Multiple aspects of mineralocorticoid selectivity

NICOLETTE FARMAN AND MARIE-EDITH RAFESTIN-OBLIN
Institut National de la Santé et de la Recherche Médicale U-478, Faculté de Médecine
X. Bichat-Institut Fédératif de Recherches 02, 75870 Paris Cedex 18, France

Farman, Nicolette, and Marie-Edith Rafestin-Oblin. Multiple aspects of mineralocorticoid selectivity. Am J Physiol Renal Physiol 280: F181–F192, 2001.—Aldosterone regulates renal sodium reabsorption through binding to the mineralocorticoid receptor (MR). Because the glucocorticoid receptor (GR) is expressed together with the MR in aldosterone target cells, glucocorticoid hormones bound to GR may also intervene to modulate physiological functions in these cells. In addition, each steroid can bind both receptors, and the MR has equal affinity for aldosterone and glucocorticoid hormones. Several cellular and molecular mechanisms intervene to allow specific aldosterone regulatory effects, despite the large prevalence of glucocorticoid hormones in the plasma. They include the local metabolism of the glucocorticoid hormones into inactive derivatives by the enzyme 11β-hydroxysteroid dehydrogenase; the intrinsic properties of the MR that discriminate between ligands through differential contacts; the possibility of forming homo- or heterodimers between MR and GR, leading to differential transactivation properties; and the interactions of MR and GR with other regulatory transcription factors. The relative contribution of each of these successive mechanisms may vary among aldosterone target cells (epithelial vs. nonepithelial) and according to the hormonal context. All these phenomena allow fine tuning of cellular functions depending on the degree of cooperation between corticosteroid hormones and other factors (hormonal or tissue specific). Such interactions may be altered in pathophysiological situations.

aldosterone; glucocorticoid hormones; corticosteroid receptors; kidney; 11β-hydroxysteroid dehydrogenase; sodium transport

CORTICOSTEROID HORMONES (aldosterone and glucocorticoid hormones) are important regulators of sodium homeostasis, thus controlling volemia and blood pressure. The mineralocorticoid hormone aldosterone promotes renal sodium reabsorption (15, 46, 54, 114, 115) and potassium secretion (86); such effects occur in the distal tubule and the collecting duct of the nephron. Because the increase in sodium reabsorption occurs after 1- to 2-h hormone administration, it was inferred that transcriptional gene activation was necessary (Fig. 1). Numerous studies have documented these effects. Glucocorticoid hormones also affect sodium transport; however, it remains difficult to dissect the respective effects of mineralo- vs. glucocorticoid hormones, mainly because these two classes of hormones seem to act in a complementary manner.

Both corticosteroid hormones bind to intracellular receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). These receptors are members of the nuclear receptor family that includes receptors for steroid and thyroid hormones, vitamin D₃, and retinoic acids, as well as numerous orphan receptors for which no ligand is known (6, 32, 72, 94, 111). These receptors have a modular structure comprising five to six regions (Fig. 2). The NH₂-terminal A/B region harbors an autonomous activation function. The central C domain (DNA binding domain; DBD) is highly conserved and composed of two zinc fingers involved in DNA binding and receptor dimerization. A hydrophilic D region forms a hinge between the DBD and the COOH-terminal ligand binding domain (LBD). The
LBD mediates numerous functions, including ligand binding, interaction with heat-shock proteins and transcriptional coactivators, dimerization, nuclear targeting, and hormone-dependent activation (23, 32, 72, 94, 111). Although there is 15% homology between MR and GR within the NH2-terminal region, the LBD and the DBD are relatively or very homologous (57% homology in the LBD and 94% in the DBD; see Fig. 2). In the absence of hormone, the MR is part of a large protein complex, in which it interacts with the 90-kDa heat shock protein (HSP90). Ligand binding promotes conformational changes in the receptor and HSP90 release. Then, the receptor acts as a ligand-dependent transcription factor (Fig. 1). MR and GR bind as dimers to common glucocorticoid-response elements, and no specific mineralocorticoid-response element has been identified so far. Receptor-induced activation of target genes determines synthesis or repression of proteins, which are ultimately responsible for the physiological effects of the hormones (Fig. 1). Aldosterone increases sodium entry at the apical membrane of the cells of the distal nephron through the amiloride-sensitive sodium channel (ENaC); sodium is then extruded toward the extracellular and blood compartments by Na-K-ATPase, located in the basolateral membrane of the cells (49). It is now clear that ENaC and Na-K-ATPase are not early aldosterone-induced proteins, and an active search for such aldosterone-induced proteins is now undertaken. Among them, the serum and glucocorticoid-regulated kinase (sgk) emerged as a primary aldosterone-induced gene (21, 80). The efforts to characterize molecular events involved in early aldosterone action have been reviewed recently (114, 115) and will not be developed here.

Glucocorticoid hormones act in a broad variety of cells, whereas mineralocorticoid action is restricted to fewer cell types. Classic aldosterone-sensitive tissues include epithelia with high electrical resistance, such as the distal parts of the nephron, the surface epithelium of the distal colon, and salivary and sweat gland ducts. More recently, other MR-expressing cells have been identified, either epithelial, as in epidermal keratinocytes (57), or nonepithelial, as in the neurons of the central nervous system (27, 59, 75), the cardiac myocytes (11, 66, 70, 88), and the endothelial and smooth muscle cells (102) of the vasculature (large vessels). Functions of MR in nonepithelial cells are not fully understood. Disturbances in the aldosterone-MR system may, however, have important pathological consequences in these tissues, as recently evoked in the pathogeny of cardiac fibrosis (105). Neuronal and cardiac effects of aldosterone are beyond the scope of this review.

All MR-expressing cells also express the GR (39). GR and MR have different but overlapping patterns of expression along the nephron. A general agreement exists to point on the distal nephron as aldosterone-specific target cells. Evidence has been provided for MR expression at the mRNA (30, 107) and the protein level (39, 68) in the distal tubule, the connecting tubule, and all along the collecting duct. Specific nuclear binding sites for aldosterone exist from the thick ascending limb of Henle’s loop (cortical part) to the end of the collecting duct (36, 41–43). The situation is less clear for the GR: its mRNA (30, 107) has been found all along the nephron, at approximately similar levels between tubular segments. Surprisingly, immunodetection of GR was negative in the proximal tubule (39), whereas all other tubular segments were positive. In addition, specific nuclear binding sites for dexamethasone have
been evidenced in the glomerulus and all along the tubule, except for the whole proximal tubule (42). These results suggest that the other form of GR [beta isoform (26)], distinct from the classic GR, may exist in this epithelium; this form is not recognized by the GR antibody and is unable to translocate into the nucleus, in experimental conditions where the neighboring cells of the thin descending limb of Henle’s loop (following the pars recta) do show nuclear binding. The nature of the GR expressed in the proximal tubule remains to be clarified.

An important finding that issued from expression studies of MR is that the receptor is not ligand selective. Indeed, both aldosterone and glucocorticoid hormones bind MR with similar high affinity [dissociation constant ($K_d$): 0.5–2 nM]. Furthermore, plasma concentrations of glucocorticoid hormones are 100- to 1,000-fold higher than those of aldosterone (0.1–1 nM), and each hormone level varies according to different stimuli (see Table 1). The large prevalence of glucocorticoid hormones in the plasma should thus lead to permanent maximal occupancy of MR, leading to sustained maximal sodium reabsorption, precluding any regulatory role of aldosterone. Because this is obviously not compatible with the well-known physiological mineralocorticoid action of aldosterone, efforts were made to understand how this hormone could act selectively in its target cells. Major progress in the dissection of the cellular and molecular mechanisms of mineralocorticoid selectivity has emerged over the last 12 years. Interestingly, each of these mechanisms is likely to vary quantitatively among cell types and will also be influenced by the cell context. Because they occur at different steps of aldosterone action, these mechanisms appear mutually interdependent, constituting a cascade of successive dynamic equilibriums (34, 35, 45).

**PHYSIOLOGICAL RESPONSES TO ALDOSTERONE AND GLUCOCORTICOID HORMONES ARE DIFFERENT, BUT PARTIALLY OVERLAPPING**

Numerous experiments showing the differential effects of aldosterone and glucocorticoid hormones have been performed in the kidney and the colon. In the latter epithelium, it has been proven unambiguously that each hormone has distinct effects on ion movements. In the distal part of the colon, aldosterone increases active electrogenic sodium absorption and potassium excretion, whereas the glucocorticoid receptor agonist RU-28362 does not modify these active transports (112).

In the kidney, results have sometimes been conflicting because of the complexity of this organ. Indeed, whereas aldosterone action is restricted to the distal nephron (including the cortical part of the thick ascending limb of Henle’s loop), glucocorticoid hormones increase the glomerular filtration rate and affect tubular functions all along the nephron (16). Therefore, interpretations of clearance data are sometimes complex. Infusion of low doses of aldosterone to rats results in a delayed (1- to 2-h) increase in net renal sodium reabsorption (occurring at the level of the collecting duct); the synthetic glucocorticoid hormone dexamethasone may also promote sodium reabsorption, as shown initially in the reports of Campen et al. (20) and Horisberger and Diezi (53). Our purpose here is not to examine in detail the numerous subsequent reports (for example, see Refs. 54, 84, 106). Although there is general agreement from which to state that aldosterone increases renal sodium reabsorption, its effect on potassium secretion remains more controversial (86). Glucocorticoid hormones affect both sodium absorption and potassium secretion, and this latter effect may be attributable to the increase in glomerular filtration rate elicited by the hormone, resulting in a flux-dependent potassium secretion. In fact, many reports on the renal effects of aldosterone, corticosterone, cortisol, or dexamethasone have been published, with sometimes conflicting results. These contradictions may arise from the variety of experimental protocols used, susceptible to interference with other regulatory pathways. Most experiments have been performed in normal, adrenalectomized, and hormone-infused rats, in rabbits, and in animals subjected to various diets (low-or high-Na/K intake). For example, adrenalectomy suppresses both aldosterone and corticosterone secretion (as well as catecholamines of adrenal origin), a situation that results in a decrease in glomerular filtration rate, in sodium losses, and in contraction of extracellular volume. Thus several hormonal systems are likely activated to compensate for the disequilibrium induced by adrenalectomy. Among these factors, an increase in arginine vasopressin (AVP) probably plays an important role, because it controls both water and sodium renal reabsorption. Conversely, thyroid hormone levels are reduced after adrenalectomy (Farman, personal observations), thus affecting tubular functions [synergism between aldosterone and thyroid hormone effects has been reported in the collecting duct (10)]. In an attempt to illustrate the hormonal changes that occur in experiments aimed at manipulating plasma aldosterone levels, Table 1 shows the variations in glucocorticoid hormones and AVP along with those of aldosterone. This view is restrictive, of course, because many other hormonal systems are likely involved (among them, atrial natriuretic factor,

### Table 1. Variations in plasma hormone concentrations in experimental situations designed to manipulate plasma aldosterone levels

<table>
<thead>
<tr>
<th>Aldosterone</th>
<th>Glucocorticoid Hormones</th>
<th>AVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomy</td>
<td>↓ ↓ or ←</td>
<td>↑</td>
</tr>
<tr>
<td>High-Na diet</td>
<td>↓ or ←</td>
<td>↑</td>
</tr>
<tr>
<td>Low-K diet</td>
<td>↑ ←</td>
<td>↑</td>
</tr>
<tr>
<td>Low-Na diet</td>
<td>↑ ←</td>
<td>↑</td>
</tr>
<tr>
<td>High-K diet</td>
<td>↑ ←</td>
<td>↑</td>
</tr>
<tr>
<td>Water restriction</td>
<td>↑ ←</td>
<td>↑</td>
</tr>
<tr>
<td>Stress</td>
<td>↑ ←</td>
<td>↑</td>
</tr>
</tbody>
</table>

AVP, arginine vasopressin. Arrows indicate observed changes in hormone levels: normal (←), increased (↑), or decreased (↓).
dopamine, catecholamines, adrenomedullin), and they can modify sodium transport in the distal nephron.

It is striking to see how well rodents (rats, mice) can survive in the absence of adrenals (with a sole salt supplement), in contrast to the dramatic and life-threatening disturbances observed in humans suffering from adrenal insufficiency. Thus one can wonder whether murine models can provide completely satisfying answers to these questions.

A great number of physiological studies have also been performed in amphibians (toad bladder, frog skin), and the use of amphibian cell lines (A6 or TBM cell lines) has allowed important progress; however, there is now general agreement to consider the effects of aldosterone in A6 cells as relevant to the occupancy of GR rather than MR (73, 100). A similar situation may also occur in mammalian cells in culture (61, 81), in which the expression of MR is much less than that in native collecting duct cells. Recently, an aldosterone-sensitive collecting duct mouse cell line, which increases its sodium transport in the presence of low doses of aldosterone (12), has been characterized. It may be a powerful tool in exploring aldosterone-related cellular events in mammalian cells. Nevertheless, our purpose here is not to review the different cellular models that are now available.

Another difficulty is that physiological effects (on ion excretion) are evidenced several hours after hormone exposure, and little is known, at the present time, about the molecular events involved in the early phase of aldosterone action. The search for aldosterone or early-induced glucocorticoid proteins will likely depend on the model chosen; efforts are presently being made to identify such proteins (114), and most models used (cultured cells) might make one unable to distinguish between MR and GR transcriptional effects.

**ARE MR AND GR REDUNDANT?**

Because MR and GR are homologous, and because their expression pattern shows overlapping, it was reasonable to ask whether they might be redundant. A nice, negative answer to this question was provided by work with their respective knockouts. Genetic invalidation of the GR results in mice that die a few hours after birth (22). They exhibit profound impairment of lung maturation and lack of activation of genes of key gluconeogenic enzymes. The phenotype of MR-knockout mice is different (13): during the first days of the postnatal period, there is a progressive syndrome of sodium and water loss, with weight loss, hyperkalemia, and hyponatremia (close to human pseudohypoaldosteronism), leading to death. Interestingly, the MR-knockout mice can be rescued by sodium chloride injections (14). These experiments clearly show that each of these receptors has specific functions that cannot be overcome by the remaining receptor.

**A MAJOR STEP IN MINERALOCORTICOID SELECTIVITY IS ENZYMATIC**

The high plasma glucocorticoid levels that reach the mineralocorticoid target cells are somewhat reduced by their binding to plasma albumin and to corticosteroid binding globulin: only 10% of cortisol is free in the plasma, whereas aldosterone circulates mainly in a free form. Twelve years ago (29, 47), it was demonstrated that an enzyme, 11β-hydroxysteroid dehydrogenase (11β-HSD), plays a critical role in preventing major glucocorticoid access to the cells. The enzyme belongs to the short-chain alcohol dehydrogenase family, and the isoform responsible for MR protection (11β-HSD2) was cloned and characterized functionally (1, 2, 85). Detailed reviews on 11β-HSD2 properties have been published recently (for example, see Refs. 85 and 103). Several aldosterone target cells express 11β-HSD2, which transforms glucocorticoids (cortisol in humans, corticosterone in rodents) into metabolites (cortisone, 11-dehydrocorticosterone) that have weak or no affinity for the MR (and the GR as well). Thus, in cells coexpressing MR and 11β-HSD2 (16, 82, 99), permanent occupancy of MR by glucocorticoid hormones is largely prevented, allowing concentration-dependent binding of aldosterone to MR and regulation of sodium reabsorption. 11β-HSD2 catalytic activity has been evidenced initially by Bonvalet et al. (16): high activity is present in the loop, distal tubule, and connecting tubule, and all along the collecting duct of the rabbit kidney. Some minor variations for this expression pattern exist among species, i.e., rats, rabbits, and humans (55, 56). After the identification of the two molecular forms of 11β-HSD (11β-HSD1, a NADP-dependent oxidoreductase, and 11β-HSD2, a NAD-dependent dehydrogenase), it appeared that the form of the enzyme responsible for MR selectivity (11β-HSD2) was restricted to the distal nephron, whereas the proximal tubule exhibits 11β-HSD1 activity (4, 82, 99). Similar results were reported by using specific tools for these two enzyme forms at the mRNA and protein levels (18, 60, 96). Thus tubular cells expressing the MR (distal nephron) do have high levels of 11β-HSD2, allowing selective aldosterone action.

The major role of 11β-HSD2 is highlighted by clinical situations (79, 118) in which the enzyme is inactive, because of mutations [syndrome of apparent mineralocorticoid excess (AME)] or to its inhibition (by glycyrhetinic acid, a derivative of licorice): patients exhibit hypertension, hypokalemia, and very low levels of renin and aldosterone. These clinical features are due to permanent occupancy of MR by endogenous glucocorticoids. Recently, a mouse model for AME has been produced by inactivation of the 11β-HSD2 gene. Mice lacking 11β-HSD2 (+/− mice) develop marked hypertension, hypokalemia, and profound suppression of plasma renin activity and aldosterone levels (58). Unexpectedly, this phenotype was not observed in heterozygote mice (11β-HSD2 +/−), at variance with human inactivating mutations of 11β-HSD2 producing AME.

Little is known about regulation of 11β-HSD2; however, such regulations are potentially of great pathophysiological interest, because they will likely modulate the amounts of glucocorticoids that will go through this major selectivity filter and thus bind to corticoste-
roid receptors. Incubation of isolated cortical collecting ducts with AVP results in a stimulation of 11β-HSD2 catalytic activity (through the protein kinase A (PKA) pathway). Interestingly, this acute in vitro AVP effect on 11β-HSD2 was observed only in tubules originating from adrenalectomized rats treated in vivo with aldosterone (48 h), whereas the phenomenon was absent when rats were infused with corticosterone or dexamethasone for the same period of time (3). This indicates that chronic treatment with aldosterone specifically modifies the status of the collecting duct cells to allow AVP stimulation of 11β-HSD2 activity. These data suggest that aldosterone and vasopressin pathways coordinately interact to upregulate 11β-HSD2, thus reinforcing mineralocorticoid selectivity in the collecting duct.

The 11-dehydro metabolites produced in the kidney by 11β-HSD2 were initially considered inactive. However, recent data indicate that they behave as low-affinity aldosterone antagonists (78). Other steroid hormones are also metabolized in the kidney and may interfere with corticosteroid action in this organ. For example, progesterone and its metabolites have been shown to inhibit 11β-HSD2 (89).

Although it is clear that 11β-HSD2 plays a pivotal role in MR protection, it is likely that some glucocorticoid hormones remain unmetabolized (the enzyme does not function at 100% of its capacity) and thus can reach the MR and the GR. A great deal of recent data indicates that the MR itself can play a significant role in mineralocorticoid selectivity. Because this notion is relatively recent and complex, it will be evoked in detail in the section that follows.

ROLE OF THE MINERALOCORTICOID AND GLUCOCORTICOID RECEPTORS IN HORMONAL SELECTIVITY

Evidence has been accumulated that MR displays different properties depending on the nature of the ligand. Studies have revealed that both the MR conformation and the MR nuclear translocation are dependent on the ligand (24, 44, 67, 108). The kinetics of MR-ligand interaction are also very different: aldosterone dissociates more slowly from MR than does cortisol, indicating that the stability of the aldosterone-MR complex is higher than that of the cortisol-MR complex (51, 52, 69). From this observation it can be suggested that the off-rates of aldosterone and cortisol to MR are also different, as the on-rates of aldosterone and cortisol to MR are (51, 52, 69). The kinetics of MR-ligand interaction are also very different: aldosterone dissociates more slowly from MR than does cortisol (24, 76, 97, 108). The MR transactivation activity measured in cis-trans cotransfection assays is also highly dependent on the ligand: the aldosterone concentration required to induce 50% of the maximal MR activity (ED\textsubscript{50}) is ~100-fold lower than that of cortisol (5, 51, 69, 98). Thus the slow off-rate of aldosterone from MR is responsible for its high efficiency in stimulating MR transcriptional activity. In other words, the stability of interaction is critical to determine transactivation properties. Major progress in the understanding of the molecular interactions between steroid hormones and their receptors has appeared recently, because of a combination of structural analyses and targeted mutagenesis of receptors, together with functional analysis of mutant receptors. We have chosen to detail here the most recent knowledge on the distinct interactions between the MR or GR and aldosterone or glucocorticoid hormones.

The crystal structure of several nuclear receptor ligand binding domains is now available, revealing a common fold with 11–12 α-helices (numbered H1-H12) and 1 β-turn arranged as an antiparallel α-helical “sandwich” in a 3-layer structure (17, 19, 93, 104, 113, 116, 119). The human MR and GR LBDs (hMR- and hGR-LBDs) have not been purified, but their structures have been modeled (33, 91). The positioning of aldosterone within the hMR model, validated by mutagenesis analysis, has revealed two polar sites located at each extremity of the ligand binding cavity anchoring the two polar extremities of aldosterone: at 1 extremity, Gln\textsuperscript{776} and Arg\textsuperscript{817} anchor the 3-ketone function of aldosterone, and, at the opposite side of the cavity, Cys\textsuperscript{942} contacts the 20-ketone function and Asn\textsuperscript{770} the 21-hydroxy group (33, 71), as illustrated in Fig. 3. Glucocorticoid hormones adopt the same orientation within the hGR-LBD, with the three-ketone contact to Gln\textsuperscript{570} and Arg\textsuperscript{611} (91).

The first step in the MR and GR activation process triggered by ligand binding is a receptor conformational change (24, 76, 97, 108). Our knowledge concerning ligand-induced conformation changes has been improved by structural data of nuclear receptor LBDs (77). The major difference between the ligand-free and ligand-associated receptors is the positioning of H12, which contains a motif critically required for the li-
gand-dependent activation function. Agonist binding results in a major transition of H12, placing it like a lid over the ligand binding pocket, where it contributes to the surface required for coactivator interaction. Antagonist binding modifies H12 positioning, as H12 adopts a distinct position that does not allow receptor interaction with transcriptional coactivators. The way by which ligand binding triggers the repositioning of H12 is not well understood. As far as the hMR is concerned, a network of contacts is required for the stabilization of the active conformation; it involves contacts between the 21-hydroxyl group of aldosterone and the amino acid Asn$^{770}$ of the hMR and also contacts between the amide group of Asn$^{770}$ and the backbone oxygen atom of Glu$^{655}$, in the loop connecting H11 and H12 (52). Conversely, the antagonist effect of progesterone or spirolactones (see formulas in Fig. 4A), two small molecules that dissociate more rapidly from the hMR than aldosterone (90), has been related to the loss of these critical ligand-protein contacts (33).

One can wonder about the mechanisms involved in the preferential recognition of aldosterone and cortisol by their cognate receptors, as the C21 hydroxyl group is common to both aldosterone and cortisol and the

![Fig. 4. A: structural formulas of the corticosteroids. B: transactivation properties of hMR and hGR in response to various corticosteroids: aldosterone (Aldo); deoxycorticosterone (DOC); corticosterone (B); cortexolone (S), cortisol (F), or dexamethasone (Dex). Results are expressed as %receptor activities in response to 10$^{-9}$ M aldosterone (hMR; left) and 10$^{-8}$ M dexamethasone (hGR; right). Data are from Ref. 51.](http://ajprenal.physiology.org/)

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Several putative phosphorylation sites have been identified in the sequence of steroid receptors. The influence of receptor phosphorylation status has been evaluated in detail for the GR (117); it may affect the transcriptional response of the GR and likely intervenes in regulating the level of expression of the receptor (117). Although very limited information is available on this topic for the MR, indirect experiments suggest that the MR also undergoes phosphorylation (48). A detailed analysis of the nature of the effects of MR phosphorylation should bring interesting new information.

**DIMERIZATION OF STEROID RECEPTORS**

Dimerization of steroid receptors is required for binding to hormone-response elements and activation of gene transcription (64, 109, 110). The MR can homodimerize (MR-MR) or associate with the GR (MR-GR). Experiments in which variable proportions of each receptor cDNA were transfected to test their efficiency on a reporter gene revealed that transactivation activity was dependent on the nature of the dimer formed. In some cases, transcription synergy was evidenced on MR and GR cotransfection, whereas, in other cases, it was shown that MR inhibits GR transcriptional activity (64, 65, 109, 110). These interactions appear to be dependent on the cellular context and/or on the promoter used for driving the reporter gene (62, 63). In addition, recent studies revealed that heterodimers might be formed with only one receptor entity in a liganded state, increasing the diversity of steroid hormone effects. This is, namely, the case for GR-β, the nonhormone binding splice variant of hGR, that exerts an inhibitory effect on GR-α activity and for...
which physical association with GR-α has been clearly demonstrated (26, 83). GR-β also reduces the transcriptional activity of hormone-activated MR, consistent with the formation of an heterodimer between GR-β and the MR (9). As the two entities of the heterodimer MR-GR-α may be liganded by aldosterone or glucocorticoid hormones, the steroid context might also be of crucial importance, because the stability of a steroid-receptor complex is highly dependent on the nature of the steroid. Another example of a possible heterodimer formation is observed with the molecular form of the progesterone receptor (PR-A) that is functionally inactive in some specific cellular contexts. PR-A functions as a hormone-dependent inhibitor of MR and GR, and progesterone ligands differ in their ability to facilitate the inhibitory function of PR-A (74).

It is quite difficult to evaluate the in vivo relevance of homodimers (MR-MR) vs. heterodimers (MR-GR) in particular because all MR-expressing cells also express the GR. In an attempt to gain some insight into this question, we have collected some data concerning the estimated relative number of MR and GR (as binding sites) in different cell types (Table 2). For example, in collecting duct cells, there are 10,000 aldosterone binding sites/cell (41, 43) and about twice that number of glucocorticoid binding sites (42), resulting in an MR/GR ratio of 1:2. This ratio is usually overestimated in biochemical studies (40), which compare the maximal number of binding sites of each steroid as measured in whole kidney: indeed, GR are found all along the nephron (except, perhaps, in the proximal tubule), whereas MR is restricted to the distal nephron (10% of kidney cells), so that the whole kidney MR/GR ratio largely reflects the low abundance of distal nephron cells within the organ. On the whole, it appears that glucocorticoid binding sites are slightly (2-fold) more abundant than that those for MR in renal collecting duct cells and in the surface epithelium of the distal colon (101). In the heart (11) and arterial smooth muscle cells (102), the number of glucocorticoid binding sites seems to be much higher than those for MR (resulting in a MR/GR ratio close to 1:30). Thus it is likely that, in these two categories of tissues, the proportion of MR-GR heterodimers varies greatly, resulting in distinct transactivation properties. Tissue-specific in vivo alterations of this ratio (by mouse genetic engineering) should bring new information on the relevance of such dimers in organ physiology.

### Table 2. Variable proportions of aldosterone (MR) and glucocorticoid (GR) binding sites among tissues

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>MR</th>
<th>GR</th>
<th>MR/GR</th>
<th>Ref. No(s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal collecting duct</td>
<td>10,000/cell</td>
<td>20,000/cell</td>
<td>1/2</td>
<td>40–43</td>
</tr>
<tr>
<td>Colonic epithelium</td>
<td>7,000/cell</td>
<td>21,000/cell</td>
<td>1/3</td>
<td>101</td>
</tr>
<tr>
<td>Brain hippocampus</td>
<td>100 fmol/mg protein</td>
<td>100 fmol/mg protein</td>
<td>1/1</td>
<td>59</td>
</tr>
<tr>
<td>Arterial smooth muscle cells</td>
<td>1,000/cell</td>
<td>30,000/cell</td>
<td>1/30</td>
<td>102</td>
</tr>
<tr>
<td>Cardiac myocytes</td>
<td>10 fmol/mg protein</td>
<td>300 fmol/mg protein</td>
<td>1/30</td>
<td>11</td>
</tr>
</tbody>
</table>

Estimates of the relative abundance of mineralocorticoid (MR) and glucocorticoid (GR) receptor, measured by binding experiments, vary greatly among cell types. Data have been taken from the literature and are expressed as either number of binding sites per cell or femtomoles of hormone bound per mg protein.
transcripts are unaffected by either corticosteroid hormone in the neighboring pyramidal neurons of the parietal cortex (37). The subunits of ENaC also have tissue-specific distinct hormonal sensitivity. In the kidney (collecting duct), aldosterone (not dexamethasone) upregulates the mRNA encoding for the α-subunit of ENaC (whereas β- and γ-subunits are unchanged) (7, 31). Conversely, in the colon, both hormones increase β- and γ-transcripts (α remains unchanged) (7, 31). In the lung, glucocorticoid hormones increase the level of expression of all three ENaC subunits whereas aldosterone has no effect (92). Thus it appears that MR and GR likely interact with tissue-specific factors (as yet unidentified) to exert effects that differ among tissues.

Cooperation of corticosteroids with other hormones may also be relevant to such interactions. It is well established that AVP acts in synergy with aldosterone or glucocorticoid hormones in kidney cells (50). AVP activates the cAMP-PKA pathway to modulate short-term sodium reabsorption in the collecting duct, and such a response is augmented in the presence of aldosterone; this phenomenon has been well documented, using physiological approaches, in particular by Hawk et al. (50), although its precise molecular mechanism remains unknown. AVP can also promote, after several hours, a delayed stimulation of sodium transport, as demonstrated in cultured collecting duct cell (28); such an effect involves a de novo increase in transcripts and protein synthesis encoding the β- and γ-subunits of ENaC (not α). It is likely reminiscent of cAMP-dependent activation of transcription factors such as cAMP response element binding protein (CREB) or cAMP response element modulator (CREM), which bind to cAMP-responsive elements present in the promoter region of regulated genes. It is conceivable that full regulation of sodium transport by aldosterone (which upregulates the α-ENaC subunit) and by AVP (acting on β- and γ-ENaC subunits) involves nuclear protein-protein interactions between the MR and CREB/CREM (or other PKA-sensitive transcription factors). Such a mechanism may also be relevant in the cooperation between the GR and the AVP-cAMP pathways that exists in the thick ascending limb of Henle’s loop to modulate concentration of urine (8).

In conclusion, we have described here the main determinants of mineralocorticoid selectivity. We have documented the complementary and sequential contribution of the enzyme 11β-HSD2 and the MR. The MR intervenes at numerous steps as the ligand modulates the receptor transconformation and likely the homo- or heterodimerization with the GR and the interactions with other transcription factors. Each of these cellular events will ultimately influence the nature and/or the magnitude of the response of the tissues after aldosterone and/or glucocorticoid exposure. It is important to realize that these mechanisms are ordered so that a small change in the earlier ones will affect the dynamic equilibrium of the following downstream steps. For example, any modification of 11β-HSD2 activity will affect the relative proportion of aldosterone vs. glucocorticoid hormones bound to the MR and/or GR and thus influence the rest of the cascade. It is necessary to realize that in vitro studies of each step of mineralocorticoid selectivity are performed in the most appropriate model. It is not known at the present time how these models fit closely to in vivo situations. For example, from cis-trans cotransfection assays, it is clear that MR activation requires 100-fold more glucocorticoid hormones than aldosterone, as measured on an artificial mouse mammary tumor virus-glucocorticoid-response element promoter-reporter gene construct. It is likely that this figure may vary somewhat when receptors are facing other promoters. Future experiments should examine the transactivation properties of MR-ligand complexes on endogenous target genes (early aldosterone-induced proteins). Along the same line, it has been shown that the nature of the cell context is an important determinant of the response (45). Here again, it will be important to use several cell types (collecting duct, colon, heart) to understand our standing of the tissue-specific in vivo relevance of the role of the MR itself. Another important issue is that the relative importance of each step contributing to mineralocorticoid selectivity varies among aldosterone target tissues. Although 11β-HSD2 represents the main selectivity filter in the kidney, this is probably not the case in the heart, where 11β-HSD2 is 100 times less active than in the collecting duct. Thus post-11β-HSD2 events are likely very important in cardiac myocytes, where the specific transcriptional properties of ligand-bound MR and GR, and their variable combinations, may play a very significant physiological role.

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