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.1 Similarly, the finding of evidence against a primary renal role for aldosterone/MR selective MR antagonist eplerenone has been interpreted as between the antihypertensive and electrolyte effects of the MR blockade despite normal aldosterone levels.

There is no question that elevated aldosterone levels inappropriate for sodium status are followed by marked cardiovascular pathology, both experimentally and clinically. Rocha and Funder7 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.9 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])9, and as monotherapy in essential hypertension,1,10,11 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 rats12 and subsequently for recombinant human MR13 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
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\textsuperscript{14,15} proposed that epithelial aldosterone selectivity was conferred by the coexpression of MR and the enzyme 11\textbeta-hydroxysteroid dehydrogenase (11\textbeta-HSD2), which converts cortisol and corticosterone to their MR-inactive 11-keto congeners cortisolone 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\textbeta-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\textbeta-HSD2 is to prevent glucocorticoid occupancy of epithelial MR.

Like all great lies,\textsuperscript{16} 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 99 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,\textsuperscript{17} and over a decade ago was demonstrated experimentally not to be the case,\textsuperscript{18} in studies that incidentally address the question of relative intracellular levels of aldosterone and glucocorticoids.

Adrenalectomized rats were injected with [\textsuperscript{3}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 [\textsuperscript{3}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\textbeta-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\textsuperscript{13} 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 [\textsuperscript{3}H] aldosterone is shifted in comparison with the other tissues.

If the action of 11\textbeta-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\textbeta-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 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 cortisol 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 11βHSD2 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.19

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 11βHSD2 and MR) but does not
offer an explanation of how glucocorticoids might activate
cardiomycyte MR in RALES and EPHEBUS, eg, in the
absence of 11βHSD2. 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.20 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 al21 in recent
ischemia-reperfusion studies. In rat heart Langendorff prep-
arations, administration of aldosterone increases infract 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 infract 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
confused with oxidized glutathione, however, cortisol be-
comes an MR agonist, mimicking the effect of aldosterone.22

It appears then that MR, which are largely constitutively
occupied by normal glucocorticoid levels, respond in both
11βHSD2-expressing tissues (vascular smooth muscle cells
and endothelial cells) and nonexpressing tissues, eg cardio-
myocytes, to changes in intracellular redox state. The exper-
imental 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)α, 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 Fe2+/2 and Fe3+/2 in the heme moiety.23

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,24,25 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.26 Corti-
osterone stoichiometrically blocks the aldosterone effect at
equivalent or similar doses,27 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 11βHSD2 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 (presum-
ably 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.29–31 What has remained un-
explored 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 contra-indication: in patients with normal renal function and with antagonist titrated to effect, there appears to be only a very modest increase of ≤0.3 meq/L in plasma [K⁺], even at high doses (200 mg) of eplerenone.¹ This said, the dangers of hyperkalemia in elderly patients with a reduced creatinine clearance are real,² 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 of 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.¹,³,⁵,⁹,¹¹

From a variety of microarray studies (commonly in response to other steroids) it is clear that receptor agonists induce/press an overlapping but not identical portfolio of genes: in human liver cells, eg, of a total of ~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.

References

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