Syndromes of glucocorticoid and mineralocorticoid resistance

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Introduction

Since the discovery of high-affinity (type I) and low-affinity (type II) corticosteroid receptors, it has become apparent that glucocorticoids and mineralocorticoids constitute a two-hormone two-receptor system, in which variations/modulation of hormone concentration, receptor occupancy and specificity-conferring mechanisms allow a variety of physiological effects to be exerted and multiple gene networks to be regulated. Besides stricto sensu glucocorticoid and mineralocorticoid actions exerted through their cognate receptors, corticosteroids have overlapping physiological effects in some epithelial target tissues. In other tissues in which cortisol binds to both receptors, a clear distinction between the two pathways becomes even more difficult, and functional antagonism may be observed between glucocorticoid and mineralocorticoid receptor-mediated effects. This is particularly evident in the brain, where cortisol is able to mediate antagonistic effects on hippocampal ion regulation and transmitter responsiveness, but also in other brain regions involved in volume regulation and food intake, as well as in the immune system and the colon.

The clinical observation and molecular study of hormone resistance syndromes has been fundamental to the advance of our knowledge on the mechanism of action of glucocorticoid and mineralocorticoid hormones. The purpose of this review is to present recent acquisitions of knowledge in terms of physiopathology and the molecular basis of glucocorticoid and mineralocorticoid resistance syndromes, placed in the context of molecular events underlying the cellular actions of corticosteroid hormones.

The glucocorticoid and mineralocorticoid receptors

Most of the known effects of cortisol and aldosterone are exerted by interaction with specific intracellular receptors belonging to the nuclear receptor superfamily, which includes the other steroid hormone receptors as well as thyroid hormone, vitamin D and retinoid acid receptors and a large group of orphan receptors (1). Nuclear receptors are highly specialized ligand-dependent transcription factors, which interact with specific DNA sequences on hormone-responsive genes. The existence of multiple receptor subtypes, encoded by different genes, and of a large number of receptor isoforms, generated from the same gene by virtue of alternative splicing, alternative promoter or start codon utilization, allows diversification of hormonal responses and increases their repertoire of regulatory functions. Members of the superfamily display both amino acid and structural homology, and their cDNAs are highly conserved. In particular, the glucocorticoid and mineralocorticoid receptors (GR and MR respectively) are closely related, and together with the androgen and progesterone receptors they form a subfamily, which shares more than 90% amino acid homology in the central cysteine-rich region of the protein (2). GR and MR, as well as the other nuclear receptors, are composed of different functional domains (Fig. 1) (3, 4). The N-terminal part of the protein, or A/B domain, is variable in length and nucleotide sequence, and is responsible for target gene activation, probably via interaction with components of the transcriptional machinery, with co-activators and/or other transcription factors. In GR, the A/B domain contains a strong transactivation function (3, 5, 6); the same region of MR, in contrast, has only weak transactivating activity (7). The cysteine-rich central DNA-binding domain (DBD, region C) is the best characterized region of nuclear receptors. This small segment of approximately 90 amino acids is the most conserved among nuclear receptors (3). DBD folds into two zinc fingers, in which each zinc atom is coordinated by four cysteines (8). These structures are responsible for interaction with DNA and receptor dimerization (9–11). The sequence located between the DBD and the ligand-binding domain (LBD) is called the hinge region (region D), and appears to be important for protein bending or conformational changes of the receptor molecule (12). In GR this region also contains a nuclear localization signal (6). The C-terminal part of the receptor protein (region E) constitutes the LBD. This relatively large region (~250 amino acids) is functionally complex and contains signals for dimerization and transactivation (11, 13). A second, hormone-dependent, nuclear localization signal is also present in the LBD of the GR (14). Finally, LBD is essential for interaction of receptors with heat shock protein 90 (hsp90) and other associated proteins (15–17).
Both the human GR and MR (hMR and hGR) genes have recently been cloned (18, 19). In both genes, which are located on chromosomes 5 and 4 respectively, eight exons encode the functional domains of the receptor protein. Their 5' and 3' regions, however, appear to be structurally different. While the hGR gene contains a unique 5'-untranslated exon, two hMR mRNA variants (hMR\textalpha{} and hMR\textbeta{}) containing different 5'-untranslated sequences are generated by utilization of alternative promoters. Use of alternative promoters allows tissue-specific and developmentally regulated gene expression, and indeed evidence is accumulating for distinct basal and hormonal regulation of hMR regulatory regions (20, 21). In contrast, hGR is under the control of a unique promoter, but two mRNA isoforms (hGR\textalpha{} and hGR\textbeta{}) and different proteins are generated by alternative use of two different 3'-exons. Whereas hGR\textalpha{} can bind hormone and is transcriptionally active, the non-binding hGR\textbeta{} was proposed to act as a dominant negative regulator of hGR\textalpha{} activity (22), although its actual physiological importance remains controversial (23, 24).

It has previously been shown that GR and MR are localized in the cytoplasm in the absence of hormone and translocate into the nucleus after hormone binding (14, 25, 26). Nuclear localization signal is recognized by a specific receptor and nuclear entry occurs through the nuclear pore complex (27, 28). More recently, data have accumulated pointing towards bidirectional nucleocytoplasmic receptor shuttling (29). While receptor import into the nucleus is ATP-dependent, nuclear GR export does not require energy (30). It appears therefore that intracellular localization of corticosteroid receptors depends on the balance between nuclear import and export, which might be modulated by hsp90 (31).
Furthermore, phosphorylation may influence intracellular trafficking of GRs (42).

Inside the nucleus, ligand-bound GR and MR exert their transcriptional effects by binding as homodimers to specific DNA sequences known as glucocorticoid-responsive elements (GREs) (Fig. 2). These elements are composed of two inverted palindromes of the recognition motif AGAACA separated by three nucleotides (43). Frequently, GREs diverge to a variable extent from this sequence; receptor binding is then achieved at lower affinity and may involve accessory transcription factors to stabilize the complex. While several GREs have been characterized in glucocorticoid-responsive genes, no specific mineralocorticoid-responsive element has yet been identified. MR, however, is able to activate transcription from GRE localized in the mouse mammary tumor virus (MMTV) long terminal repeat, although to a lesser extent than GR, reflecting the high sequence identity in the DBD of the two receptors (44, 45). Both the progestosterone and the androgen receptors also bind to the same DNA-recognition motif (43). It is interesting to note that, on a more complex composite responsive element of the proliferin promoter, GR, but not MR, can block transactivation induced by activating protein-1 (AP-1) (46). Recent data suggest that GR and MR interact with hormone-responsive elements not only in the form of homodimers. Indeed, binding of GR tetramers has been demonstrated on the aspartate aminotransferase promoter (47), and there is evidence for heterodimerization of MR and GR leading to either synergy or inhibition of transactivation depending on the promoter and cellular context (20, 48, 49).

Glucocorticoids can also negatively regulate transcription as a result of interaction with negative GREs, as described in the pro-opiomelanocortin promoter (50) and in the prolactin promoter (51). Furthermore, interaction of GR with part of the response element of other transcription factors, e.g. AP-1 or NF-κB, as well as formation of intranuclear complexes with heterodimers of the AP-1 transcription factors c-Jun and c-Fos can result in inhibition of transcriptional activation induced by the same transcription factors (52, 53).

The molecular mechanisms by which corticosteroid receptors modulate transcription after binding to hormone-responsive elements are largely not known. Steroid receptors are believed to stimulate gene expression by facilitating the assembly of basal transcription factors into a stable pre-initiation complex, which may be achieved by direct binding to basal transcription factors or by interaction with transcription accessory factors, co-activators or co-repressors. A large number of nuclear receptor co-activators/co-repressors have recently been cloned, which act as molecular bridges between the receptors and the transcriptional machinery (54). Steroid receptor co-activator 1 (SRC-1) (55) is able to interact with GR and to increase its transcriptional properties; proteins called GRIP1 (56) and GRIP172 (57) have also been shown to interact with GRs. In the same context, factors capable of modifying chromatin structure have been shown to increase the affinity of GR for DNA (58, 59). Finally, the role of chromatin structure on transcriptional activation by GR has been studied in detail on the MMTV promoter (43). Interaction of ligand-bound GR with GRE on this promoter allows access of other transcription factors to their DNA-binding sites and activation of transcription.

**Mineralocorticoid specificity**

While GR expression is ubiquitous, MR is expressed in a restricted number of tissues. Classical aldosterone target tissues are sodium-transporting epithelia, such as the distal parts of the nephron and colon and salivary and sweat glands (60). MR is also expressed in many areas of the forebrain, with very high levels of expression reported in the rodent hippocampus (61). More recently, MR has been identified in a large variety of non-epithelial target tissues, including heart and large blood vessels (62–64), mononuclear leukocytes (65) and epidermis (66). In kidney, MR mediates aldosterone effects on sodium, potassium and hydrogen ion balance in the distal parts of the nephron. In some areas of the brain, in contrast, the MR responds to diurnal fluctuations in cortisol levels, thus providing, together with GR, a system capable of responding to normal and elevated cortisol concentrations respectively (7).

Indeed, while aldosterone binds with high affinity to MR, natural glucocorticoids have higher affinity for MR than for GR. Mineralocorticoid specificity is achieved mainly by regulating intracellular hormone availability (Fig. 3). Glucocorticoids, which in plasma are largely bound to corticosteroid-binding globulin, are excluded from epithelial target tissues by the action of the enzyme 11β-hydroxysteroid dehydrogenase 2 (11βHSD2), which converts cortisol into its inactive metabolite cortisone (67–69). Aldosterone, in contrast, is not a substrate for the enzyme because of a highly reactive C-11,18-hemiketal group. Thus, despite circulating glucocorticoid
levels 1000-fold higher than aldosterone, MR is selectively occupied by aldosterone in tissues expressing 11\(\beta\)HSD2. However, even in non-epithelial target tissues, which do not express 11\(\beta\)HSD2, distinct roles for MR and GR have been described. In particular, glucocorticoid effects on inflammation, bone turnover and glucose metabolism are independent of MR, and aldosterone effects on central blood pressure regulation or cardiac fibrosis are not reproduced by glucocorticoids (70, 71). Recent data indicate that, independently of 11\(\beta\)HSD2 activity, MR is able to discriminate between glucocorticoids and mineralocorticoids in terms of ligand-dependent transactivation. When activated with different ligands, MR is more sensitive to aldosterone than to cortisol, which appears to be in relation to their respective dissociation rates (72) and to different conformational changes induced by the two hormones. Additional cellular factors that discriminate between GR and MR also play an important role in modulating ligand sensitivity (73). Finally, several lines of evidence suggest that additional mechanisms, e.g. specific membrane transporters, may play a role in regulating intracellular accumulation of steroid hormones. In particular, it has been shown that members of the family of ABC (ATP-binding cassette) membrane transporters are capable of selectively transporting corticosteroids (74–77), and may be associated with cellular resistance to glucocorticoid-induced apoptosis (78).

For both glucocorticoids and mineralocorticoids there is evidence for rapid non-genomic effects (79 and references therein). Such effects have been observed in vascular smooth muscle cells for both aldosterone and cortisol; in the case of aldosterone, effects on ion fluxes in human lymphocytes and on intracellular pH in kidney cells have also been reported. However, none of the putative membrane steroid receptors has yet been purified or cloned, and their nature and distribution as well as their physiological role remain to be determined.

**Glucocorticoid resistance**

Glucocorticoids are involved in many physiological processes, including lipid and carbohydrate metabolism and modulation of immune response and stress response in the brain. In man, response to glucocorticoids is variable; some individuals are hypersensitive and others are quite resistant (80, 81). This is reflected by different responses to corticosteroid treatment, with certain individuals being more susceptible than others to developing adverse effects at therapeutical doses. The molecular mechanisms underlying these differences are not known, although preliminary data indicate that certain GR polymorphisms may be associated with glucocorticoid sensitivity (82). Clinical syndromes of glucocorticoid resistance can be familial or acquired, generalized or tissue-specific. Besides generalized inherited glucocorticoid resistance (GIGR), which will be discussed in detail below, acquired generalized glucocorticoid resistance is observed in a subgroup of patients with acquired immunodeficiency syndrome (83). Tissue-specific glucocorticoid resistance includes glucocorticoid-resistant asthma, rheumatoid arthritis, osteoarthritis, lymphoid tumors, eutopic or ectopic adrenocorticotropin (ACTH) production, Crohn’s disease, ulcerative colitis and nephrotic syndrome (80, 81). A reduced number of GRs or other GR abnormalities

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**Figure 3** Mechanisms of mineralocorticoid specificity. Multiple factors influence MR response to aldosterone. 1. Circulating glucocorticoids are largely bound to corticosteroid-binding globulin. 2. 11\(\beta\)HSD2 converts cortisol into its inactive metabolite cortisone. 3. ABC membrane transporters are capable of selective transport of corticosteroids. 4. MR possesses an intrinsic mineralocorticoid specificity in terms of ligand-dependent transactivation. 5. Rapid non-genomic effects are mediated by membrane receptors.
have been reported in these patients, which may therefore be linked to the underlying mechanism.

GIGR, also referred to as primary glucocorticoid resistance, was first described by Vingerhoeds et al. in 1976 (84). It is characterized by greatly increased serum cortisol concentrations and resistance to adrenal suppression by dexamethasone, without signs or symptoms of Cushing’s disease. The diurnal rhythm of cortisol and ACTH is intact, although at a higher level, and there is normal response of ACTH, cortisol and growth hormone to insulin-induced hypoglycemia (85). Negative feedback on the hypothalamic–pituitary–adrenal axis is decreased, with higher levels of ACTH and cortisol compensating for the generalized tissue resistance to glucocorticoids. The clinical presentation of GIGR is variable and may differ even among members of the same family. Although elevated cortisol levels appear to compensate for the end-organ insensitivity, excess ACTH secretion results in increased production of adrenal steroids with salt-retaining or androgenic activity. Symptoms include hypertension, hypokalemic acidosis and chronic fatigue. Women suffer from male pattern baldness, acne and hirsutism, menstrual irregularities and infertility; isosexual precocity, abnormal spermatogenesis and infertility have been described in men (85). Patients benefit from low-dose dexamethasone therapy (1–1.5 mg/day), which has been shown to efficiently suppress ACTH and androgen production (85, 86).

Only a few unrelated probands affected with GIGR have been described to date. However, it has been suggested that the syndrome may be more common than previously thought. It may be possible that among women presenting with hirsutism there is a subgroup with familial cortisol resistance, which can be recognized by elevated levels of circulating cortisol and ACTH, lower sensitivity to dexamethasone suppression, in the presence of a normal diurnal rhythm of cortisol (85). With the limited numbers it is difficult to make a definitive statement on inheritance of the disease. In cases in which the underlying molecular defect has been elucidated, transmission appears to be autosomal dominant; homozygous patients are more severely affected than heterozygous ones, who may present only biochemical abnormalities (87, 88). This is in agreement with the observation of a close correlation between receptor concentration and target cell responsiveness (89), predicting that heterozygotes will have an intermediate phenotype compared with homozygotes.

Different GR abnormalities have been detected in affected kindreds, including decreased ligand-binding affinity, decreased receptor concentration and increased thermolability. The molecular mechanisms underlying GIGR have recently been elucidated (Fig. 4). In one family in which familial glucocorticoid resistance was first described, the severely affected index case was homozygous for a non-conservative Asp-641 to Val substitution in the LBD of the GR (87). His mildly affected son and nephew were heterozygous for the same mutation. In vitro expression studies showed that the Val-641 mutant GR had reduced ligand-binding affinity and impaired ability to activate gene expression.

In another kindred, analysis of dexamethasone binding to mononuclear leukocytes revealed 3-fold decreased affinity and normal binding capacity in a boy who presented with primary cortisol resistance and isosexual precocious pseudopuberty, and in his asymptomatic mother (90). A homozygous Val-729 to Ile substitution was detected in the GR LBD, for which the mother was a heterozygous carrier. The mutated GR had a 2- to 3-fold decreased affinity for glucocorticoids, whereas maximum binding capacity was not modified. The 4-fold decrease in apparent potency of dexamethasone to stimulate gene transcription via the Val-729 mutant correlated well with the daily dose of dexamethasone required to suppress ACTH in the index case (88). Finally, Karl et al. (91) studied the structure and function of the GR in a Dutch kindred, in which the index case was a woman presenting with a clinical picture of hyperandrogenism, whereas her two brothers were asymptomatic. The number of GRs on mononuclear leukocytes was decreased in all three patients, while binding affinity for dexamethasone was normal. In a functional test, dexamethasone suppressibility of mitogen-stimulated incorporation of tritiated thymidine in mononuclear leukocytes was reduced. A heterozygous 4 bp deletion was identified at the 3′-boundary of exon 6 in all three affected subjects, removing a donor splice site in one allele (91). The absence of transcripts derived from the allele harboring the deletion suggested that only transcripts from the wild-type allele were transcribed. As transcriptional responses mediated by GR appear to be strongly correlated with receptor number, the reduction of GR protein levels by 50% fully accounts for the glucocorticoid resistance in this kindred. The same authors recently reported a heterozygous Ile to Asn change at codon 559, which abolished detectable ligand binding in a patient who presented with hypertension and oligospermia (92). This mutation also completely abolished dexamethasone-induced transactivation from the MMTV promoter. No mutation
affecting the DBD of the receptor molecule has yet been reported in humans.

Recently, de Lange et al. (93) studied the capacity of the mutants described above to regulate transcription from different promoters in vitro. Decreased dexamethasone binding to these GR variants correlated well with reduced capacity to activate transcription from the MMTV promoter. However, the Val-641 variant had normal capacity to repress a negative GRE of a prolactin promoter element, and repression of NFκB-induced transcription was even more efficient than wild-type GR. Moreover, all variants were able to repress AP-1-mediated transcription from the collagenase promoter, although with 10-fold lower potency than wild-type GR. These results indicate that different GR mutations can affect the different GR pathways of transcriptional regulation in different ways, explaining the phenotypic differences observed in patients with glucocorticoid resistance.

Mineralocorticoid resistance

Mineralocorticoid resistance, or type I pseudohypoaldosteronism (PHA), is a rare inherited disease, presenting in the newborn with failure to thrive, salt loss and dehydration. While the clinical presentation is heterogeneous, including severely affected as well as asymptomatic patients, biological abnormalities include invariably high urinary sodium despite hyponatremia, hyperkalemia and metabolic acidosis, high plasma and urinary aldosterone levels, and high plasma renin activity (94). Diagnosis requires proof of renal unresponsiveness to exogenous mineralocorticoid therapy. Sodium supplementation allows recovery of a positive sodium balance, although the extent and duration of treatment varies considerably. Generally, treatment can be discontinued after a few months (95–97); occasionally, the metabolic defect and need for treatment may persist. Note that elevated aldosterone and renin levels may persist after clinical recovery throughout adulthood (98). Clinical manifestations are most severe in children under 10 weeks of age; with time, improvement occurs, and after age 4 salt wasting is rare, although growth retardation may persist.

Since the first description of PHA in 1958 by Cheek & Perry (99), more than a hundred kindreds have been reported. Accurate examination of their clinical history, evolution and mode of inheritance has suggested the existence of two subtypes of PHA (100). In most cases, the clinical picture of PHA is not severe, and aldosterone unresponsiveness seems to be restricted to the renal tubule; this form is transmitted as an autosomal dominant trait; alternatively, it might be sporadic. The autosomal recessive form of PHA, in contrast, is characterized by multiple target organ resistance to aldosterone; patients present a more protracted course of the disease with recurrent life-threatening episodes of salt loss.

Type I PHA must be distinguished from transitory forms of mineralocorticoid resistance, which have been observed during certain nephropathies, such as acute pyelonephritis (101, 102), chronic interstitial nephropathy (103), renal transplantation (104), familial juvenile nephrolithiasis (105), or secondary to massive bowel resection (106, 107). Differential diagnosis includes other forms of salt loss, in particular renal or gastrointestinal infections, and abnormalities in aldosterone biosynthesis.

The search for molecular abnormalities in PHA patients has been a challenge over the last decade. Indeed, in analogy with glucocorticoid and other steroid hormone resistance syndromes, a receptor defect had been postulated as being responsible for PHA. The discovery of decreased aldosterone binding in peripheral blood mononuclear leukocytes of several unrelated patients (108, 109) has prompted a series of molecular studies to characterize the defect. However, analysis of MR in seven different patients, including both renal and generalized forms, familial autosomal dominant or recessive transmission and sporadic cases, has shown that no major rearrangement was present in the hMR gene (110–113). Furthermore, hMR was shown to be expressed in apparently normal amounts in peripheral mononuclear cells, and no abnormality was found in the sequence of the hMR coding region and portions of the 5′-untranslated and regulatory regions.

Two different studies confirmed that hMR is not involved in PHA. Using linkage analysis, Chung et al. (114) have excluded the locus for autosomal recessive PHA from the MR gene region on chromosome 4. Subsequently, two different groups reported linkage between autosomal recessive PHA and regions on chromosomes 12p13.1-pter and 16p12.2-13.11 containing the loci of the α and β–γ subunits of the amiloride-sensitive epithelial sodium channel (ENaC) respectively (115, 116). The amiloride-sensitive ENaC constitutes the limiting step for sodium reabsorption in tight epithelia. ENaC is a hetero-oligomer composed of three subunits α, β, γ, each of them possessing an intracellular N- and C-terminal domain, two hydrophobic transmembrane domains and a large extracellular loop (117–119) (Fig. 5). Increased sodium reabsorption through polarized epithelia is mainly due to an increase in luminal passive sodium permeability induced by activation/induction of amiloride-sensitive ENaCs. Although aldosterone is believed to regulate ENaC by activating pre-existing sodium channels and by chronic induction of new channels (120), transcriptional regulation of ENaC subunits appears to be tissue-specific (121), and no modification of ENaC mRNAs has been found in mice inactivated for the MR (122). Additional studies are required to elucidate molecular mechanisms by which aldosterone regulates sodium channel activity.

Different mutations of ENaC subunits have subsequently been described in patients with autosomal...
recessive PHA (Fig. 5). In the α subunit of ENaC, Chang et al. (123) have identified a homozygous 2 bp deletion at codon 168 in three Saudi kindreds, introducing a frameshift mutation before the first transmembrane domain, with a termination codon ending the translation at amino acid 144. In another family, a single base substitution changing codon Arg-508 from CGA to TGA introduces a premature stop codon in the extracellular domain; the resulting protein lacks the second transmembrane domain and the intracytoplasmic C-terminus (123). A homozygous Gly to Ser substitution was found at position 37 of the β subunit in another family. This residue is localized in a region highly conserved among members of the ENaC family; when expressed in Xenopus oocytes, a significant reduction in channel activity is observed, as the result of a gating mode characterized by short-open and long-closed times (124). Strautnieks et al. (125) identified a homozygous 3' splice site mutation (318–1 Gly to Ala) in the γ subunit of ENaC in three families originating from the Indian subcontinent. Abnormal splicing results in generation of two different mRNAs: one arises from utilization of an adjacent cryptic splice site which leads to substitution of three highly conserved residues in the extracellular domain by a novel amino acid (KYS 106–108 to N); in the other case, skipping of the downstream exon results in a protein truncated from 649 to 134 amino acids.

In contrast with the recessive kindreds, no mutations of the amiloride-sensitive ENaC have been found in patients affected by the autosomal dominant form of PHA (126). Two mutations have been reported in patients presenting as sporadic cases of PHA (127). One patient was homozygous for a Leu to Phe substitution at position 34 of the γ subunit; another patient was a compound heterozygote for an Asn-207 to Asp and an Asn-257 to Tyr substitution in the β subunit and an Ala-595 to Ser substitution in the γ subunit. However, clinical evidence of multiple tissue resistance to aldosterone, and the fact that mutations were found on both alleles, strongly argues against sporadic mutations and rather supports autosomal recessive transmission of PHA in these patients.

**Insights from animal models**

Besides that of guinea pigs, prairie voles and New World primates, all of which present general resistance to glucocorticoids because of differences in their GR LBD (80, 81), involvement of GRs and MRs in normal physiology and development has recently been investigated using gene targeting in mice (122, 128). GR-deficient mice develop to term, but most of them die shortly after birth, indicating that GR mutations lead to perinatal lethality. Indeed, GR¹⁻/⁻ mice display acute respiratory distress at birth associated with severe lung atelectasia, due to reduced expression of the amiloride-sensitive ENaC and impaired synthesis of surfactant proteins. Mice inactivated for the GR gene have impaired function of hepatic gluconeogenic enzymes, enlarged and disorganized adrenal glands lacking adrenergic chromaffin cells, and severely impaired feedback regulation via the hypothalamo–pituitary–adrenal axis. It appears therefore that integrity of GR signaling is critical for lung maturation and normal adrenal gland development. Animals containing only one functional copy of the GR gene survive and have increased plasma levels of glucocorticoids and ACTH and adrenal cortical hyperplasia.

MR-deficient mice all die around day 10 after birth; death is preceded by weight loss which is correlated with hematocrit elevation (122). As a consequence of

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**Figure 5** Schematic representation of the α, β and γ subunits of the amiloride-sensitive ENaC. Amino acid substitutions reported in patients with type I PHA are indicated.
impaired sodium reabsorption, activity of the renin–angiotensin system is increased, with elevation of plasma levels of angiotensin II and aldosterone. In contrast, heterozygous MR+/− mice appear to be normal.

Recently, the murine αENaC locus has been inactivated by homologous recombination (129). In αENaC−/− mice, embryonic and fetal development was not significantly impaired; however, all −/− mice die within 40 h of birth from severe respiratory distress due to failure to absorb fetal lung liquid and unopposed liquid secretion by neonatal lung. The renal phenotype of PHA was not observed, probably because neonates die too early to manifest electrolyte abnormalities. Genetic rescue of the perinatal lethal pulmonary phenotype in αENaC−/− mice partially restored Na+ transport in renal, colonic and pulmonary epithelia (130). At days 5–9, these transgenic animals presented with clinical features of severe PHA, with urinal salt wasting, growth retardation and metabolic acidosis, thus establishing a useful animal model for mineralocorticoid resistance.

It can be deduced from these models that gross alterations of GR, MR and ENaC functions are not compatible with life, and that only mutations that result in partial loss of function are detected in GIGR and PHA, which would explain the rarity of the diseases compared with other hormone resistance syndromes. It would be interesting to generate animals in which MR mutations produce receptors with reduced ligand affinity, in analogy with GIGR patients, and to study the resulting phenotype. Supported by the recent discovery of MR expression in brown adipose tissue (131) together with accumulating data on mineralocorticoid effects in the cardiovascular and central nervous systems, it appears more and more evident that aldosterone effects are not restricted to water and electrolyte homeostasis, and it is tempting to speculate that MR alterations could result in a clinical picture involving mineralocorticoid actions in non-epithelial target tissues.

**Concluding remarks**

Although clinical, biochemical and molecular studies have provided substantial insights into the physiopathological mechanisms of glucocorticoid and mineralocorticoid resistance, there still remain some important questions to be addressed. In particular, molecular defects underlying the autosomal dominant or sporadic forms of PHA remain to be elucidated. It is worth noting that PHA represents the first steroid hormone resistance syndrome in which the molecular abnormality lies at the post-receptor level. In this context it might be postulated that alterations of other aldosterone-induced proteins or putative sodium channel-regulating proteins may play a role in the pathogenesis of the disease. Given the multiplicity of intervening factors and the complexity of the regulatory cascade involved in mediating hormone response, it becomes clear that several other mechanisms may be responsible for the observed hormone insensitivity. Identification of such mechanisms in PHA, as well as those involved in tissue-specific or acquired glucocorticoid resistance, will be of major interest in unraveling molecular functioning of corticosteroid receptors and their interaction with other signaling pathways.

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