Mineralocorticoids, Glucocorticoids, Receptors and Response Elements

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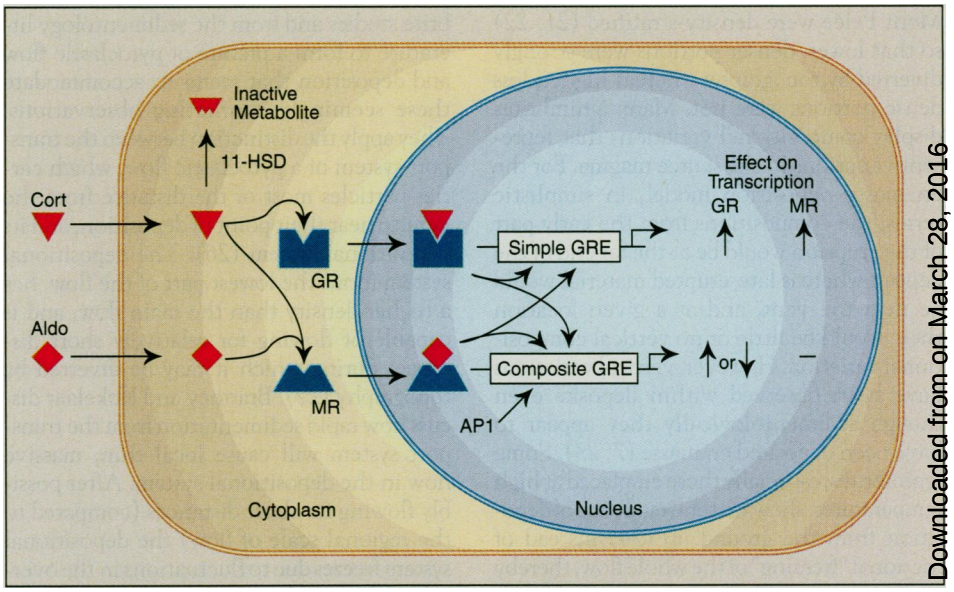
Receptors for steroid hormones differ markedly from membrane receptors in that they are intracellular and act primarily by regulation of DNA transcription. Like membrane receptors, they are members of a large family that includes receptors for thyroid hormone, retinoic acid and 9-cis retinol (vitamin A derivatives), and numerous "orphan" receptors, often precisely regulated but for which no ligands have yet been described. In this issue of *Science* (*1*), Pearce and Yamamoto offer some insight into how a degree of transcriptional specificity can be achieved for two closely related receptors, the mineralocorticoid and glucocorticoid receptors.

All members of this extended receptor family can be viewed as having three domains—an NH_2-terminal sequence of highly variable length and <15% amino acid identity between receptors, a DNA binding domain of constant length (66 or 68 amino acids) and >40% identity across the family, and a COOH-terminal ligand binding domain, of similar length but highly variable (<15 to 57%) amino acid identity. The mineralocorticoid, glucocorticoid, progesterone, and androgen receptors subfamily (MR, GR, PR, and AR, respectively) is characterized by relatively high amino acid identity in the ligand binding domain (50%) and very high levels in the DNA binding domain (90%).

Given that only 4 to 6 of the 66 amino acids in the DNA binding domain differ between the four members, it should come as no surprise that they appear to share a common hormone response element (HRE), comprising or approximating the nucleotide sequence GCTACAnnnTGTTCT, to which the activated receptors bind as homodimers with high affinity. Indeed, single (or tandem repeat) HREs have been shown to respond similarly to activated MR, GR, PR, and AR. GR are expressed in all nucleated cells; how can signal specificity be achieved in a system with such a promiscuous final common pathway?

What Pearce and Yamamoto have shown is that although MR and GR are equivalently active at a variety of simple HRE, they mediate different effects at plfG, a 25-nucleotide "composite response element" containing both a low-affinity HRE and an AP1 binding site (see figure). The latter site binds heterodimers of c-Fos and c-Jun (or c-Jun homodimers); c-Fos and c-Jun are proto-oncogene products that are expressed in response to a variety of stimuli and with wide-ranging modulatory effects. An activated (ligand-bound) GR can block c-Jun–c-Fos–enhanced transcription from the composite response element, but an activated MR cannot. Through studies of receptor chimeras and mutants, the authors show that a segment of the NH_2-terminal domain of GR (amino acids 105 to 438) is required for this activity, and they speculate that specificity may be conferred by interaction of this segment with accessory (nonreceptor) factors.



Transcriptional regulation by MR and GR. Glucocorticoid hormones, such as cortisol and corticosterone (Cort), and mineralocorticoids, such as aldosterone (Aldo), enter the cell and bind to MR or GR; Cort activates both MR and GR, whereas at physiologic concentrations Aldo activates only MR. In cells expressing 11-HSD, Cort is selectively metabolized and inactivated. Activated receptors enter the nucleus and bind to DNA sequences termed glucocorticoid response elements (GREs) (MR is shown here with Aldo bound; Cort is equally effective). MR and GR can each bind to simple and composite GREs, and both receptors enhance transcription from simple GREs. In contrast, GR but not MR alters transcription from the composite GRE discussed in the text; the direction of GR regulation depends on the subunit composition of transcription factor AP1, which also binds to the composite element. Hence, response elements are either promiscuous or selective in mediating the activities of different bound receptors. In addition, the pattern of regulation by a given response element is shaped by ligand availability, by 11-HSD expression, and by the functional composition of nonreceptor factors (such as AP1) that interact with the receptor.

We are accustomed to thinking of aldosterone as the physiologic ligand for MR, but in most tissues MR are occupied by circulating glucocorticoids (cortisol, or corticosterone in rat and mouse), reflecting their much higher circulating concentrations and an affinity for MR equivalent to that of aldosterone. It might plausibly be argued that the ability to synthesize aldosterone has emerged as a relatively late evolutionary event, possibly by duplication of the gene coding for 11β-hydroxylase, a glucocorticoid-specifying enzyme in the adrenal cortex. Aldosterone synthase has 95% amino acid identity with 11β-hydroxylase and is the product of an adjacent gene on human chromosome 8 (*2*). In contrast, human MR and GR are products of separate chromosomes (4 and 5, respectively) and have—except in the short DNA binding domain—far less sequence similarity than do 11β-hydroxylase and aldosterone synthase.

The physiologic mineralocorticoid role of aldosterone appears to reflect the activity of both aldosterone synthase, which confers on aldosterone the highly reactive aldehyde group at C-18, and 11β-hydroxysteroid dehydrogenase (11-HSD) in aldosterone target tissues. The latter enzyme metabolizes cortisol and corticosterone into their receptor-inactive 11-keto congeners; in aldosterone, however, the aldehyde at C-18 cyclizes with the C-11 hydroxyl group and thus protects it from dehydrogenation and inactivation. The operation of these two enzymes allows aldosterone to occupy MR in physiologic mineralocorticoid target tissues, despite receptor nonselectivity and the much higher plasma glucocorticoid levels (*3*).

For MR and GR, there may be multiple specificity-conferring mechanisms in miner-

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alocorticoid target tissues. Normally, 11-HSD excludes glucocorticoids from both MR and GR in MR-containing cells in the kidney; aldosterone itself has very low affinity for GR. In renal cortical-collecting tubules, activated MR and activated GR have been shown to have similar effects on sodium transport under conditions in which glucocorticoid can access the receptor (4). In the colon, this mechanism is less well established, with evidence for differential MR and GR effects on ion transport (5); protein-protein interactions of the type proposed by Pearce and Yamamoto may thus be particularly important in this tissue. Specificity may also be conferred by membrane receptors for aldosterone. These recently described receptors (6) are insensitive to cortisol and spirolactone and are linked with the Na^+-H^+ antiporter, acting as additional discriminants of mineralocorticoid and glucocorticoid action.

Since what we term "MR" in most tissues act as glucocorticoid receptors (because there is nothing to exclude the much higher circulating concentrations of glucocorticoids), any consideration of their potential physiological roles needs to factor in their much higher (> tenfold) affinity for cortisol and corticosterone than classical GR. The differential transcriptional activity of GR and MR (1) may thus have particular relevance in nonepithelial tissues such as the hippocampus, where similar concentrations of the two receptors are found in the same cells and where both classes of receptor are glucocorticoid-responsive. In the hippocampus there is clear evidence for different effects mediated by the two receptors—differences impossible to reconcile with a simple difference in receptor affinity for ligand.

If the mechanism described by Pearce and Yamamoto is operant in the hippocampus and if activated MR bind but do not repress AP1 activity at composite response elements, then clearly a control system with at least six interacting factors (glucocorticoids, GR, MR, c-Fos, c-Jun, and c-Jun–like factors) would appear responsible for modulating hippocampal responses to adrenal steroids. Most likely, this system operates not only in response to chronic stress but also in response to normal circadian variation in glucocorticoid concentration.

Parenthetically, the present findings also provide an entirely plausible explanation for an otherwise puzzling feature of the retinoic acid receptor (RAR) family. When human RAR α, β, and γ sequences are aligned, they show ~90% amino acid identity in the ligand binding and the DNA binding domains but minimal identity elsewhere. However, when the human and murine RAR sequences are compared, several of the minimal identity regions show 96 to 99% conservation between species (7). This observation suggests that the major determinant of RAR activity may be the particular accessory factor binding sites in the NH_2-terminus, with retinoic acid acting as a broad-spectrum receptor activator and the DNA binding domain as a docking mechanism.

Finally, these studies on MR and GR, and those recently published by Robins and coworkers (8) on GR and AR, suggest a novel mechanism whereby transcriptional control may be modulated by inactive "antagonists" competing for receptor binding sites on response elements. The demonstration by Robins that activated GR can block androgen-specific induction of an androgen-specific enhancer (C'Δ9) and the probability that MR may similarly modulate GR action open the possibility that such antagonism is a major integrative negative regulatory mechanism in steroid action.

In transfection studies receptors are commonly overexpressed, which may cause difficulties in extrapolation to native cells. If receptors in vivo are also in excess vis-à-vis total HRE (canonical plus low-affinity HRE, of the type on the plfG fragment), then response element occupancy by an inactive hormone-receptor complex (GR in the case of C'Δ9) may be anticipated to blunt the effects of the activated androgen receptor, with occupancy being determined by activated receptor concentrations and the relative affinity with which they bind to C'Δ9. The affinity with which each receptor binds to the HRE includes, of course, the affinity of binding to DNA as well as interaction with nearby nonreceptor factors.

If, on the other hand, the concentration of HRE in vivo exceeds the total concentration of MR, GR, AR, and PR, then the potential antagonist activity of any one receptor on the action of any other receptor is diminished, in line with the extent of the concentration differences. For example, if a hippocampal neuron contains 10,000 MR, 10,000 GR, and 100,000 total HRE, GR occupancy of any particular nuclear binding site will be minimally influenced by the state of MR activation. In this case, however, the physiologic relevance of the GR-induced repression of AP1 activity must also be questioned. Even if the presence of a c-Fos–c-Jun dimer at the AP1 site were to increase the plfG affinity for GR to that displayed by a canonical glucocorticoid response element, at best 10% of such sites would be occupied by GR in a cell with a total of 100,000 HRE and 10,000 GR, leading to a maximal repression of 10%. To achieve the repression reported by Pearce and Yamamoto in vivo, the increase in affinity of plfG for GR consequent upon the interaction of GR, c-Fos, and c-Jun would need to be about three orders of magnitude. This is not impossible but, in the absence of such very marked positive cooperativity, GR repression of AP1-enhanced induction of transcription can only occur with concentrations of receptor and response element that allow the possibility of competition between receptors for response elements—that is, the possibility of physiologic antagonism between receptors for different steroid hormones.

It is too early to second-guess the physiological implications of this distinction between MR and GR. The studies call for a reevaluation of glucocorticoid action in the nervous system and reconsideration of steroid specificity and ion transport, particularly in the colon. They also provide a model that would account, at the transcriptional level, both for signal specificity and the possibility of physiological antagonism between different classes of steroid hormone receptors.

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