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Symposia

Corticosteroid Receptors and the Central Nervous System

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In mammalian systems, the physiological mineralocorticoid is aldosterone (aldo), and the physiological glucocorticoid cortisol (F), or corticosterone (B) in rats and mice. Receptors (MR) with high affinity for aldo, B and F are found in both epithelia and the central nervous system (CNS); receptors (GR) with lower affinity for F and B, and still lower for aldo, are found in essentially all cells. Both MR and GR bind to and activate canonical pentadecamer response elements in transfected cells and in epithelia, wherein MR aldo, B and F all act as agonists. *In vivo*, in epithelial cells a low K_m, NAD-dependent, 11β hydroxysteroid dehydrogenase (11βOHSD) converts B and F, but not aldo, to receptor-inactive 11-keto congeners, thus allowing aldo to occupy epithelial MR and produce sodium retention. The CNS differs markedly in terms of MR/GR in a number of ways: (i) most but not all MR in the CNS are functionally unprotected, despite the presence of a low K_m, NADP-preferring 11βOHSD, so that they operate as high-affinity GR; (ii) in such CNS 'MR', aldo antagonizes the effects of B, and vice versa, in contrast with epithelia; (iii) also in contrast with epithelia, activated GR in the CNS do not mimic activated MR, suggesting considerable if not total specificity at the response element level. These differences suggest that glucocorticoids have two distinct domains of action in the CNS, mediated by 'MR' at low B/F concentrations, and GR at higher concentrations; secondly, they suggest that the nuclear recognition and response elements mediating these effects are other than canonical pentadecamer sequences.

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When the cloning of the human mineralocorticoid receptor (MR) was first reported from Evans' laboratory, Arriza *et al.* [1] noted two at first sight unexpected properties of the newly characterized receptor. First, when mRNA from a range of rat tissues was run on Northern blots and probed with human MR cDNA, message levels were clearly much higher in the hippocampus than the kidney. Second, in studies of the binding of [^{3}H]aldosterone by recombinant MR, the physiological glucocorticoids cortisol and corticosterone were found to have affinity equal to that of aldosterone itself. Through the identity of rat kidney MR and hippocampal 'corticosterone-preferring' sites had been previously noted [2], as had the inability of both rat [2] and human [3] MR to distinguish aldosterone from the physiological glucocorticoids, the cloning of the human receptor provided a powerful impetus to seeking answers to the two questions raised above—of the physiological role of 'MR' in the central nervous system (CNS), and of how epithelial MR exclude the far higher circulating levels of glucocorticoid hormones to allow the physiological action of aldosterone.

To date, there appear to be more coherent answers to the second question than the first. In the process of establising the mechanism of epithelial action of MR, a number of clear distinctions between the operation of these receptors in epithelial and the CNS have emerged. These will be presented in some detail, as a context in which physiological actions of corticosteroids via CNS MR need to be considered.

Even though neither recombinant human [1] nor rat tissue extract MR [2] can distinguish aldosterone from the physiological glucocorticoids *in vitro*, such sites in epithelial tissues—kidney and colon, but not hippocampus—are clearly aldosterone-selective *in vivo*. When adrenalectomized rats are injected with [^{3}H]aldosterone or [^{3}H]corticosterone in the presence of excess RU28362 to exclude tracer from glucocorticoid receptors (GR),

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and the rats are killed 15 min later, binding in hippocampal extracts is high, and equivalent for both steroids [4]. In extracts of kidney and colon from the same animals binding of [^{3}H]aldosterone is also high; however binding of [^{3}H]corticosterone is very much lower, suggesting the operation of prereceptor mechanisms conferring *in vivo* aldosterone selectivity on otherwise non-selective MR in epithelial tissues.

In large part, at least, this specificity-conferring mechanism appears to reflect the operation in aldosterone target tissues of the enzyme 11β hydroxysteroid dehydrogenase (11βOHSD). In adrenalectomized rats pretreated with carbenoxolone, an inhibitor of 11βOHSD, the pattern of uptake and retention of rats injected *in vivo* with [^{3}H]aldosterone or [^{3}H]corticosterone is indistinguishable from that in pretreated rats; in epithelial aldosterone target tissues, however, binding of [^{3}H]corticosterone rises to equal (kidney, parotid) or approach (colon) that of [^{3}H]aldosterone [5]. In parallel studies on kidney slices, inhibition of 11βOHSD by glycyrrhetinic acid, the active principles of licorice, was similarly shown to be followed by [^{3}H]corticosterone occupancy of otherwise 'protected' receptors [6]. Whereas cortisol and corticosterone are equipotent with aldosterone in terms of MR occupancy, the products of their dehydrogenation (cortisone, 11-dehydrocorticosterone) have $<1\%$ of the affinity of their parent compounds for MR. Aldosterone is not similarly metabolized, reflecting the presence of its unique and highly reactive aldehyde group on C18, which cyclizes with the hydroxyl at C11 to form a very stable 11,18 hemiketal.

In addition to their much reduced affinity for MR, cortisol and corticosterone similarly have very low affinity for GR. Thus 11βOH excludes the physiological glucocorticoids not only from MR in aldosterone target tissue cells, but also from GR. Exclusion of glucocorticoids from both MR and GR is important in physiological activation of distal tubular sodium retention in response to aldosterone, since glucocorticoids appear able to act as agonists in terms of sodium retention via either MR or GR occupancy. When adrenalectomized rats are pretreated with carbenoxolone, corticosterone elevates urinary K^+/Na^+ in the presence of RU486, a GR antagonist, but not in the combined presence of RU486 and RU28318, a very selective MR antagonist [7]. Equally, GR activation can lead to a mineralocorticoid response: in carbenoxolone-pretreated adrenalectomized rats the highly specific GR agonist RU28362, which neither binds to nor activates MR [8], causes a urinary K^+/Na^+ response similar to that of aldosterone. Similarly, in immunodissected cortical collecting tubule cell preparations, cultured on a grid separating two chambers and grown to confluence, aldosterone, dexamethasone and RU28362 have indistinguishable effects on Na^+ and K^+ fluxes, and the resulting potential difference between the two chambers measured as a short-circuit current [9].

The interpretation of these data is that all the specificity, in terms of MR-activated epithelial mechanisms, appears vested in two enzymes—target-tissue 11βOHSD, and adrenal glomerulosa aldosterone synthase, which confers upon aldosterone its unique C18 aldehyde group. Both aldosterone and corticosterone are agonist when they occupy MR; both GR and MR, when appropriately activated, can produce a mineralocorticoid response. *In vitro* systems, in which similar levels of vectors producing GR or MR are cotransfected into receptor negative cell lines with an MMTV-LTR-luciferase reporter sequence, resemble the renal distal tubular cell, in that corticosterone produces an equivalant response via MR or GR [10]. Though the particular genes which respond *in vivo* to aldosterone remain to be directly characterized, it would *prima facie* appear that MR action in the renal tubule, and elsewhere in epithelial tissues, reflects transcriptional activity via classical pentadecamer regulatory elements, similar to the consensus sequence GGTACAnnnTGTTCT.

This does not appear to be the case for MR in the CNS, at least in large part. Though there is good evidence that the effects of aldosterone on salt appetite are mediated via aldosterone-selective MR, 'MR' in hippocampus and elsewhere in the circumventricular area appear unprotected by 11βOHSD by both binding [5] and effector studies [11, 12]. Occupancy of 'MR' and GR does not produce similar or identical effects [13], but often in fact apparently opposite effects [14]. Finally, occupancy of CNS 'MR' by aldosterone or glucocorticoids does not produce equivalent effects, as is the case in epithelia or cotransfection systems, but opposite effects; where corticosterone is agonist aldosterone is antagonist [11], and vice versa [12]. To illustrate these differences between MR-mediated mechanisms in epithelia and those in the CNS, the studies of Gomez-Sanchez and her colleagues [12, 13] on the effects of steroids on blood pressure will be presented in some detail.

When aldosterone is administered by the intracerebroventricular (ICV) route to uninephrectomized rats maintained on 1% NaCl solution to drink blood pressure rises progressively over 2–4 weeks. The dose required (10 ng/h) is approximately two orders of magnitude less than the equivalently effective peripheral dose and the effect can be blocked by the concomitant administration of MR antagonists. Infusion of the highly specific GR agonist RU26988 neither mimics or blocks the response to aldosterone. ICV infusion of corticosterone alone, at 10–20 ng/h, does not affect blood pressure compared with control (infusion of artificial cerebrospinal fluid); in contrast with RU26988, however, infusion of corticosterone at 10–20 ng/h progressively blocks the hypertensive effects of coadministered aldosterone. From these studies, then, it would appear that aldosterone is acting via specific 'MR'; that GR activation does not produce the

same effects as MR activation; that the 'MR' are not protected against glucocorticoid occupancy; and that; finally, although the affinity of the 'MR' for aldosterone and corticosterone appears identical, as is the case in epithelia, there must be some additional mechanism or factor determining that in the CNS one ligand is agonist and the other antagonist.

One possible mechanism whereby activated MR and GR may have different effects at the transcriptional level has recently been demonstrated by Pearce and Yamamoto [10]. In their studies, whereas MR and GR were equivalently active via a canonical pentadecamer regulatory element, the receptors differed in the effects mediated via a 25-nucleotide composite response element, containing both a steroid receptor binding sequence (with, however, only one tenth of the affinity of the pentadecamer sequence) and an AP-1 site. Activated GR were able to block transcription stimulated by c-*fos*/c-*jun* binding to the AP-1 site on the composite response element; unoccupied GR, and activated or unoccupied MR, were shown unable to block AP-1 induced transcription. Activated MR were, however, able to occupy the steroid binding site on the composite response element, and thus at least theoretically might attenuate the effects of GR via such a site, particularly at low ligand levels when the higher affinity MR are much more likely to be activated [15]. Such a mechanism may also be relevant to those situations [14] in which essentially opposing effects of GR and MR occupancy have been described, in contrast with effects on blood pressure [12] or serotonin receptors [11]. The extent to which such a mechanism underlies some or all of the MR-specific effects of steroids in the CNS, and the nature of other specificity-conferring mechanisms at the postreceptor level, remain to be determined.

Similarly remaining to be determined is how 'MR' in the CNS see corticosterone as agonist and aldosterone as antagonist [11], or vice versa [12]. The evidence for corticosterone being the physiologic occupant of such unprotected receptors in the rat is overwhelming. Adrenalectomy lowers hippocampal serotonin binding, a decrease which is reversed by corticosterone but not dexamethasone, evidence for an action via 'MR'. The restorative effect of corticosterone is not mimicked by aldosterone, but substantially lowered by an equal dose, coadministered [11], just as the hypertensive effect of ICV aldosterone is blocked by similar doses of corticosterone. Although there appear to be a variety of start sites for the initiation of MR transcription, and thus the possibility of tissue specific differences in terms of transcriptional activation [16], there do not appear to be between-tissue variants in terms of expressed receptor protein. One possible way to reconcile the unchanged binding affinity with the agonist/antagonist activity of corticosterone and aldosterone in MR is to invoke the involvement of a tissue-specific accessory factor in many CNS sites. Preliminary evidence for the existence of such a factor, binding to MR and modifying receptor activation but not steroid binding, has come from fast protein liquid chromatographic analysis of renal hippocampal and renal cytosols. Labeled with either [^{3}H]aldosterone or [^{3}H]corticosterone, peak binding of tracer consistently eluted 1–2 fractions earlier in hippocampal cytosol extracts; in contrast GR labeled with [^{3}H]dexamethasone eluted identically in the two tissues [17].

To date, what has been covered has largely been a review of MR specificity and action, primarily drawn from existing findings on aldosterone and glucocorticoid actions in both epithelial tissues and the CNS. To provide some sort of balance, the paper will now address three more contemporary findings related to glucocorticoid actions on the CNS via GR, and the various ways that steroids may modulate the stress response at a regional, local or cellular level.

First, Komesaroff and Funder [18] have shown that plasma adrenaline levels—presumably reflecting adrenal medullary adrenaline release—are affected by ambient glucocorticoid levels in some types of stress but not others. When sheep were exposed to a barking dog for 5 min their plasma adrenaline levels showed a rapid peak, indistinguishable between four groups of sheep on different glucocorticoid administration regimes. In contrast, the higher the level of glucocorticoids (as judged by plasma ACTH measurement) the lower the adrenaline response to insulin hypoglycaemia. We interpret these studies as evidence for the existence of a glucocorticoid inhibitable relay between the cells responsible for sensing cerebral glucopenia and the splanchnic nerve outflow. Noradrenaline release—presumably reflecting largely sympathetic activity in various vascular beds—was similar for all four groups of animals, in response to either audiovisual or metabolic stressors.

Second, in recent studies Engler and coworkers [19] have injected noradrenaline or adrenaline ICV, and then charted levels of CRF and AVP in pituitary portal blood over the subsequent 4 h, and of ACTH and cortisol in peripheral blood. Portal venous samples were obtained from conscious undisturbed animals via a portal access cannula placed in an artificial ethmoid sinus at surgery 1–2 days before; on the day of the study the animals were heparinized, a stilette passed to cut a modest percentage of the superficial pituitary portal vasculature, and blood samples collected each 5–10 min. Noradrenaline given ICV was followed by a prompt elevation of CRF, AVP, and ACTH and cortisol, a rise which was maintained over the 4 h of subsequent observation. We interpret these data as evidence for the abrogation by noradrenaline of fast feedback pathways normally suppressing ACTH release in response to elevated levels of glucocorticoid hormones. This action is presumably at or above the level of the hypothalamus, as high levels of noradrenaline did not suppress ACTH secretion by dispersed preparations of sheep anterior pituitary cells.

Third, Autelitano and Sheppard [20] have explored the very different effects of glucocorticoids on proopiomelanocortin (POMC) synthesis and β-endorphin release, depending whether or not AtT20 (a mouse pituitary tumour cell line) cells have been previously exposed to CRF. Prior exposure to CRF makes AtT20 cells insensitive to dexamethasone added even 10–15 min later, and also acutely elevates c-*fos* levels. Transfection of AtT20 cells with antisense sequences to c-*fos* mRNA, however, completely reverses the glucocorticoid insensitivity otherwise seen after CRF stimulation. These data suggest a key role for the coordinate transcriptional regulation of POMC and c-*fos* under physiological circumstances, allowing the possibility of prolonged secretion of ACTH and glucocorticoids in response to prolonged stress.

As a final comment on the roles of corticosteroids in the CNS, it is important to try and dispel the ambiguity inherent in referring to the high affinity 'MR' in the brain as mineralocorticoid receptors. Lifton *et al.* [21] have shown that the syndrome of glucocorticoid remediable aldosteronism reflects the activity of a chimaeric enzyme transcribed from a chimaeric gene, the product of an unequal cross-over between aldosterone synthase and 11β hydroxylase. Aldosterone synthase is responsible for the unique aldehyde group on C18 that characterizes aldosterone, and allows it to escape metabolism by 11βOHSD; 11β hydroxylase, in contrast, specifies glucocorticoid. The crossover can occur at a variety of sites 5′ to Exon 3, so that the chimaeric gene contains the upstream elements conferring ACTH responsivity and responsible for expression throughout the adrenal (like 11β hydroxylase), plus the sequence coding for aldosterone synthase enzymatic activity. The reason that such a crossover can occur is that the two genes are normally adjacent on 8q22, and that the sequences are 95% identical, evidence strongly suggesting a relatively recent (in evolutionary terms) gene duplication. Compare this with the extent of homology between GR and 'MR', 94% amino acid identity in the short (66aa) DNA binding domain, 57% identity in the ligand binding domain, and <15% in the N-terminal domain. Though clearly related, MR and GR are in evolutionary terms much more distant than 11β hydroxylase and aldosterone synthase. The inference of this comparison is that the 'MR' appears to have emerged earlier in evolution than the gene responsible for aldosterone synthesis. If this is the case, then what we term a MR in primordial terms is clearly a high affinity GR, pressed into service in epithelia to affect ion flux in response to the very much more recently evolved aldosterone. We might then profitably focus on the possible mechanisms of action of 'MR' in non-epithelial tissues, including and perhaps primarily in the CNS, in the hope of elucidating their role in the physiology of glucocorticoid hormones in such tissues.

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