone, ‘ACTH’). These animals developed symptoms that mimicked the phenotype of the glucocorticoid-excess (Cushing) syndrome. In addition, they had impaired behavioural responses to external stressors and displayed reduced anxiety, indicating that the brain GR plays roles in emotional behaviour and cognitive functions of the brain.

MR-deficient mice died in the second week after birth, because of high renal salt wasting and hyponatraemia, hyperkalaemia and acidosis. They had very high plasma renin activity and increased plasma aldosterone levels. This phenotype of the MR-knockout mice is similar to that of PHA1 syndrome in humans [7]. The activity of the ASSC, whose defect is known to be a major cause of human PHA1, was almost undetectable in their kidneys, while the mRNA levels of the three ASSC subunits were preserved, indicating that MR may affect translational or post-translational steps in the expression of functional ASSC proteins.

The MR functions as a GR in the brain, since it is exposed to relatively high concentrations of glucocorticoids due to no expression of 11β-hydroxysteroid dehydrogenase type 2, which in the kidney converts active cortisol/corticoesterone into inactive cortisone. MR-knockout mice, whose electrolyte deficits were corrected by exogenous NaCl administration, demonstrated a decreased number of hippocampal granular cells and decreased neurogenesis, while brain-specific GR-knockout mice had a normal expression of hippocampal granular
cells and neurogenesis [8]. These MR-mediated brain functions may contribute to the development of pathological changes in the hippocampus that appear with normal aging, in subjects exposed to chronic stress, and in patients with affective disorders.

**Natural physiological steroid hormone ‘resistance’ in animals**

**New World monkeys**

Some animal species demonstrate generalized (whole-body) insensitivity to several steroid hormones. New World monkeys (infraorder Platyrhini, superfamily Ceboidea), such as the owl (*Aotus*), titi (*Callicebus*) and squirrel (*Saimiri*) monkeys, have elevated levels of cortisol, which compensate for the low binding affinity of their GRs to glucocorticoids [9,10]. These animals also demonstrate increased levels of other steroid hormones, such as progesterone, oestrogen, testosterone and aldosterone, indicating that they have pan-steroid resistance, possibly via a common mechanism shared by these affected receptors [10]. Since squirrel monkey GR in cultured cell lines has only a mildly decreased affinity to glucocorticoids *in vitro* and since the cytoplasmic fraction from squirrel monkey cells reduces ligand-binding activity of human GR, it has been suggested that some unknown cytosolic factor(s) might regulate ligand-binding potency of GR in affected New World monkeys.

Recently, FK506-binding protein (FKBP) 51, an immunophilin that inhibits the association of GR with heat-shock protein 90 and thus reduces the affinity of GR to ligands, was postulated to cause glucocorticoid insensitivity in New World monkeys [11]. Indeed, immunophilin FKBP51 is expressed at large amounts in all affected New World monkeys tested and administration of FK506, which binds and activates FKBP51, reverses the glucocorticoid-insensitivity state of squirrel monkey cells *in vitro*.

**Guinea pigs**

Guinea pigs also demonstrate generalized glucocorticoid ‘resistance’ associated with high levels of glucocorticoids [12]. Similarly to New World monkeys, their GR shows low affinity to ligands, but the cause of such a change may be due to the sequence alteration of the receptor itself. Indeed, the LBD of guinea pig GR has five amino acid substitutions, which are preserved in many other species. The alteration of these amino acids reduces the affinity of the guinea pig GR to glucocorticoids, possibly by inducing a conformational change in the ligand-binding pocket.

**Prairie voles**

Prairie voles (*Microtus ochrogaster*) also demonstrate extremely high plasma glucocorticoid concentrations in the absence of any apparent negative causes of glucocorticoid excess [13]. They have a significantly higher adrenal/body-
weight ratio, 5–10-fold greater basal plasma corticosterone and 2–3-fold greater basal plasma corticotropin concentrations than montane voles (*Microtus montanus*) and rats. Their plasma corticosterone levels are responsive to both stress and circadian cues but are resistant to the administration of the synthetic glucocorticoid, dexamethasone. In agreement with these physiological results, GR in their brain and liver shows significantly lower affinity to glucocorticoids compared with that in the same tissues of rats. These pieces of evidence indicate that resistance to glucocorticoid hormones in peripheral tissues, possibly due to lower affinity of their GR to glucocorticoids, may account for their high glucocorticoid levels.

### Pathological changes of GR and MR activities in humans

Humans also develop generalized insensitivity/hypersensitivity to single or several steroid hormones that are caused by mutations in steroid hormone receptors. Below, we explain the genetic and biological changes seen in patients with GR or MR mutations.

#### GR mutations

Familial/sporadic glucocorticoid resistance syndrome was first described in 1976, as a disorder characterized by hypercorticosolism without Cushingoid features. Since then, over ten kindreds and sporadic cases with abnormalities of the number of binding sites for glucocorticoids, affinity for glucocorticoids, stability and translocation into the nucleus have been reported [2,14]. However, to date, the molecular defects have been elucidated in five kindreds and three sporadic cases (Figure 2, Table 1). The index propositus, a patient through whom the particular mutation(s) is/are found in his or her family, of the original kindred was a homozygote for a single non-conservative point mutation, replacing aspartic acid with valine at amino acid 641 in GR LBD; this mutation reduced binding affinity for dexamethasone by 3-fold and caused a concomitant loss of transactivation activity [15].

The proposita of the second family had four-base deletion at the 3′ boundary of exon 6, removing a donor splice site [16]. This resulted in complete ablation of one of the GR alleles in affected members of the family. The propositus of the third kindred had a single homozygotic point mutation at amino acid 729 (valine to isoleucine) in LBD, which reduced both the affinity and transactivation activity of the GR [17]. There was also an interesting sporadic case of a man with a *de novo* germline heterozygotic GR mutation at amino acid 559 (isoleucine to asparagine) also in LBD close to nuclear-localization signal 1. This mutant GR bound no ligand but exerted dominant-negative activity on the wild-type receptor by preventing or retarding the translocation of the wild-type receptor into the nucleus [18,19].

Study of a fifth case/kindred with glucocorticoid resistance and a heterozygotic GR mutation in the LBD (amino acid 747, replacing isoleucine...
with methionine) was recently completed [20]; the mutant receptor had mildly reduced affinity for dexamethasone and markedly decreased transactivation activity; interestingly, it also had dominant-negative activity on the wild-type receptor. The mutation was located just a few amino acids before the helix 12 of the GR LBD. This mutant receptor could not bind to the nuclear co-activator signature motif LXXLL of the p160 type nuclear receptor co-activator but still associated with this co-activator through its intact activation function 1 domain. Overexpression of p160 co-activator diminished the dominant-negative activity of the mutant receptor, suggesting that defective interaction of the mutant receptor with p160 co-activator might explain its dominant-negative activity on the wild-type receptor.

The sixth and seventh sporadic cases were also found as heterozygous mutations with histidine replacing arginine at amino acid 477, and a glycine-to-serine change at amino acid 697, respectively [21]. The former is located in the second zinc finger in the DBD. This mutant receptor has no transactivation activity due to impaired binding to GREs. The latter mutation is located in the LBD, outside of the ligand-binding pocket. This mutation caused 50% reduction of ligand-binding affinity with comparable reduction of the transactivation activity. Since these two mutant receptors were found in the heterozygotic condition, they might also behave as dominant-negative mutants to the wild-type receptor, suppressing its activity.

The proposita of the eighth case, a girl born with ambiguous genitalia, had a homozygotic point mutation replacing valine with alanine at amino acid 571

Figure 2. Location of the known mutations of the GR in its genomic (A) and protein (B) structures

ATG, start codon; TGA, stop codon.
<table>
<thead>
<tr>
<th>Position of mutation</th>
<th>Amino acid</th>
<th>Biochemical phenotype</th>
<th>Genotype/transmission</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-2054→T</td>
<td>Asp-64→Val</td>
<td>Affinity ↓</td>
<td>Homozygote/autosomal recessive</td>
<td>[15]</td>
</tr>
<tr>
<td>G-2317→A</td>
<td>Val-729→Ile</td>
<td>Number ↓</td>
<td>Heterozygote/autosomal dominant</td>
<td>[16]</td>
</tr>
<tr>
<td>G-2317→A</td>
<td>Val-729→Ile</td>
<td>Number ↓</td>
<td>Heterozygote/autosomal recessive</td>
<td>[17]</td>
</tr>
<tr>
<td>T-1808→A</td>
<td>Ile-559→Asn</td>
<td>Number ↓</td>
<td>Heterozygote/sporadic</td>
<td>[18,19]</td>
</tr>
<tr>
<td>T-2373→G</td>
<td>Ile-747→Met</td>
<td>Affinity ↓</td>
<td>Heterozygote/autosomal dominant</td>
<td>[20]</td>
</tr>
<tr>
<td>G-1430→A</td>
<td>Arg-477→His</td>
<td>Transactivation ↓</td>
<td>Heterozygote/sporadic</td>
<td>[21]</td>
</tr>
<tr>
<td>G-2035→A</td>
<td>Gly-679→Ser</td>
<td>Transactivation ↑</td>
<td>Heterozygote/sporadic</td>
<td>[21]</td>
</tr>
<tr>
<td>A-1844→T</td>
<td>Thr-57→Cys</td>
<td>Affinity ↓</td>
<td>Homozygote/autosomal recessive</td>
<td>[22]</td>
</tr>
</tbody>
</table>

**Table 1. Pathological mutations in the GR gene**
in the LBD [22]. The mutant receptor had a 6-fold reduction in its binding affinity for dexamethasone and 10–50-fold lower transactivation activity than the wild-type receptor. Interestingly, the proposita was also a carrier of 21-hydroxylase deficiency, suggesting that association with this congenital disorder exacerbated the hyperandrogenism and virilization potential of the glucocorticoid-resistance syndrome.

Pathophysiology of glucocorticoid-resistance syndrome

A complex negative-feedback system exists in the human CNS that regulates glucocorticoid homoeostasis. The regulatory circuit for glucocorticoid secretion in the CNS detects and integrates external signals through numerous parts of the CNS, and such inputs are transduced to the paraventricular nucleus of the hypothalamus, which produces CRH, the major stimulator of corticotropin from the anterior lobe of the pituitary gland, and arginine vasopressin (AVP). Axons from this nucleus project to the median eminence and secrete CRH into the hypophyseal portal blood system, which then circulates to the anterior pituitary gland and stimulate corticotropin production and secretion. Glucocorticoids exert negative-feedback effects on both hypothalamic CRH and AVP secretion and inhibit pituitary corticotropin secretion itself. In addition, glucocorticoids influence the activity of suprahypothalamic centres, including the paraventricular nucleus, that control the activity of CRH and AVP neurons [2].

This complex regulatory system is adjusted to higher levels in patients with loss-of-function GR mutations, since the mutated GRs need more glucocorticoids to exert normal biological effect, including suppression of the regulatory system. Thus the mutations result in compensatory increases in corticotropin and cortisol secretion (Figure 3). 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 corticotropin 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 hypokalaemic alkalosis, which is caused by compensatory increase of the H⁺ secretion from the renal tubules in an attempt to recover secreted K⁺ from the filtrated fluid due to mineralocorticoid excess. On the other hand, the latter causes varying manifestations of hyperandrogenism, such as acne, hirsutism, male-pattern baldness, menstrual irregularities and infertility in women. Precocious puberty has been seen in a boy due to early and excessive prepubertal adrenal androgen secretion and ambiguous genitalia in a genetic female due to excessive production of adrenal androgens by the fetal adrenal zone. 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 broad, as a large number of subjects are asymptomatic and show only biochemical changes.

Treatment of glucocorticoid-resistance syndrome
Patients are treated with high doses of synthetic glucocorticoids with low mineralocorticoid activity. The goal is to suppress the increased levels of corticotropin, which cause overproduction of mineralocorticoids and androgens (Figure 3) [2]. As all cases described to date have had partial inactivation of GR activity, synthetic potent glucocorticoids (e.g. dexamethasone) in minimal intrinsic mineralocorticoid activity is a rational approach. These steroids achieve activation of the mutated GR in homozygous cases or of the wild-type receptor in heterozygous cases sufficient to suppress the compensatory increases of corticotropin, and hence the production of the

© 2004 The Biochemical Society
adrenal mineralocorticoids and androgens causing the clinical manifestations of the condition. The patients should be treated with high, individualized doses of oral dexamethasone (1–3 mg/day). Dexamethasone indeed suppresses corticotropin and therefore endogenous cortisol, deoxycorticosterone, corticosterone and adrenal androgen secretion, correcting the mineralocorticoid and androgen excess states of these patients.

**MR mutations**

**Inactivating mutation of the MR**

The mechanism whereby 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. The phenotype of patients with loss-of-function mutations of the MR mimics that of patients with defects in the subunits of the ASSC who represent the bulk of patients with PHA1 [23,24].

Cheek and Perry first reported PHA1 in an infant with severe salt-wasting syndrome in 1958; PHA1 was subsequently reported in more than 70 patients [25]. 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. Approx. one-fifth of these cases are familial. 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.

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 failed to find pathological mutations in the MR gene in our sporadic and familial cases with autosomal-recessive PHA1, and concluded that, most probably, this condition was due to a defect in a post-MR step of aldosterone action [26–28]. Indeed, in 1996, PHA1 was found to be caused by loss-of-function mutations in genes encoding subunits of the ASSC [23,24]. However, Geller et al. [2a] identified heterozygotic MR gene loss-of-function mutations in one sporadic case and four autosomal-dominant cases of PHA1 (Figure 4, Table 2) [2a]. 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. Subsequently, Tajima et al. [29] reported the fifth family of the pathological mutation in the MR gene from the patients with the autosomal-dominant PHA1 [29]. The propositus had a single heterozygotic point mutation at amino acid 924 (leucine to proline) in LBD of MR. The mutation
completely abolished the transactivation activity of the mutant MR on the murine mammary tumour virus promoter. Since the mutation is located in helix 11 of the MR LBD that forms the ligand-binding pocket with helices 3, 4 and 12, the mutant receptor might lose transcriptional activity through its inability to bind ligands. The sixth inactivating MR mutation was found as a sporadic PHA1, harbouring a heterozygous frame-shift mutation that inserted a cytosine at position 3094 in exon 9 that resulted in a nonsense protein from 958 and a first stop codon at position 1012 [30]. The seventh pathological mutation of the MR gene was found in a German family as a heterozygotic mutation in exon 2 replacing serine at amino acid 163 to stop codon (Ser-163→stop) [31]. The propositus demonstrated a clear phenotype of autosomal-dominant PHA1, while his father had no clinical signs of PHA1 in his entire life, indicating that phenotype of the MR mutation is heterologous even within a family, possibly due to their genetic background and/or developmental influences. Recently, Sartorato et al. [32] reported a large study analysing 14 families with an autosomal-dominant PHA1 phenotype and found six heterozygotic mutations. They reported two frame-shift mutations in exon 2 (insertion of Thr-1354, deletion of 8 bp at position 537) and one nonsense mutation in exon 4 (Cys-2157→Ala, Cys-645→stop), which produce several truncated MR molecules devoid of the entire LBD. They also reported three missense mutations (Gly-633→Arg, Gln-776→Arg and Leu-979→Pro); the Gly-633→Arg mutation, situated in the DBD, demonstrated attenuated transactivation activity possibly due to reduced binding activity of the mutant receptor to DNA. The Gln-776→Arg and Leu-979→Pro mutations, also in the LBD, had reduced or absent binding activity to
Table 2. Pathological mutations in the MR gene

<table>
<thead>
<tr>
<th>Position of mutation</th>
<th>Amino acid</th>
<th>Genotype/transmission</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔG-1226</td>
<td>Frame shift</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[2a]</td>
</tr>
<tr>
<td>ΔT-1597</td>
<td>Frame shift</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[2a]</td>
</tr>
<tr>
<td>C-1831→T</td>
<td>Arg-537→stop</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[2a]</td>
</tr>
<tr>
<td>ΔA at the 3’ boundary of exon and intron 5</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[2a]</td>
<td></td>
</tr>
<tr>
<td>C-2651→T</td>
<td>Ser-810→Leu</td>
<td>Heterozygote</td>
<td>Hypertension</td>
<td>[33]</td>
</tr>
<tr>
<td>T-2993→C</td>
<td>Leu-924→Pro</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[29]</td>
</tr>
<tr>
<td>Insertion of C-3094</td>
<td>Frame shift</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[30]</td>
</tr>
<tr>
<td>C-488→T</td>
<td>Ser-163→stop</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[31]</td>
</tr>
<tr>
<td>Δ8 bp at 537</td>
<td>Frame shift</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[32]</td>
</tr>
<tr>
<td>Insertion of 1357T</td>
<td>Frame shift</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[32]</td>
</tr>
<tr>
<td>G-2119→A</td>
<td>Gly-633→Arg</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[32]</td>
</tr>
<tr>
<td>C-2157→A</td>
<td>Arg-645→stop</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[32]</td>
</tr>
<tr>
<td>A-2549→G</td>
<td>Gln-776→Arg</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[32]</td>
</tr>
<tr>
<td>CT-3158→C</td>
<td>Leu-979→Pro</td>
<td>Heterozygote</td>
<td>PHA1</td>
<td>[32]</td>
</tr>
</tbody>
</table>
aldosterone and corresponding blunted or absent transactivation capacity. Leu-979→Pro also functioned as a transdominant-negative mutant to the wild-type MR on its transactivation of responsive genes.

As indicated, all reported MR mutations causing PHA1 phenotype were found in the heterozygote state, i.e. in only one of the two alleles of the MR gene. They consisted of a frameshift, insertion of a premature termination codon or a point missense mutation, indicating that PHA1 phenotype can be caused by haploinsufficiency, a condition harbouring the heterozygotic mutation. The patients developed PHA1 in an early stage of their life and their clinical condition improved with age, although plasma aldosterone concentrations and renin activity remained high. These clinical findings suggest that the intact MR gene is required for the reabsorption of salt at least in infancy, and some other yet unknown mechanisms may overcome defects of MR function in older ages. Further work is necessary to address this issue.

Treatment of PHA1 is done by the supplementation of patients with NaCl. The amounts of salt may be different from patient to patient depending on their degree of salt wasting. The salt-administration requirements may decrease with advancing age.

Activating mutation of the MR
The first activating MR mutation was found in a patient with early-onset hypertension that was markedly exacerbated in pregnancy (Figure 4, Table 2) [33]. The propositus had a heterozygotic point mutation, Ser-810→Leu. This mutation is localized in helix 5 of the MR LBD: the leucine side chain projects into the ligand-binding pocket, potentially forming additional van der Waals interactions with Ala-773 of helix 3 and the carbon-19 methyl group of steroid hormones. Therefore, this mutation may change the ligand specificity of the mutant receptor, conferring increased binding to progesterone in addition to mineralocorticoids and glucocorticoids. Thus a patient with such a mutation may have worsening hypertension in pregnancy due to the activation of the mutant receptor by progesterone, secreted from the placenta or by the physiologically increased levels of cortisol in pregnancy. In addition, it was recently reported that cortisone and 11-dehydrocorticosterone bind to the mutant receptor with high affinity and may thus cause early-onset hypertension in affected men and non-pregnant women [34].

Conclusions
We have described the molecular defects observed in the GR and MR genes, the pathophysiological mechanisms resulting in disease and have suggested rational therapeutic interventions. Although these mutations are rare, they provide strong insight into the physiological importance of hormonal actions of glucocorticoids and mineralocorticoids, and may provide clues to unknown important functions of these hormones.
Summary

- Adrenal corticosteroids, i.e. glucocorticoids and mineralocorticoids, play important roles in human physiology. The former are necessary for the maintenance of CNS function and cardiovascular, metabolic and immune homeostasis, while the latter play a critical role in the retention of salt in the kidney, salivary glands, sweat glands, and colon.
- The actions of these hormones are mediated by intracellular receptor molecules, the GR and MR, which function as hormone-dependent transcription factors. Ligand-activated receptors modulate the transcription rates of responsive genes by interacting with responsive elements in the promoters of these genes and/or by influencing the activities of other transcription factors, via protein–protein interactions. The biological activities of these receptors were examined recently in animals and human patients whose genes for these receptors are influenced by genetic modifications.
- Natural physiological steroid hormone ‘resistance’ in animals has been reported in New World monkeys, Guinea pigs and prairie voles, with distinct mechanisms affecting the biological actions of several steroid hormone receptors.
- In human patients, the familial/sporadic glucocorticoid-resistance syndrome is characterized by partial insensitivity to glucocorticoids with concomitant hypercortisolism, but without Cushingoid features. This syndrome is caused by loss-of-function mutations of the GR gene and is associated with hyperandrogenism and/or hypermineralocorticoidism.
- In human patients, inactivating mutations of the MR cause pseudo-hypoaldosteronism type 1, which presents in infancy with urinary salt wasting and failure to thrive, in spite of high levels of circulating aldosterone. Recently, an activating mutation of the MR was reported as early-onset hypertension that was markedly exacerbated in pregnancy. This mutation changed the ligand specificity of the receptor, conferring increased binding to progesterone in addition to aldosterone and cortisol.

References


© 2004 The Biochemical Society


the nucleus: importance of the ligand-binding domain for intracellular GR trafficking. J. Clin. Endocrinol. Metab. 86, 5600–5608

© 2004 The Biochemical Society