GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS: Biology and Clinical Relevance

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ABSTRACT
Mineralocorticoid and glucocorticoid receptors act as homodimers via canonical pentadecamer hormone response elements to regulate transcription. Glucocorticoid, but as yet not mineralocorticoid, receptors have been shown also to modulate AP-1- and NFκB-induced transcription by direct protein-protein interactions. The role of 11β–hydroxysteroid dehydrogenase in conferring aldosterone specificity on epithelial mineralocorticoid receptors has been proven by the demonstration of sequence mutations in all cases of apparent mineralocorticoid excess examined to date. The autosomal form of aldosterone resistance (pseudohypoaldosteronism) has been shown to reflect loss-of-function mutations in epithelial sodium channel subunit sequence. (Patho)physiological roles for aldosterone and glucocorticoid membrane receptors, and for the recently described nuclear receptors for 11-ketosteroids in 11β–hydroxysteroid dehydrogenase–protected epithelia, remain to be established.

Background
The human glucocorticoid receptor (GR) was first cloned and expressed in 1985 (1) as two forms: a ligand-binding GRα of 777 amino acids; and a 742–amino acid β isoform, which differs only in the last 15 amino acids and which does not bind active glucocorticoids. Long dismissed as a cloning
artefact, GRβ are expressed at modest but varying levels in a range of tissues and act as ligand-independent negative regulators of glucocorticoid action (2). The cloned and expressed GRα showed high affinity for dexamethasone, modest affinity for the physiologic steroids cortisol and corticosterone, and low affinity for aldosterone, deoxycorticosterone, and the sex steroids, consistent with previous in vivo and in vitro studies. The human mineralocorticoid receptor (MR), of 984 amino acids, was cloned and expressed two years later (3). It has 57% amino acid identity with GRα in the ligand binding domain (LBD), and 94% in the DNA binding domain (DBD). The cloned and expressed MR showed high and equivalent affinity for corticosterone, aldosterone, and cortisol, confirming previous studies on MR in rat kidney and hippocampus (4); subsequently, MR have been shown in addition to have equivalent high affinity for progesterone (5).

Progesterone receptors (PR) and androgen receptors (AR) show significant amino acid homology (LBD, ~50%; DBD, ~90%) with MR and GR. Together, these four receptors constitute a subfamily within the steroid/thyroid/retinoid/orphan receptor (STRO) superfamily, which currently lists over 150 members (6). In common with estrogen receptors but in contrast with other STRO superfamily members, MR/GR/PR/AR in the unliganded state are associated with a complex of chaperone proteins, including the heat shock protein Hsp 90 and the immunophilin Hsp 56, which maintain the receptors in an inactive form with high affinity for hormone. Upon appropriate ligand binding, the associated chaperone proteins are shed, exposing nuclear localization signals in MR and GR, which are thus enabled to access and be retained in the nucleus.

**MR and GR as Transcription Factors**

Reflecting the high degree of homology within their DBD, MR/GR/AR/PR bind to common nuclear hormone response elements (HRE), with a consensus 15-nucleotide sequence of AGAACAnnnTGTTCT. Regulation of transcription is dependent on interaction not only with the HRE, but also with the transcription initiation complex, an assembly of transcription factors and RNA polymerase II. In addition, largely from studies of other members of the STRO superfamily, there is increasing evidence for the existence of coactivators, which act as bridging factors between activated receptor and the transcription initiation complex (7), and similarly for the existence of corepressors, both ligand-dependent (8) and independent (9).

Perhaps not surprisingly, given the inverted palindrome sequence of HRE, members of the MR/GR/PR/AR subfamily appear to act as dimers, either homodimers or heterodimers. For MR and GR, heterodimers have been reported to enhance (10) or lower (11) transcriptional activity, a difference
probably reflecting the number of HRE used in the reporter constructs. There is also in vitro evidence for heterodimerization of MR and GR with the A form of PR (12, 13). Given the essentially ubiquitous distribution of GR, such heterodimers would not be unexpected in MR- or PR-containing cells; the in vivo occurrence and physiologic relevance of such heterodimers, however, are yet to be established.

**Distinguishing GR- and MR-Mediated Responses**

In in vitro cotransfection systems, GR and MR appear to have equivalent transcriptional activity at a classical (canonical) HRE when activated by an appropriate ligand (14); moreover, MR are equivalently activated by cortisol and aldosterone in some studies (14), although others (15) have claimed aldosterone to be more potent, equivalence in affinity notwithstanding. There are clear-cut physiological and clinical differences in mineralocorticoid and glucocorticoid action, however, suggesting that in vivo mechanisms other than those established in MR/GR HRE cotransfection studies operate to ensure specificity.

To date, no unambiguously specific mineralocorticoid response elements have been demonstrated, despite an intensive search in appropriate aldosterone target tissues. The recent demonstration of the aldosterone-selective increase in expression of the γ-subunit of the epithelial Na⁺ channels (16) may allow a more focused exploration of this area, although in unpublished reports MR knockout (MRKO) mice show normal amiloride-sensitive Na⁺ channel activity (TJ Cole, personal communication). In fact, specific mineralocorticoid response elements may be more profitably sought in MR-regulated genes in nonepithelial tissues rather than classical aldosterone target tissues, as discussed in detail below.

At the HRE level, one distinction between MR and GR is the ability of the latter to self-synergize, i.e. to produce more than additive effects via multiple HRE, an N-terminal–dependent phenomenon; MR share <15% sequence identity in this region and do not self synergize (17, 18). A second difference is the ability of GR, but not MR, to inhibit induction of AP-1–dependent genes by Fos-Jun heterodimers (14). This is not an isolated interaction, in that GR and Jun-Jun homodimers clearly synergize rather than mutually repress (19); whether or not MR-specific heterodimer formation with other transcription factors underlies the MR-specific actions in nonepithelial tissues, for example, awaits exploration.

Glucocorticoids affect the transcriptional activity of NFκB, another very important mediator of the inflammatory response, in at least two ways. First, GR, via a classical genomic action, increase the levels of 1kB, which traps NFκB in the cytoplasm (20, 21). Second, GR interact with p65, one of the
transcriptionally active subunits of NFκB, by a protein:protein interaction similar to that for Fos-Jun (22). The evidence for such protein:protein interactions being independent of the genomic actions of GR is strong, in that the effects on both NFκB- (23) and AP-1–dependent genes (24) are seen with transactivation-deficient GR mutants.

To date, the clearest evidence for MR-specific interactions comes from studies on isolated neonatal cardiomyocytes (25), where activation of MR by aldosterone increases [3H]leucine incorporation, whereas activation of GR by dexamethasone predictably lowers incorporation. Activation of protein kinase C in response to increased medium glucose lowers the threshold and increases the MR response to aldosterone, but it has no effect on the GR response; the mechanisms underlying this selective synergy are yet to be explored.

**Epithelial and Nonepithelial MR**

MR not only have equivalent affinity for aldosterone and cortisol, they are also expressed at considerable levels in tissues such as hippocampus and heart (1, 26), which are not classical aldosterone targets. The question of how aldosterone can occupy MR in epithelial target tissues has been answered, at least in part, by the recent cloning and expression of the enzyme 11β-hydroxysteroid dehydrogenase 2 (11-HSD2) from human kidney (27), and by the demonstration of a range of mutations in all cases of the syndrome of apparent mineralocorticoid excess studied to date (28–30).

As shown in Figure 1, in epithelial aldosterone target tissues, cortisol is normally excluded from both MR and GR by the operation of 11-HSD2, which can be inhibited by licorice or carbenoxolone ingestion. Aldosterone is not a substrate for 11-HSD2, because its 11-OH group is protected by cyclization with the unique aldehyde group at C18 to yield a very stable 11,18 hemiketal. If the enzyme is blocked or deficient, cortisol can occupy MR (for which it has ∼10-fold higher affinity than for GR) and mimic the aldosterone effect on ion transport. Importantly, from studies using the GR-specific agonist RU28362, activation of GR in these cells is followed by effects on ion flux indistinguishable from those of aldosterone via MR (31, 32), strong evidence for an action via a nondiscriminating HRE.

In nonepithelial tissues MR, although apparently the same gene product, differ operationally in a number of ways. Figure 2 depicts the actions of MR in the circumventricular region of the brain and their role in blood pressure elevation in response to aldosterone. Such MR are not protected and therefore are not aldosterone-selective. Also, when they are occupied by aldosterone, given either intracerebroventricularly or systemically in rat studies (33, 34), blood pressure rises. Corticosterone, the physiologic glucocorticoid in the rat, is not an MR agonist as it is in the kidney (32) but blocks the effect of
coinfused aldosterone. The GR-specific agonist RU26988 infused intracerebroventricularly neither blocks or mimics the aldosterone response, evidence for MR-specific actions distinct from those of GR, again in contrast with the demonstrated equivalence of MR and GR on renal ion fluxes (31, 32). Finally, intracerebroventricular infusion of the MR-selective antagonist RU28318 can block the central hypertensive action of peripherally infused aldosterone, with no change in various indices of peripheral aldosterone action (35, 36).

Other nonepithelial MR-mediated effects of aldosterone have been described, most notably in producing experimental cardiac hypertrophy and fibrosis (37). Like the central effects of aldosterone on blood pressure, these effects are mediated via unprotected MR and are blocked rather than mimicked by corticosterone occupancy of MR (38). The extent to which this mechanism may be operant in the cardiac fibrosis of congestive cardiac failure has yet to be established.

Steroid Resistance Syndromes

Over the last 15 years, sporadic and familial cases of glucocorticoid resistance, manifest as hypertension and hyperandrogenization, have been reported; in the
majority of these cases, GR mutations have been established as the molecular basis for the syndrome (39–41). More recently, in a study of over 200 nonselected subjects given a dexamethasone suppression test, a relatively high incidence (\(\sim 10\%\)) of allelic variation was found, with the majority having a variant consistent with some degree of glucocorticoid resistance (42). Mineralocorticoid resistance was first described in 1958 (43) in association with pseudohypoaldosteronism (PHA); subsequently, sporadic and autosomal recessive cases have been documented, and reduced or absent MR in peripheral mononuclear leucocytes have been described (44). On the other hand, in neither the sporadic nor the more seriously affected familial form could any MR coding abnormality be established, despite exhaustive studies on three continents (45–47). Recently, linkage analysis of family members in kindred with the autosomal recessive form of PHA provided no evidence for involvement of the region of chromosome 4 containing the MR gene (48); even more recently, the defect in such patients was shown to be a loss-of-function mutation in sodium channel subunits (i.e. the opposite of Bartter’s syndrome) (49). The mechanisms of MR down-regulation in this severe form of PHA and whether an MR defect is primary in sporadic cases have not yet been resolved.

Considerable insight into steroid resistance syndromes—and perhaps into aspects of steroid action, including, for example, MR:GR heterodimers—can

![Figure 2](image-url)
be expected from the recent production of GRKO and MRKO mice. Homozygous (−/−) GRKO mice are born at the expected Mendelian frequency, but they usually die within hours of birth of atelectasis. A minority, however, survive, showing very high levels of adrenocorticotropin and corticosterone (50). Heterozygous (+/−) GRKO mice show adrenocorticotropin and corticosterone levels intermediate between those of −/− homozygotes and wild type. These latter data suggest that normal GR levels are necessary to maintain a normal set of the pituitary-adrenal axis. On the other hand, heterozygotes show normal adrenal medullary development, in contrast with −/− homozygotes in which the adrenal medulla is vestigial, and the glucocorticoid-regulated enzyme PNMT, which catalyzes the conversion of epinephrine to norepinephrine, is not expressed. MRKO mice have been produced (TJ Cole, personal communication), again at expected Mendelian ratios after heterozygote matings, but they lose weight from approximately four days postpartum and die on days 8–11, presumably reflecting their inability to retain sodium and its relatively low content in milk. Further studies characterizing the MRKO mouse are currently in progress.

Non-MR, Non-GR Corticosteroid Receptors

The demonstration of rapid hormone effects with a time course inconsistent with genomic action suggests that through physiologic mineralocorticoids and glucocorticoids additional sites may exist. For glucocorticoids, at least, the demonstration of a range of protein:protein interactions involving classical GR should serve as a caution against overinterpreting rapid time-course data. For both glucocorticoids and mineralocorticoids, however, additional specificity data support actions via other than classical receptors.

In the amphibian, corticosterone has been shown to have high potency in rapidly extinguishing the clasp reflex and to bind with nanomolar affinity to neuronal membrane preparations; the rank order of in vivo potency is well correlated with the ability to displace labeled corticosterone from the membranes, with dexamethasone and RU28362 having low potency on either index (51). Aldosterone has similarly been shown to have very rapid effects on ion fluxes in a variety of cells, an effect that is not spironolactone-inhibitable and that clearly occurs via receptors other than the classical MR (52). In both instances, however, isolation and characterization of the putative membrane-bound receptors have proven elusive.

Very recently, evidence for the existence in aldosterone-target tissues of a classical intracellular receptor specific for 11-keto steroids has been reported (53). Given the exclusion of glucocorticoids from both MR and GR in such tissues by their conversion to their 11-keto congeners, such a receptor would represent a mechanism for physiologic actions of circulating glucocorticoids.
on aldosterone target tissues. More detailed characterization should be possible if the receptor can be cloned and expressed; its presence in GRKO mice would appear to exclude GRβ from such a role.

Literature Cited

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