Mineralocorticoid selectivity: Molecular and cellular aspects

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Mineralocorticoid selectivity: Molecular and cellular aspects. Aldosterone acts in mineralocorticoid-sensitive cells by binding to the mineralocorticoid receptor (MR). Because the MR displays similar affinity for aldosterone and glucocorticoid hormones and because these latter hormones are 100-1000-fold more abundant than aldosterone in the plasma, mechanisms are required to avoid permanent illicit occupancy of MR by glucocorticoid hormones. The main mechanism of mineralocorticoid selectivity is enzymatic: the 11β hydroxysteroid dehydrogenase (HSD2) metabolizes glucocorticoid hormones into derivatives with a low affinity for MR. The cell biology and regulation of HSD2 are reviewed in this article, as well as its implications in human hypertension. Other factors play a role in mineralocorticoid selectivity: the MR itself, the possibility to form homodimers (MR-MR), or heterodimers (with the glucocorticoid receptor). All of these cellular events participate to successive dynamic equilibriums, which allow fine tuning of transcriptional regulation of target genes, depending on the target tissue and the hormonal status.

It has long been known that corticosteroid hormones are important for the maintenance of renal sodium reabsorption, volemia, and blood pressure levels [1, 2]. The mineralocorticoid hormone aldosterone promotes sodium absorption in the renal distal nephron, the colon, and the ducts of sweat and salivary glands. Glucocorticoid hormones (cortisol in humans and corticosterone in rodents) also play an important role in the control of ion reabsorption and the glomerular filtration rate. These two hormones appear to act in a complex, distinct but complementary pattern, which is not fully understood at the present time. Both bind intracellular receptors [1-3], the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR; Fig. 1). These receptors are members of the superfamily of steroid hormone receptors that act as ligand-dependent transcription factors [4]. They interact (directly or indirectly) with glucocorticoid response elements (GREs) in the promoter regions of regulated genes. GR is found in all aldosterone target cells and largely prevails over MR [5, 6]; There are about 100,000 GRs per cell in the renal collecting duct and 10,000 MRs (Fig. 1).

Because the MR displays the same affinity for aldosterone and glucocorticoid hormones (about 0.5 to 1 nmol/L) and because these latter hormones are much more abundant (100-1000-fold) in the plasma, as compared with aldosterone (Fig. 1), it is obvious that some mechanisms are necessary to protect the MR against permanent occupancy by glucocorticoid hormones [7, 8]. The main mechanism of mineralocorticoid selectivity depends on an enzyme [7, 8], 11β hydroxysteroid dehydrogenase (HSD), which metabolizes cortisol (or corticosterone) into derivatives (cortisone or dehydrocorticosterone) with little or no affinity for MR (and GR as well). Thus, in the presence of HSD2, glucocorticoid hormones entering the cell are metabolized into 11-dehydro derivatives, and the MR will be occupied by aldosterone as a function of its plasma levels. In the absence of HSD2, the MR will be occupied by glucocorticoid hormones, and a permanent maximal sodium reabsorption will occur, without any possible regulation by aldosterone. Such a situation is achieved when the enzyme is inhibited (hypertension caused by licorice ingestion) or genetically inactive [hypertension from the syndrome of apparent mineralocorticoid excess (AME)]. Several recent reviews have been published on the subject [9-13].

11β-hydroxysteroid dehydrogenase is coexpressed with MR and GR in renal aldosterone target cells [1], as illustrated in Figure 2. The MR is expressed selectively in distal tubules and all along the collecting duct of the rabbit [5, 6] and presumably in the mouse and in humans. In the rat, however, MR expression extends to the cortical part of the thick ascending limb of Henle’s loop (TAL) [14]. HSD2 activity is high in distal tubules and collecting ducts of the rabbit, the mouse, and humans [15-21]. As was observed for MR, high HSD2 activity extends to the TAL in rats [17, 18]. Besides renal coexpression of MR and HSD2, other (putative) aldosterone target tissues have been examined. Coexpression has been documented, as expected, in sweat gland ducts [22] and in the epithelium of distal colon [23]. In nonepithelial cells, however, the situation is more complex. Evidence
Table 1. Comparison of mineralocorticoid receptor (MR) and 11β-dehydroxysteroid dehydrogenase-2 (HSD2) abundance

<table>
<thead>
<tr>
<th></th>
<th>MR receptor per cell</th>
<th>HSD2 activity fmol/mg protein/10 min</th>
<th>HSD2 MR ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collecting ducts</td>
<td>10,000</td>
<td>50,000</td>
<td>5</td>
</tr>
<tr>
<td>Cardiac myocytes</td>
<td>500–1000</td>
<td>70</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The HSD2:MR ratio shows that cardiac myocytes MR are 50 times less protected (by HSD2) than collecting duct cells. Data are from [5, 18, 24–26].

for MR expression (and specific aldosterone binding) has been provided in cardiac myocytes [24, 25] in the rabbit and humans, and HSD2 activity has been evidenced in cardiac biopsies [25], at levels lower than those observed in classic aldosterone cells such as the collecting duct (Table 1) [26]. A comparison of the amounts of HSD2 available to protect MR in these two cell types shows that the collecting duct cell MR is much better protected by HSD2 than the cardiomyocyte MR (50 times less efficient protection; Table 1). One can wonder whether the cardiac MR-HSD2 system is sufficiently tight to avoid binding of glucocorticoid hormones or whether a significant proportion of MR may be occupied by these latter hormones in vivo. In the brain (particularly in the hippocampus), MR is expressed at high levels [27–30], and there is a general agreement to consider that HSD2 is very low in these tissues. This would mean that MR in brain is permanently occupied by glucocorticoid hormones. However, it remains to be understood why aldosterone and corticosterone exert distinct effects, at least in some regions of the brain (article by de Kloet et al, in this issue of *Kidney International*, p. 1329).

**CELL BIOLOGY OF HSD2**

11β-hydroxysteroid dehydrogenase (HSD) activity has been studied for a long time and was characterized first from liver microsomal preparations [31]. Initial cloning revealed a form of the enzyme (named HSD1) that was absent from mineralocorticoid-sensitive tissues [32]. The MR-protecting enzyme (named HSD2) was subsequently cloned [33, 34], and the differences between these two forms are listed in Table 2. Several excellent reviews have been published on this subject [11–13, 31, 35]. Here, we would like to emphasize some aspects of the cell biology of HSD2. First, the enzyme HSD2 is consistently found as a unidirectional enzyme (dehydrogenase activity only); this unexpected behavior is unexplained. Another peculiarity of the HSD2 is the wide range of substrate concentrations (0.1 nmol/L to 1 μmol/L) where the enzyme is active [26], which is not compatible with simple michaelian enzyme kinetics. This aspect has been misregarded by most investigators, who considered
Table 2. Differences between HSD1 and HSD2

<table>
<thead>
<tr>
<th></th>
<th>HSD1</th>
<th>21% identity</th>
<th>HSD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity for cortisol</td>
<td>Low (100 nm)</td>
<td></td>
<td>High (1–10 nm)</td>
</tr>
<tr>
<td>Cofactor</td>
<td>NADP</td>
<td></td>
<td>NAD</td>
</tr>
<tr>
<td>Enzymatic properties</td>
<td>Dehydrogenase and reductase</td>
<td></td>
<td>Dehydrogenase</td>
</tr>
<tr>
<td>Expression</td>
<td>Ubiquitous</td>
<td></td>
<td>Aldosterone-sensitive cells</td>
</tr>
<tr>
<td>Function</td>
<td>Local regulation of glucocorticoid action</td>
<td></td>
<td>Protection of MR</td>
</tr>
<tr>
<td>Molecular wt</td>
<td>34 kD (290 aa)</td>
<td></td>
<td>44 kD (400 aa)</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>Important for activity</td>
<td></td>
<td>Not required for activity</td>
</tr>
</tbody>
</table>

Abbreviations are: HSD, 11β-hydroxysteroid dehydrogenase; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; MR, mineralocorticoid receptor.

it as a michaelian enzyme [12, 13, 19, 21]. However, its functioning may be more complex (nonmichaelian), involving a series of equilibriums between multimers of the enzyme (for example, dimers and tetramers). It has been suggested that HSD2 exists as a dimer, and other members of the short-chain alcohol dehydrogenase (SCAD) superfamily also function as multimers [13]. The SCAD superfamily, to which both HSD1 and HSD2 belong, includes more than 100 members; the three-dimensional structures of some of them have been solved by x-ray crystallography. Interestingly, the 3α-20β-HSD is a tetramer, and it has been suggested that the 11β-HSD enzymes exhibit protein folding very similar to those of 3α-20β-HSD and 17β-HSD1 [13, 35].

HSD2 has a hydrophobic N-terminal domain that is considered important for anchoring in the membrane of the endoplasmic reticulum [13]. However, it has been shown that deletion of this region does not modify its activity and does not release the enzyme in the cytoplasm [13, 36]. Elegant studies with green fluorescent protein (GFP)-tagged HSD2 transfected in Chinese hamster ovary (CHO) cells indicated that HSD2 is localized in the endoplasmic reticulum [37], whereas other studies [13, 23] found nuclear localization of the enzyme, as demonstrated by immunolocalization. Further studies are needed to clarify the subcellular localization of HSD2.

Little information is available on putative post-translational modifications of HSD2. Despite the presence of one potential N-glycosylation site, such a phenomenon does not seem to be effective, since the molecular weight of HSD2 is not influenced by tunicamycin or N-glycosidase treatment [13, 38]. Other post-translational modifications, such as phosphorylation or myristoylation may exist, but experimental investigations have not yet been performed to test these possibilities.

11β-HYDROXYSTEROID DEHYDROGENASE 2 AND HYPERTENSION

Clinical evidence for the major role of HSD2 came from observations of some forms of arterial hypertension, where the enzyme appeared to be abnormal [12, 13, 39]. The syndrome of apparent mineralocorticoid excess (AME) is a rare form of congenital hypertension, with severe hypertension, hypokalemia, and low levels of renin and aldosterone. Genetic analyses of the kindreds showed mutations in HSD2 as being responsible for inactivation of the enzyme [13, 39]; in these children, cortisol permanently occupies the MR and promotes sustained sodium reabsorption. A mouse model for AME has been obtained recently by inactivation of HSD2 using homologous recombination [40]; these knockout mice develop hypertension, hypotonic polyuria, and low levels of plasma aldosterone. The enzyme may also be inhibited by glycyrrhetinic acid, the active metabolite of licorice [12, 13]. Hypertension, which develops after chronic ingestion of licorice, is due to HSD2 inhibition, and normalization of blood pressure occurs after licorice withdrawal.

Other situations (for example, subgroups of essential hypertension) may be reminiscent of abnormal HSD2 activity, although definite information is actually lacking [12, 13], probably because of the difficulty to estimate the in vivo activity of the enzyme. A good estimate of the efficiency of the enzyme can be obtained by the plasma or urine ratio of the THE/THF, that is, the tetrahydro-derivatives of cortisone (E) and cortisol (F). Abnormally low ratios have been reported in patients with AME [39], but a search for milder involvement of the enzyme deficiency in essential hypertension has been disappointing using this test (as well as the search for mutations of HSD2). It should be taken into consideration that the ratio of reduced versus dehydrogenated cortisol metabolites indeed depends on the activity of renal HSD2 (dehydrogenase) as well as the hepatic HSD1, which is a bidirectional enzyme (dehydrogenase and reductase). Thus, a partial defect in renal HSD2 (leading to abnormal sodium reabsorption) may be masked in blood or in urine by a partial compensation by hepatic HSD1. In order to gain more precise information from human aldosterone target cells, a microassay of HSD2 activity has been developed using sweat gland ducts [22]. These ducts may be obtained by microdissection from skin biopsies and allow an accurate measurement of the catalytic activity of the enzyme. Sweat gland
ducts also express the MR, and their level of HSD2 activity is similar to that of the collecting duct [22]. A search for an abnormal function of HSD2 in hypertensive patients is currently under investigation using this approach.

**REGULATION OF 11β-HYDROXYSteroid Dehydrogenase 2**

An important issue is to know whether or not HSD2 is subject to regulation. Surprisingly little information is available on this point. Some studies showed inhibition of the enzyme by several natural components. Besides glycyrrhetinic acid and carbenoxolone, inhibitors were found in grapefruit juice, cotton seeds, and others [13]. Some bile acids also reduce the activity of HSDs, and recently it has been suggested that endogenous inhibitors of HSD2, named GALF (for glycyrrhetinic acid-like factor) exist in the plasma and may be increased in essential hypertension (discussed in the article by D. Morris in this issue of *Kidney International*). The search for stimulatory factors has been scarce. In vivo changes in corticosteroid status in rats (deprivation by adrenalectomy and selective treatment by aldosterone or glucocorticoid hormones) showed a moderate increase [41] or no change [26] in HSD activity. No effect of corticosteroid hormones was evidenced on HSD2 transcripts. Protein kinase A may be involved in the regulation of HSD2, since arginine-vasopressin (or cAMP) stimulates HSD2 activity in vitro in isolated cortical collecting ducts [41]. Interestingly, such an effect requires aldosterone; the effect is observed only in tubules from normal animals or adrenalectomized rats receiving a substitutive aldosterone treatment. Thus, it appears that the two main hormones that exert a fine tuning of renal sodium reabsorption [42]—aldosterone and vasopressin—act coordinately to enhance HSD2 activity, thereby reinforcing mineralocorticoid selectivity.

**OTHER MECHANISMS OF MINERALOCORTICOID SELECTIVITY**

The MR itself has intrinsic properties that discriminate between aldosterone and glucocorticoid hormones [43–46]. Indeed, the MR displays the same apparent affinity for these ligands. However, it is important to remember that the affinity constant at equilibrium ($K_a$) is the ratio of $K_{on}$ and $K_{off}$, if both are increased (for example, in the case of glucocorticoid hormones binding to the MR), the apparent affinity is unchanged. It has been shown that the interaction of the MR with aldosterone has a more prolonged half-life [45], as compared with glucocorticoid hormone–MR complexes, reflecting distinct molecular ligand–MR interactions. The compaction of the MR bound to aldosterone [47] differs from that occurring when MR is bound to glucocorticoid hormones (discussed in the article by M.E. Oblin in this issue of *Kidney International*). Such distinct properties of MR, depending on its ligand, ultimately modify its transactivation capacities [45, 48]. As already noted by Arriza et al., MR-induced transactivation is much more efficient in the presence of aldosterone, the half maximal transactivation occurring at $5 \times 10^{-10}$ mol/L with aldosterone and $5 \times 10^{-8}$ mol/L with dexamethasone [48]. It should be noted that this kind of study is usually performed using a classic GRE [from mouse mammary tumor virus (MMTV)] linked to a reporter gene. It will be interesting to determine whether the aldosterone-bound MR (and the cortisol-bound MR) behaves similarly on the promoters of endogenously regulated genes, such as early aldosterone-induced proteins (most of which have not yet been characterized).

Despite the remarkable efficiency of HSD2 to metabolize most of the glucocorticoid hormones entering the aldosterone target cell, it is likely that some glucocorticoids escape this inactivating mechanism and thus are able to occupy MR (and GR) to some extent [1, 9]. This is probably a very significant regulatory pathway in these cells because it may allow the variation of the proportion of each receptor bound to each ligand. Furthermore, because of the possibility to form homodimers (MR-MR or GR-GR) or heterodimers (MR-GR), which have distinct transactivation properties [49], transcription of target genes can be controlled in a very specific pattern, which will vary with the corticosteroid status [1, 9]. Interesting information should be provided by systematic analysis of the transactivation efficiency of these homodimers/heterodimers in the presence of varying proportions of aldosterone and glucocorticoid hormones as ligands.

Because inactivation of the bulk of glucocorticoid hormones by HSD2 precedes the formation of liganded homodimers/heterodimers, any change in HSD2 activity will likely modify this series of dynamic equilibriums by affecting the downstream steps, that is, the relative proportion of receptors occupied by each ligand [9].

Transcription of target genes will also depend on interactions between MR (or MR-GR) and other transcription factors [9] such as tissue-specific factors, cAMP response element-binding proteins, or members of the Jun-Fos family. Such interactions have not yet been fully documented, but they are highly probable in view of the differential effects of aldosterone in distinct tissues. For example, aldosterone up-regulates the transcripts encoding for the sole α subunit of the epithelial sodium channel (ENaC) in renal collecting duct cells (β and γ unchanged), while affecting the β and γ subunits (not α) of ENaC in the epithelial cells of the distal colon [50]. Along the same line, aldosterone and vasopressin cooperate to up-regulate ENaC subunits transcripts in kidney cells, with
alderosterone affecting the α subunit, and vasopressin the β and γ subunit [50, 51].

**HOW CAN GLUCOCORTICOID HORMONES ACT IN CELLS EXPRESSING HSD2?**

As stated earlier, 11-dehydro metabolites also have little affinity for GR. Thus, in cells expressing MR, GR, and HSD2 (such as those of the renal collecting duct), mineralocorticoid selectivity is indeed ensured, but it is difficult to understand how glucocorticoid hormones can act through their own receptor [1]. Specific actions of glucocorticoid hormones on renal distal tubular function have been described, such as an increase in potassium and proton excretion [52]. Some of these effects (K+ movements) may be secondary to the glucocorticoid-induced increase in glomerular filtration rate or its effects on proximal tubule and Henle’s loop and may represent flux-dependent changes rather than primary effects [1, 52]. In addition, it has repeatedly been found that glucocorticoid hormones potentiate the effect of aldosterone, for example, for the stimulation of Na,K-ATPase [1, 52]. Further studies should help us understand how the direct effects of glucocorticoid hormones can develop in cells expressing HSD2.

**SPECULATIONS ON THE ROLE OF HSD2 IN THE REGULATION OF BLOOD PRESSURE**

Reduction of HSD2 activity is clearly responsible for an increase in blood pressure. We have seen that HSD2 may be mutated (AME) or its activity may be impaired by exogenous factors, such as licorice derivatives, or endogenous factors (GALFs). An important clinical issue will be to determine whether some drugs used for other purposes could reduce HSD2 activity after long-term treatment (for example immunosuppressive drugs) and thus participate in the maintenance of high blood pressure levels.

Alternatively, it can be proposed that HSD2 may be stimulated, under some circumstances, and that this could protect against hypertension. Activation of the cAMP-protein kinase A pathway, as observed in vitro after incubation with vasopressin [41], leads to a transient rise in HSD2 activity. Whether such a phenomenon is effective over the long-term is unknown. The search for HSD2-regulatory proteins should also provide important information. Finally, activating mutations of HSD2 (or of regulatory proteins) may exist and may be important for preventing the development and/or the maintenance of high blood pressure levels (protection from hypertension).

**REFERENCES**


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