Nuclear Receptors
II. Intestinal corticosteroid receptors

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MINERALOCORTICOID AND GLUCOCORTICOID hormones are predominantly products of the adrenal cortex and collectively are known as corticosteroid hormones. Historically, the distinctions between these steroids are based on effector criteria. For example mineralocorticoids modulate unidirectional transepithelial sodium transport, whereas glucocorticoids affect glycogen deposition in the liver. Because this early classification of steroid is based on action, it is clear that these definitions are too stringent. It is now known that glucocorticoids mediate a myriad of responses including modulating the stress response (30), immune system (26), and development (9). Mineralocorticoids, in addition to their epithelial effect on sodium transport, have non-epithelial actions including a central role in modulating salt appetite and blood pressure (20). Unlike peptide hormones and growth factors, which bind to cell surface receptors, the lipophilic nature of steroid hormones allows them to pass through the cell membrane and bind to their cognate receptor. Steroid receptors are located in the cell cytoplasm or nucleus, and on steroid binding, they undergo an allosteric change resulting in heat shock protein dissociation, receptor dimerization, and binding of the receptor to specific DNA elements, which, in turn, modulates gene transcription. Cloning of the steroid receptors provided identification of a common structure consisting of a highly conserved DNA binding domain, a COOH-terminal region that encompasses the ligand binding site, and a variable NH2-terminal domain. In addition to these cloned receptors, other corticosteroid receptors have recently been identified in intestine. Steroid binding studies have identified two novel putative corticosteroid receptors in intestinal epithelia, and molecular cloning studies have detected two low-affinity receptors in small intestine that are activated by corticosteroids and induce CYP3A gene expression. This article focuses on the identification of these novel corticosteroid receptors and the potential role they may play in intestinal physiology.

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CORTICOSTEROID RECEPTORS AND SPECIFICITY

To date, two high-affinity receptors for corticosteroids have been cloned: the mineralocorticoid receptor (MR), or type I corticosteroid receptor, and the glucocorticoid receptor (GR), or type II corticosteroid receptor. The initial distinction between these two receptors was demonstrated in rat kidney slice studies in which aldosterone was shown to bind with high affinity to type I sites and with much lower affinity to type II sites. Corticosterone (the endogenous rat glucocorticoid) had a lower affinity to both sites that was interpreted as evidence that these were MR (17). Two types of GR were then demonstrated in rat hippocampus; one site had high affinity for the synthetic glucocorticoid dexamethasone, whereas the other had a higher affinity for corticosterone and was called the corticosterone-prefering site (11). Subsequent biochemical studies and eventual cloning of these receptors demonstrated that the kidney type II site was the classic GR and had high affinity for the synthetic glucocorticoid dexamethasone and the kidney type I receptor (MR) was identical to the hippocampal corticosterone-prefering site and intrinsically has high affinity for both cortico-

rone and aldosterone. Thus MR are unique in that they have two distinct physiological ligands, depending on the cell and tissue in which they are expressed. In epithelial cells, or mineralocorticoid target tissue such as kidney and colon, aldosterone specificity is conferred on MR by 11β-hydroxysteroid dehydrogenase (11βHSD) (14, 19). The 11β-dehydrogenase activity of this enzyme converts corticosterone and cortisol to their MR and GR inactive 11-ketometabolites, 11-dehydrocorticosterone and cortisone, and thus allows aldosterone access to MR. In tissues that express MR but do not possess 11β-dehydrogenase activity, the higher circulating levels of endogenous glucocorticoids compared with aldosterone and the equivalent affinity of MR for these steroids result in MR binding glucocorticoids and mediating glucocorticoid effects (12).

Two isoforms of 11βHSD have been isolated, cloned, and characterized (1, 2). The first of these, 11βHSD1, is NADP preferring and bidirectional, although it acts predominantly as a reductase in vivo, potentiating glucocorticoid action by forming active glucocorticoids from inactive 11-ketometabolites and thus increasing the local tissue concentration of endogenous glucocorticoids. 11βHSD1 is not present in intestinal epithelia (36). The second, 11βHSD2, operates as an exclusive 11β-dehydrogenase for endogenous glucocorticoids, and, given its colocalization with MR in sodium-transporting epithelia and the increase in sodium retention when enzyme activity is compromised (37, 39), it is this enzyme that confers aldosterone specificity on MR. This isoform is present in intestinal epithelia, with high expression in colon and ileum (36).

**MR, GR, AND 11βHSD IN INTESTINAL CELLS**

In intestinal epithelial cells, glucocorticoids appear essential for cellular differentiation (6) and maintaining electroneutral sodium absorption (5). In the small intestine, there is good evidence that glucocorticoids regulate several aspects of electrolyte transport, whereas mineralocorticoids have minor or no effect. In contrast, in the colon, both mineralocorticoids and glucocorticoids stimulate sodium and potassium transport (29). Agreement with these functional studies is the relatively high expression of GR and low expression of MR in duodenum and jejunum and the high expression of both these receptors in colon (36). The cellular response to endogenous corticosteroids is dependent on many factors, two of which are receptor concentration (7) and the presence of 11βHSD (13, 18). The low level of MR in duodenum and jejunum suggests that these receptors may not be capable of inducing a major response. The minimal 11βHSD2 activity in these segments of the small intestine plus the observation that corticosterone binding to MR or GR is not significantly increased when 11βHSD2 activity is inhibited (36) suggest that in vivo, corticosterone rather than aldosterone binds MR. Thus the MR in duodenum and jejunum may resemble hippocampal MR, which bind corticosterone in vivo (32) and thus mediate physiological glucocorticoid actions (12).

MR, GR, and 11βHSD2 are present in ileum and colon, and when 11βHSD2 activity is inhibited, corticosterone binding to MR increases fourfold, whereas in contrast, binding to GR only doubles (36). These data suggest that in vivo, MR in these intestinal segments bind aldosterone and that corticosterone binding to GR is also modulated by enzyme activity. This is supported by functional studies in which both mineralocorticoids and glucocorticoids have been shown to regulate electrolyte transport in both ileum and colon (29). Given that MR have substantially higher affinity for corticosterone than do GR, the consistently enhanced occupancy of MR compared with GR in colon when 11βHSD2 is inhibited cannot be explained simply on the basis of increased corticosterone concentrations. A possible interpretation of these data is that 11βHSD2 is in much closer association with MR than with GR. This could be due to either the fact that some colonic epithelial cells express GR only so enzyme inhibition does not alter corticosterone binding to GR in these cells or an intimate intracellular association of 11βHSD2 with MR so that the local microconcentration of corticosterone is lower for MR than GR. The latter is supported by transfection studies in which cotransfection of both MR and 11βHSD2 in HEK293 cells resulted in association of both proteins with the endoplasmic reticulum membrane, which differed from the nuclear and cytoplasmic distribution of MR when transfected alone (27). In addition to 11βHSD2 modulating corticosteroid responses in intestinal epithelia, other studies have demonstrated that the cellular milieu imparts an apparent low affinity and reduced binding capacity on colonic GR for both corticosterone and the synthetic glucocorticoid dexamethasone (31). The mechanisms involved are yet to be determined, although steroid availability and an inherently lower affinity-receptor complex in intact cells may be involved.

The combination of an apparent low affinity of corticosterone for GR and the low intracellular concentration of this steroid due to 11βHSD activity questions the role GR plays in colonic epithelial cell physiology. There is good evidence that synthetic steroids such as dexamethasone and RU28362 regulate cation transport in colon via GR (3–5), but studies using the endogenous glucocorticoid corticosterone are limited. When endogenous corticosteroids are removed in rats by adrenalectomy, colonic Na\(^{+}\)-K\(^{-}\)-ATPase activity and electrolyte movement are lower than in intact animals (4, 40). Colonic ion transport is restored by the administration of low doses of dexamethasone or RU26988 but not by physiological doses of aldosterone (3, 4). Endogenous glucocorticoids therefore maintain basal colonic ion-transport activity via GR. In another study on intact animals, dexamethasone but not aldosterone increased Na\(^{+}\)-K\(^{-}\)-ATPase α1-subunit mRNA expression, an effect mimicked by 11βHSD inhibition with carbexoxolone, indicating that the effect was mediated by endogenous corticosterone via GR (16). The relatively low-affinity GR in intact colonic epithelial cells and the presence of 11βHSD2 strongly suggest that levels of circulating “free” corticosterone (~10 nM)
(21) would not significantly occupy colonic GR, even if 11βHSD activity is inhibited. The question of how endogenous glucocorticoids mediate effects in colon is moot, although the identification of a novel putative steroid receptor in colonic epithelial cells, which binds 11-dehydrocorticosterone (DHB), the 11-ketometabolite of corticosterone produced by 11βHSD2 (33), may provide the answer. This putative receptor has negligible affinity for aldosterone, dexamethasone, estradiol, RU38486, and 5α-dihydrocorticosterone, the classic ligands for the other members of the steroid receptor family. In addition, competitive inhibitors of 11βHSD2 do not compete for binding, suggesting that binding is not to this enzyme (Table 1). The putative DHB receptor colocalizes with 11βHSD2, although not all 11βHSD2-expressing cells have the receptor, further suggesting that the putative receptor is not 11βHSD2 (35). A physiological function of a DHB receptor is yet to be defined, but, given its colocalization with 11βHSD2 and the fact that it would reflect circulating levels of endogenous glucocorticoids, it is conceivable that the DHB receptor mediates endogenous glucocorticoid effects in 11βHSD2-expressing cells such as colonic epithelia. Thus a DHB receptor would allow mineralocorticoid target cells to respond to circulating glucocorticoid without compromising the aldosterone selectivity of MR. Consistent with the possibility that 11-dehydrocorticosterone via a DHB receptor mediates glucocorticoid effects is the demonstration that 11-dehydrocorticosterone can regulate cation transport in toad bladder and rat kidney (10, 28).

**NOVEL NUCLEAR CORTICOSTEROID RECEPTORS IN SMALL INTESTINE**

In addition to GR, MR, and the putative DHB receptor, another corticosteroid binding site has been described in epithelial cells of the intestine (34). In duodenum and jejunum, corticosterone binds to a nuclear localized site that has a relatively low affinity for corticosterone (50 nM), a high capacity, and a broad steroid specificity compared with both MR and GR (Table 1). The steroid specificity profile of this binding site distinguishes it from MR, GR, and other classic steroid receptors (androgen receptor, progesterone receptor, estrogen receptor), although the specificity mirrors the potency of various steroids to inhibit 11βHSD2 activity, suggesting that binding may be to the substrate binding site on an 11βHSD2 isoform. In discordance with this concept is the difference in both tissue distribution and intracellular localization between the small intestinal binding site and 11βHSD2 (34).

The role the small intestinal low affinity, high-capacity corticosterone binding sites play in vivo is yet to be determined, although the recent cloning of two orphan receptors expressed in the small intestine may provide insight into the function of this binding site. These orphan receptors, termed human steroid/xenobiotic receptor (SXR) (8, 24) and the rodent ortholog pregnane X receptor (PXR) (22), are activated by corticosteroids. They induce the expression of the MDR1 gene, (38) which encodes a transporter that protects cells from toxicity by rapidly effluxing drugs, and CYP3A genes (8, 22), which are important in the metabolism and elimination of xenobiotics and steroids. Therefore, PXR and SXR are implicated in both catabolism and clearance of xenobiotics and steroids from cells. These receptors respond to high concentrations of a diverse group of compounds including xenobiotics and both synthetic and endogenous steroids. The different steroid and xenobiotic CYP3A induction profiles between PXR and SXR is thought to reflect the differences in ingested nutrients and xenobiotics between the different species. The corticosterone binding site in the small intestine is similar to PXR and SXR in its broad steroid specificity, nuclear localization, and high expression in the small intestine. In contrast with these receptors is the 100-fold-higher affinity of corticosterone for the small intestinal binding site (50 nM compared with 5 μM), and steroid specificity appears to be restricted to C21 steroids. The small intestinal receptor may be

<table>
<thead>
<tr>
<th>Receptor/Binding Site</th>
<th>Cort Affinity In Intestinal Cells</th>
<th>Steroid Specificity</th>
<th>Negligible Affinity</th>
<th>Intestinal Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat mineralocorticoid receptor (23, 36)</td>
<td>2 nM</td>
<td>Aldo = Cort &gt; DOC &gt; Dex &gt; 11-DHB</td>
<td>RU38486, CBX</td>
<td>colon &gt; ileum &gt; duodenum = jejunum</td>
</tr>
<tr>
<td>Rat glucocorticoid receptor (36)</td>
<td>30 nM*</td>
<td>Aldo &gt; DOC &gt; Cort &gt; Dex &gt; 11-DHB*</td>
<td>RU38486, CBX</td>
<td>colon = ileum &gt; duodenum &gt; jejunum</td>
</tr>
<tr>
<td>Rat DHB receptor (31, 35)</td>
<td>10 nM</td>
<td>11-DHB = Cort &gt; Cortisol</td>
<td>Dex, Aldo, DOC, E2, DHT, RU38486, CBX</td>
<td>colon</td>
</tr>
<tr>
<td>Rat small intestinal binding site (34)</td>
<td>50 nM</td>
<td>Cort = CBX &gt; 11-DHB &gt; DOC</td>
<td>Dex, Aldo, RU38486, PCN</td>
<td>jejunum &gt; ileum &gt; duodenum</td>
</tr>
<tr>
<td>Mouse PXR (8, 22)</td>
<td>30 μM</td>
<td>PCN &gt; RU38486 &gt; Dex &gt; Cort &gt; cortisone</td>
<td></td>
<td>small intestine</td>
</tr>
<tr>
<td>Human SXR (8, 24)</td>
<td>10 μM</td>
<td>Cort &gt; cortisone &gt; RU38486 &gt; PCN = Dex</td>
<td>Aldo</td>
<td>small intestine, colon</td>
</tr>
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Note receptor affinity for corticosterone is in the absence of 11β-hydroxysteroid dehydrogenase (11βHSD) activity unless otherwise indicated. * Apparent affinity and specificity in the presence of 11βHSD2. Cort, corticosterone; Aldo, aldosterone; DOC, deoxycorticosterone; 11-DHB, 11-dehydrocorticosterone; CBX, carbenoxolone; PCN, pregnenolone 16α-carbonitrile; E2, estradiol; DHT, 5α-dihydrotestosterone; SXR, steroid/xenobiotic receptor; PXR, pregnane X receptor.
related to PXR and SXR as one of a novel branch of the nuclear receptor family in which the receptors are of low affinity, high capacity, and regulate the metabolism of a broad spectrum of compounds. The physiological role of the small intestinal receptor might be more defined than that of PXR and SXR in that it would provide an intracellular environment allowing corticosteroid receptors to respond to endogenous glucocorticoids while at the same time protecting them from ligands in ingested material. A caveat in this interpretation is the absence of the small intestinal binding site in the colon, which would also be exposed to exogenous ligands. If the DHB receptor is the physiological GR in the colon, then the restricted steroid specificity of this site compared with classic GR (Table 1) may be enough to protect it from ingested ligands so a small intestinal binding site compared with classic GR (Table 1) may be enough to protect it from ingested ligands so a small intestinal binding site would not be required under these conditions.

In conclusion, the findings discussed here have provided evidence for both classic (GR, MR) and nonclassic corticosteroid receptors in gut mucosa (Table 1). The relatively low-affinity, high-capacity receptors for corticosteroids (SXR, PXR) in intestine appear to regulate genes involved in both drug metabolism and efflux from cells. Thus these receptors are probably important in protecting gut epithelia from both ingested cytotoxic agents and ligands for the high-affinity corticosteroid receptors. The identification of the xenobiotics and steroids that activate these low-affinity receptors will be significant in minimizing adverse drug effects. The low affinity and broad steroid specificity of the small intestinal binding site suggests that it may be related to PXR and SXR, although the more restricted steroid specificity of this site indicates that it may have a more specific role. The existence of a DHB receptor in mineralocorticoid target cells is not anticipated in that it would explain how endogenous glucocorticoids can mediate glucocorticoid effects in 11βHSD2-expressing cells. Cloning of both the DHB receptor and the small intestinal receptor is required to clearly identify these binding sites as receptors and determine the role they play in intestinal physiology.

REFERENCES