

## Reconsidering the Roles of the Mineralocorticoid Receptor

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Translational research is usually taken to mean the application of laboratory discovery to clinical practice. Like languages or enzymes, however, translation is a 2-way street, and in the field of aldosterone and mineralocorticoid receptors (MR), there are a number of examples where clinical studies have prompted reconsideration of the basic biology. For example, in essential hypertensive subjects the distinction between the antihypertensive and electrolyte effects of the selective MR antagonist eplerenone has been interpreted as evidence against a primary renal role for aldosterone/MR activation in raising blood pressure.<sup>1</sup> Similarly, the finding of an S810L MR mutant receptor causing juvenile hypertension exacerbated by pregnancy<sup>2</sup> prompted a reconsideration of the order of branching of the MR/glucocorticoid receptor (GR)/progesterone receptor (PR)/androgen receptor (AR) subfamily of steroid hormone receptors from a common primordial ancestral protein.<sup>3</sup>

These studies and their implications for the basic biology of aldosterone action have been discussed elsewhere.<sup>4</sup> The present review focuses on the clinical study (Randomized ALdosterone Evaluation Study [RALES]<sup>5</sup>) that prompted this process of re-examination almost a decade ago. The rationale for this study, in patients with New York Heart Association class III heart failure, was that previous clinical studies had shown that plasma aldosterone levels could break through prolonged angiotensin-converting enzyme inhibition and angiotensin II type 1 blockade; in addition, early laboratory studies by Brilla and Weber<sup>6</sup> had shown that exogenous aldosterone plus normal saline as drinking solution produced cardiac hypertrophy and fibrosis in uninephrectomized rats.

The outcomes of RALES were remarkable. The trial was halted just more than half way through the projected period of recruitment on the basis of the divergence between the 2 groups: 1 treated with standard of care (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, etc) plus placebo and the other with standard of care plus spironolactone. Addition of a very modest dose of spironolactone (average: 26 mg/d) resulted in a 30% improvement in survival and a 35% reduction in hospitalization. The efficacy of MR blockade has widely (and perhaps too easily, if understandably) been attributed to the blockade of aldosterone from activating cardiac MR and thus for a pathophysiologic role for aldosterone in congestive heart failure.

There is no question that elevated aldosterone levels inappropriate for sodium status are followed by marked cardiovascular pathology, both experimentally and clinically. Rocha and Funder<sup>7</sup> showed in a variety of experimental models (stroke-prone spontaneously hypertensive rats [SHRSP] on normal saline, angiotensin infusion plus normal saline) that the pathological changes were abrogated by the MR selective antagonist eplerenone with no change in blood pressure; in the angiotensin-infused rats, the changes were similarly abrogated by adrenalectomy, and after adrenalectomy were reinstated by aldosterone infusion. In primary aldosteronism, the prevalence of cardiovascular pathology is considerably higher than in age-, sex- and blood pressure-matched essential hypertensive subjects.<sup>8</sup> There is thus no question that aldosterone excess, in the context of inappropriate sodium status, can produce major cardiovascular pathology.

The problem raised by RALES, however, is that plasma aldosterone levels are in the low normal range, and sodium status is completely unremarkable. Similar considerations apply to subsequent clinical studies on eplerenone, in heart failure (Eplerenone Post-acute myocardial infarction Heart failure Efficacy and SURvival Study [EPHESUS]<sup>9</sup>), and as monotherapy in essential hypertension,<sup>1,10,11</sup> where uniformly low normal plasma aldosterone levels were found. If plasma aldosterone is low, the question then is what is activating the coronary/cardiac MR, given that MR blockade is clearly of major benefit. The thrust of the review to follow is that cortisol occupies the majority of both epithelial and nonepithelial MR, normally in tonic inhibitory mode, but in the context of tissue damage becomes an MR agonist, explaining the activation of both vascular and myocardial MR in hypertension and heart failure and the efficacy of MR blockade despite normal aldosterone levels.

### Analysis and Reanalysis

A fundamental building block in any analysis of MR action was the demonstration in rats<sup>12</sup> and subsequently for recombinant human MR<sup>13</sup> of their very high affinity for physiological glucocorticoids (cortisol, and corticosterone in rats and mice). Human MR bind aldosterone and cortisol with equivalent high affinity and corticosterone with even (3 times) higher affinity. This presents an immediate problem, in that

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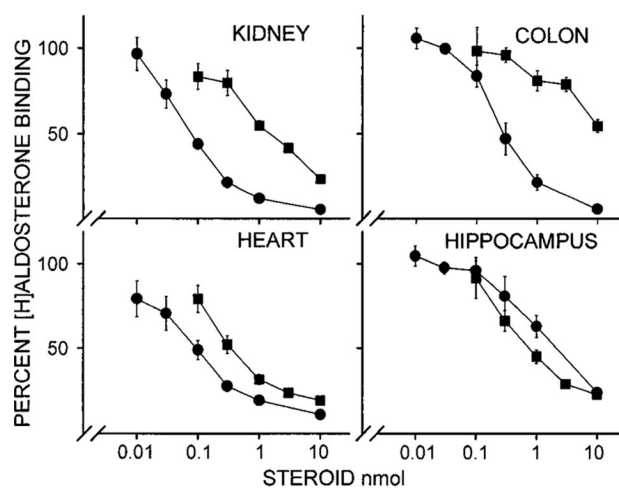
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circulating glucocorticoid levels are 1000- to 2000-fold higher than those of aldosterone. Glucocorticoids are 10-fold more highly bound in the circulation, so that plasma free levels are only 100- to 200-fold higher, still an extraordinarily high noise:signal ratio in terms of the undisputed effects of aldosterone via MR on transepithelial sodium transport. A second finding in both studies was the expression of MR at high levels in tissues which are clearly not primarily involved in sodium transport, eg, the hippocampus, heart, and blood vessels.

Twenty years ago, 2 laboratories<sup>14,15</sup> proposed that epithelial aldosterone selectivity was conferred by the coexpression of MR and the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD2), which converts cortisol and corticosterone to their MR-inactive 11-keto congeners cortisone and 11-dehydrocorticosterone. On this basis, it was proposed that conversion to receptor-inactive metabolites allows aldosterone, which is >99% cyclized in solution to the 11,18 hemiacetal and the 11-18-20 bicyclic hemiacetal forms (neither of which is an 11 $\beta$ HSD2 substrate), to preferentially occupy and activate epithelial MR. Enzyme deficiency, in the syndrome of apparent mineralocorticoid excess, or enzyme blockade with carbenoxolone or in licorice abuse, allowed cortisol to escape metabolism and thus to occupy and activate epithelial MR. Over 20 years, then, it has been received wisdom that the action of 11 $\beta$ HSD2 is to prevent glucocorticoid occupancy of epithelial MR.

Like all great lies,<sup>16</sup> however inadvertent, this is half true. There is no question that the enzyme plays a crucial role in ensuring the selectivity of the epithelial response, but the evidence is squarely against this being via denying glucocorticoid access to or occupancy of epithelial MR. If plasma free levels of cortisol are  $\geq$ 100-fold higher than those of aldosterone, *prima facie* intracellular levels will similarly be 100-fold higher. If an acceptable noise:signal ratio were 1:10, against a concentration ratio of 100:1, then the enzyme would need to convert 999 of every 1000 glucocorticoid molecules to inactive metabolites in the kidney (an organ that receives 20% to 25% of cardiac output) and thus an essentially inexhaustible supply of steroid substrate. This is theoretically highly improbable,<sup>17</sup> and over a decade ago was demonstrated experimentally not to be the case,<sup>18</sup> in studies that incidentally address the question of relative intracellular levels of aldosterone and glucocorticoids.

Adrenalectomized rats were injected with [<sup>3</sup>H] aldosterone as tracer, alone or with increasing half-logarithmic doses of nonradioactive aldosterone or corticosterone. Animals were killed after 15 minutes, tissues harvested, and the binding of tracer [<sup>3</sup>H] aldosterone determined (Figure); the displacement curves in the presence of each competitor reflect the relative tissue-specific MR occupancy by one or the other corticosteroid. The heart does not express 11 $\beta$ HSD2, and aldosterone (lower left) has 3-fold higher *in vivo* affinity for MR than corticosterone. This fits: corticosterone has 3-fold higher intrinsic affinity<sup>13</sup> but is 10-fold more highly bound in plasma. In the hippocampus, corticosterone is actually a better *in vivo* competitor than aldosterone itself, reflecting the very poor penetration of aldosterone through the blood-brain barrier: note how much to the right the standard curve for



**Figure.** Tissue-specific affinity of aldosterone (circles) and the physiological glucocorticoid corticosterone (squares) for MRs: *in vivo* studies in adrenalectomized rats injected with tracer doses of [<sup>3</sup>H] aldosterone alone to give a 100% point or with increasing half-logarithmic doses on nonradioactive aldosterone or corticosterone. Animals were killed 15 minutes after injection and specific tracer binding determined. Redrawn from Reference 18.

[<sup>3</sup>H] aldosterone is shifted in comparison with the other tissues.

If the action of 11 $\beta$ HSD2 were to metabolize 99.9% of intracellular glucocorticoid into receptor-inactive 11-keto congeners, then the corticosterone curves in the 2 classic epithelial tissues of kidney and colon would essentially flat line at 100%, ie, no competition for tracer binding. As can be seen, however, what the enzyme does is to add an order of magnitude difference to aldosterone occupancy of epithelial MR, in addition to that conferred by plasma binding. In the context of renal blood flow, this is no mean feat and reflects the very high levels of 11 $\beta$ HSD2 expression, reckoned to be 3.5 to 4.0 million molecules per principal cell (compared with those of MR,  $\approx$ 10 000 copies per cell). What it also entails, however, is that, despite this degree of metabolism, intracellular levels of glucocorticoids are still  $\geq$ 10-fold higher than those of aldosterone. However much the enzyme is crucial to the selectivity of aldosterone activation of MR in epithelial tissues under normal circumstances, it is clear that it is not by excluding glucocorticoids. In the Kagawa bioassay, adrenalectomized rats are an order of magnitude more sensitive than intact rats to injected aldosterone, evidence supporting the inference that most renal MRs are glucocorticoid occupied but not activated in intact animals. Put simply, what the enzyme does is not to block glucocorticoid occupancy of epithelial MR but the ability of glucocorticoids to act as MR agonists.

How can this be? When the enzyme is deficient or blocked, glucocorticoids indisputably act as MR agonists; when the enzyme is operant they do not, despite occupying 90% of the MR. When the enzyme is not active, glucocorticoid occupancy of MR goes from 90% to 99%, intracellular cortisone levels fall, and no reduced nicotinamide-adenine dinucleotide (NADH) is generated from nicotinamide-adenine dinucleotide (NAD), the cosubstrate for the cortisol-to-cortisone conversion. A marginal increase in MR occupancy by cortisol

appears an unlikely trigger for a change from antagonist-to-agonist activity, and cortisone has negligible affinity for MR. The answer would appear to lie with NAD, the forgotten substrate, and NAD:NADH ratio-driven intracellular redox state. For every molecule of cortisol converted to cortisone, a molecule of NAD must be converted to NADH. Although baseline levels of these dinucleotides vary between cells and intracellular compartments, at rest the ratio of NAD:NADH is commonly thought to be of the order of 600:1. What this provides, in the context of  $11\beta$ HSD2 action, is a very large pool of cosubstrate required for conversion of cortisol to cortisone, and the possibility of generating a very large increase in NADH concentration (eg, from 1 to 100) for a relatively minor change in NAD levels (600 to 501). Effects of NADH on MR-glucocorticoid complexes have yet to be demonstrated *in vitro*, but in other systems NADH has been shown, for example, to reduce the transcriptional activity of C-terminal binding protein by 2 to 3 orders of magnitude by corepressor activation.<sup>19</sup>

This may constitute a reasonable explanation of a redox-sensitive bivalent action of glucocorticoids occupying MR in epithelia (and the vessel wall, where both smooth muscle and endothelial cells coexpress  $11\beta$ HSD2 and MR) but does not offer an explanation of how glucocorticoids might activate cardiomyocyte MR in RALES and EPHEBUS, eg, in the absence of  $11\beta$ HSD2. This question was acutely posed by experimental coronary angioplasty studies in pigs dosed with placebo, aldosterone, or eplerenone for 5 days before and 28 days after angioplasty.<sup>20</sup> Eplerenone failed to affect neointima formation; what it did was to prevent luminal constriction in response to injury, thus preserving lumen diameter. This in turn raised the question of what was the antagonist blocking to achieve this effect; the animals were fed apples, cabbage leaves, etc, with no sodium supplement and had normal levels of aldosterone. Given that the bulk of their vascular wall MR would be cortisol occupied, then perhaps what eplerenone was blocking was cortisol, turned from inactive to active by the intracellular redox change consequent on tissue damage and the generation of reactive oxygen species.

That cortisol is indeed an MR agonist under conditions of tissue damage has been shown by Mihailidou et al<sup>21</sup> in recent ischemia-reperfusion studies. In rat heart Langendorff preparations, administration of aldosterone increases infarct area, consistent with previous whole animal studies on cardiac MR activation: the effect is blocked by spironolactone and by the antioxidant Tempol. Cortisol at an equal (10 nmol/L) dose similarly increases infarct area, an effect clearly via MR activation in the context of tissue damage, in that it is blocked by spironolactone but not by the GR/PR antagonist RU486. In patch-clamp studies on isolated rabbit cardiomyocytes, the same laboratory has shown that under normal conditions cortisol is an MR antagonist, stoichiometrically blocking the rapid nongenomic effects of aldosterone on transmembrane pump current. When oxidized glutathione is instilled into the cells to mimic reactive oxygen species generation and redox change, it is without effect in the absence of steroid: when coinjected with oxidized glutathione, however, cortisol becomes an MR agonist, mimicking the effect of aldosterone.<sup>22</sup>

It appears then that MR, which are largely constitutively occupied by normal glucocorticoid levels, respond in both  $11\beta$ HSD2-expressing tissues (vascular smooth muscle cells and endothelial cells) and nonexpressing tissues, eg cardiomyocytes, to changes in intracellular redox state. The experimental studies to date address pathophysiology; and whether there are physiological roles for glucocorticoid activation of MR in the vascular wall or the heart has not been squarely addressed. Such a largely constitutively occupied receptor is not unique, however: MR joins hepatic nuclear factor (HNF)-4, retinoid O receptor (ROR) $\alpha$ , and possibly liver X receptor (LXR) as presumably responding to signals other than fluctuation in levels of occupancy of the binding cleft. In terms of activation by redox change, the drosophila E-75 nuclear receptor has been shown to be overwhelmingly occupied by heme, to bind NO and CO, and to be activated or not depending on the redox state of the cell and the balance between Fe<sup>2+</sup> and Fe<sup>3+</sup> in the heme moiety.<sup>23</sup>

### Quo Vadis?

At the level of physiology, the roles of extraepithelial MR remain incompletely explored. Vascular wall MR have been shown to be responsive to aldosterone, at both the genomic and nongenomic levels,<sup>24,25</sup> for which the latter response is presumably invoked after the rapid secretion of aldosterone on orthostasis. In the brain, MR in the AV3V region of the hypothalamus have been shown experimentally to respond to intracerebroventricular aldosterone by an elevation of blood pressure, at doses without effect given peripherally.<sup>26</sup> Corticosterone stoichiometrically blocks the aldosterone effect at equivalent or similar doses,<sup>27</sup> part of the evidence against such sites being physiological aldosterone receptors, in that they are overwhelmingly occupied by glucocorticoids (for review, see Reference 28). Elsewhere in the central nervous system, the nucleus tractus solitarius is the only area in which both MR and  $11\beta$ HSD2 can be shown to be coexpressed: even in this area, however, there is little evidence for a fenestrated capillary network, which might facilitate MR occupancy by otherwise very low tissue levels of aldosterone. Elsewhere in the central nervous system, the signals (presumably neural, perhaps metabolic) that activate MR occupied by glucocorticoids remain to be explored, as they do in the AV3V region. In the heart, as opposed to blood vessels, no physiological role for MR has as yet been documented, in contrast with pathophysiologic effects noted above.

In terms of pathophysiology, MR activation by change in redox status or by aldosterone at levels inappropriate for sodium status is accompanied by vascular inflammation and end-organ damage. Clinically this is seen in the accelerated pathology of primary aldosteronism; experimentally, MR blockade has been shown to protect against not only coronary inflammation/cardiac fibrosis but also against stroke in SHR-SPs and renal damage/proteinuria; in addition, in mice, rabbits, and primates, eplerenone has been shown protective in experimental atherosclerosis.<sup>29–31</sup> What has remained unexplored in any systematic fashion is the anti-inflammatory potential for MR antagonists in a range of autoimmune disease, from rheumatoid arthritis to inflammatory bowel disease to systemic lupus erythematosus to multiple sclerosis.

It is not widely known outside neuro-ophthalmologic circles that low-dose Aldactone is not uncommonly used as adjuvant therapy in myasthenia gravis, on the back of >50 case reports published 40 years ago.

Neither is it realistic to anticipate such applications until the development of third- and fourth-generation MR antagonists. The first generation (spironolactone) and second generation (eplerenone) are suboptimal in a number of ways and are both out of patent. Spironolactone has undesirable side effects, even at modest doses, and eplerenone is expensive (except in Japan). Both have as an obligate adverse effect hyperkalemia, reflecting the renal tubular effects of MR blockade. This has been widely overinterpreted as a contraindication: in patients with normal renal function and with antagonist titrated to effect, there appears to be only a very modest increase of  $\leq 0.3$  meq/L in plasma  $[K^+]$ , even at high doses (200 mg) of eplerenone.<sup>1</sup> This said, the dangers of hyperkalemia in elderly patients with a reduced creatinine clearance are real,<sup>32</sup> particularly if dosing is overenthusiastic and/or potassium supplementation is thoughtlessly continued.

Third-generation MR antagonists would be as potent as spironolactone, as selective as eplerenone, nonsteroidal, cheap to manufacture, and with a long patent life. A fourth generation would be all of the above, plus to an extent renal tubule sparing (ie, have a lesser effect in terms of fluid and electrolytes than its effect on vascular protection). It is important that such a fourth-generation antagonist in terms of vascular/cardiac MR does not have totally absent tubular effects, which would constitute a potential hazard for patients with primary aldosteronism: hypokalemia is inherently more dangerous than hyperkalemia. Third-generation antagonists would, thus, be the MR antagonist of choice in primary aldosteronism, with fourth-generation agents used in essential hypertension, and potentially as powerful anti-inflammatory agents in an as-yet-unexplored variety of disease situations.

One final area in which reflecting on clinical experience may be worthwhile is that of the mechanism of antagonist action. It is assumed that spironolactone and eplerenone produce their effects by competing with agonists for MR occupancy. That they do this is not in question: what is in question is whether this constitutes the totality of their effect. RALES showed the remarkable efficacy of low-dose (average: 26 mg/d) spironolactone and EPHEsus a comparable dose of eplerenone (43 mg/d). In doses as low as 6.25 mg/d (one quarter of the smallest tablet), spironolactone has been shown to be remarkably renoprotective in diabetic hypertensive patients (A. Sato, personal communication, 2006). Spironolactone has a long half-life and active metabolites; the half-life in humans of eplerenone is 4 hours, and it has no active metabolites, so that between daily doses, the plasma levels fall to <2% of peak; and yet the effects of both are remarkably sustained, in experimental studies with constant aldosterone infusion and in the clinical studies cited.<sup>1,5,9–11</sup>

From a variety of microarray studies (commonly in response to other steroids) it is clear that receptor agonists induce/repress an overlapping but not identical portfolio of genes: in human liver cells, eg, of a total of  $\approx 300$  genes that are variously regulated by cortisol, corticosterone, and dexamethasone, only 25 are equivalently regulated by all 3

“gold-standard” agonists (J. Cidlowski, personal communication, 2007). Most, if not all, antagonists similarly induce/repress expression of an array of genes. The final question accordingly is this: given the very low doses of antagonist that have proven effective against MR activation by aldosterone and by cortisol in the context of tissue damage, and the persistence of the antagonist effects, is it possible that antagonists induce preemptive anti-inflammatory gene expression, at only modest levels of receptor occupancy, that contributes to their clinical efficacy over and above simple competition at the receptor level?

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### Disclosures

None.

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