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Mineralocorticoid Receptors: Distribution and Activation

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Abstract. **Mineralocorticoid receptors (MR) bind both mineralocorticoids and glucocorticoids with high affinity (deoxycorticosterone = corticosterone ≥ aldosterone = cortisol), and are found in both Na^+ transporting epithelia (e.g. kidney, colon) and nonepithelial tissues (e.g. heart, brain). MR evolved before aldosterone synthase, consistent with their acting in nonepithelial tissues as high affinity glucocorticoid receptors, essentially always occupied by normal levels of endogenous glucocorticoids. In epithelial tissues the enzyme 11β hydroxysteroid dehydrogenase Type 2 (11βHSD2) allows aldosterone to selectively activate MR, by converting cortisol to cortisone and NAD to NADH. 11βHSD2 debulks intracellular cortisol by 90%, to levels ∼10-fold those of aldosterone, so that when the enzyme is operating most epithelial MR are occupied but not activated by cortisol. When intracellular redox state is changed—by inhibition of 11βHSD2, generation of reactive oxygen species, or intracellular introduction of oxidised glutathione (GSSG)—cortisol changes from an MR antagonist to an MR agonist. This bivalent activity of cortisol appears to underlie the therapeutic efficacy of MR blockade in heart failure (RALES, EPHESUS) and in essential hypertension, providing a rationale for MR blockade in cardiovascular disease not characterized by elevated aldosterone levels. Its wider (patho)physiologic implications, particularly for neurobiology, remain to be explored.**

Key Words. **aldosterone, mineralocorticoid receptors, 11β hydroxysteroid dehydrogenase, vascular inflammation, reactive oxygen species, NADH, redox state**

Introduction

Aldosterone was characterized in 1953, on the basis of its mineralocorticoid effects on urinary Na^+/K^+ ratios [1]. Twenty years later aldosterone was shown to bind with high affinity to an intracellular site in renal cytosol preparations, initially termed the corticosteroid Type I binding site, and subsequently the mineralocorticoid receptor [2–4]. The effects of aldosterone on urinary electrolyte excretion in adrenalectomized rats could be blocked by spironolactone, with parallel decreases in aldosterone binding in the kidney [5], and its effects could also be blocked by cycloheximide and actinomycin D, evidence for a mechanism of action via DNA-directed, RNA-mediated protein synthesis.

At the same time, the binding of tritiated natural and synthetic glucocorticoids was being charted in the rat brain. After in vivo injection, [^{3}H]dexamethasone accumulated in high abundance in the pituitary, but to a much lesser extent within the brain. In contrast, [^{3}H]corticosterone was found at relatively low levels in the pituitary, but at much higher concentrations than [^{3}H]dexamethasone in the brain, most noticeably in the hippocampus, to a class of glucocorticoid receptors termed 'corticosterone-preferring sites' [6].

A decade after the initial studies on the binding of aldosterone, including demonstration of the disquietingly high affinity of the glucocorticoid corticosterone for rat kidney mineralocorticoid receptors [4], it was shown that mineralocorticoid receptors in the kidney and 'corticosterone-preferring sites' in the hippocampus were indistinguishable in terms of their affinity for a range of steroids (deoxycorticosterone = corticosterone ≥ aldosterone = cortisol), evidence for their close similarity if not identity [7]. Subsequently this has proven to be the case (the rat mineralocorticoid receptor was actually cloned from brain cDNA [8]), with a range of promoters in different tissues but no tissue-specific differences in protein sequence. It has been reported that the [^{3}H]aldosterone·MR complex consistently (10/10) runs one tube apart when hippocampal and renal cytosols were subjected to fast protein liquid chromatography, whereas [^{3}H]dexamethasone·GR complexes run indistinguishably [9]. This was interpreted as suggesting an additional factor bound to occupied MR in the hippocampus, although this proposition has not been further explored.

In addition to the kidney, mineralocorticoid receptors were also described in a variety of epithelia involved in electrolyte transport, on the basis of high-affinity, displaceable binding of [^{3}H]aldosterone. In addition to the hippocampus, mineralocorticoid receptors were characterized in non-epithelial tissues, including the A3V3 region of the brain [10], the heart [11] and the vessel wall [12], of particular relevance to the present volume. The cloning of the human mineralocorticoid receptor confirmed the findings from the prior

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binding studies on tissue extracts, and opened the way to the variety of molecular biological techniques applied in subsequent studies [13]. That said, the mineralocorticoid receptor has remained very lightly studied in terms of its cellular biology in comparison with other members of the steroid/thyroid/retinoid/sterol/xenobiotic/orphan receptor superfamily (48 family members in man, 49 in mouse). This is probably the case for a variety of reasons—receptor instability, the early availability of the antagonist spironolactone, and the now obviously too narrow view that its (patho)physiology was confined to epithelial fluid and electrolyte transport.

The cloned human mineralocorticoid receptor, like that in rat tissue studies, showed high affinity for a broad range of steroids (deoxycorticosterone, corticosterone, aldosterone, cortisol): parenthetically, progesterone was subsequently shown to have affinity equivalent to that of aldosterone [14]. When the cDNA for the cloned receptor was used to probe rat tissue extracts the highest levels of expression were found in the hippocampus, with lower levels in kidney, gut and heart. These studies thus restated, rather than answered, the two major questions in the area of mineralocorticoid receptor action. The first of these is how aldosterone can gain access to mineralocorticoid receptors in epithelia, to exert its fluid and electrolyte effects, in the face of the much higher ($\sim$3 orders of magnitude) levels of circulating glucocorticoids, which have at least equivalent affinity for mineralocorticoid receptors. The second is the role of nonepithelial mineralocorticoid receptors, and whether they are (patho)physiologic aldosterone receptors, or merely (sic) high affinity glucocorticoid receptors. The answer to the first appears to lie, in large part or in whole, in the coexpression of the enzyme 11β hydroxysteroid dehydrogenase Type II with mineralocorticoid receptors in epithelia (and in some non-epithelial aldosterone target tissues, such as blood vessels). The answers to the second question are not yet in, but some intuitions of (patho)physiologic roles have followed recent studies, primarily in the cardiovascular system, and from evolutionary considerations.

Evolution: Aldosterone and Mineralocorticoid Receptors

The clinical syndrome of glucocorticoid remediable aldosteronism, or glucocorticoid suppressible hypertension, was first characterized at the molecular level in 1992 [15], and provides a valuable insight into evolution of corticosteroids and receptors. The syndrome is familial, characterized by hyperaldosteronism, suppressed renin and hypertension, all of which can be reversed by glucocorticoid administration. It reflects the operation of a chimeric gene—5′ 11β hydroxylase, 3′ aldosterone synthase—formed by uneven crossing-over at meiosis in the ancestral founder. The 5′ 11β hydroxylase component contains both the adrenal fasciculata localization signal, and the ACTH-responsive promoter region: the chimera is thus widely expressed in the adrenal, in response to ACTH and thus out of normal feedback control. The reason that such a crossing over can occur is that the gene coding for 11β hydroxylase, the signature enzyme for glucocorticoid activity, and that coding for aldosterone synthase lie next to one another on human chromosome 8 q24, and share 94% identity in terms of DNA sequence.

The most common and plausible explanation of highly homologous genes lying side by side in the genome is that of a relatively recent gene duplication event. It is not possible from such a location to be unequivocal on which gene came first: aldosterone synthase has 11β hydroxylase activity, human 11β hydroxylase low but detectable levels of aldosterone synthase activity [16], and bovine 11β hydroxylase is responsible for both 11β hydroxylation and sequential oxidation of carbon 18 to yield aldosterone. What provides a pointer, however, is a consideration of the evolutionary appearance of cortisol and aldosterone, which strongly suggests that the primary gene product had predominant 11β hydroxylase activity. Aldosterone is first reliably found in terrestrial vertebrates, and not in fish: in contrast, fish have considerable levels of circulating cortisol, suggesting that the evolution of a dedicated aldosterone synthase is a relatively late event, presumably as part of fluid and electrolyte homeostasis in adapting to a terrestrial environment. In contrast with 11β hydroxylase and aldosterone synthase, the genes for mineralocorticoid and glucocorticoid receptors lie on different chromosomes (human chromosomes 4 and 5); in addition, the receptors show differences in homology within their constituent domains. The central DNA-binding domain of the human receptors shows 94% amino acid identity: in this region identity is $\geq$90% for all four sub-family members (mineralocorticoid, glucocorticoid, progesterone and androgen receptors). In the ligand-binding domain the across-subfamily identity is $\geq$50%, with 57% for mineralocorticoid and glucocorticoid receptors. In the long N-terminal domain, identity is $<$15%, across the whole subfamily.

The existence of three such different constituent domains, and locations on different chromosomes, is consistent with the evolutionary emergence of distinct mineralocorticoid receptors substantially before that of a dedicated aldosterone synthase. This appears to be borne out by the evolutionary record [17], and the recent demonstration of MR in fish, much more closely

aligned in terms of sequence with mammalian MR than with fish GR [18–20]. Whether such MR are mineralocorticoid receptors, mediating transepithelial electrolyte flux in response to ligands other than aldosterone, is still an area of active investigation: their abundance in non-epithelial transporting tissues, in fish as in other species, underscores potential pathophysiologic roles for nonepithelial MR as high affinity glucocorticoid receptors.

The residual conceptual problem, as yet not widely addressed, is that the affinity is so high that such nonepithelial mineralocorticoid receptors are overwhelmingly occupied by glucocorticoids over the range of diurnal variation in plasma levels, and thus not well placed to respond to glucocorticoids per se: an alternative mechanism of activation is canvassed below. Parenthetically, while mineralocorticoid receptors and glucocorticoid receptors are commonly considered (on the basis of marginally higher amino acid identity) as sharing a common 'corticoid receptor' ancestor [17], internal sequence evidence might be interpreted as glucocorticoid receptors being closer to those for progesterone and androgens than to mineralocorticoid receptors [21]. If this is the case, then mineralocorticoid receptors may be truly Type 1, and glucocorticoid receptors Type 2, in terms of evolution as well as binding of [^{3}H]aldosterone, the criterion on which the original designation was based.

Epithelial Mineralocorticoid Receptor Activation: The Role of 11β Hydroxysteroid Dehydrogenase

The syndrome of apparent mineralocorticoid excess was first described in 1977 [22]. The condition presents at a young age, sometimes with neonatal failure to thrive, in childhood but only rarely in adults, and is characterized by signs of very marked mineralocorticoid overactivity (sodium retention, hypokalemia, hypertension) in the presence of suppressed renin and low plasma aldosterone levels. Despite intensive searching, no evidence was found for abnormal levels of a known steroid able to activate mineralocorticoid receptors, or for a novel hypertensinogenic steroid. What such patients did show, however, was an abnormal ratio of cortisol to cortisone metabolites, normally around one, but with affected patients having ratios of up to 50:1. More recently, the ratio of urinary free cortisol to urinary free cortisone has been validated as a more precise discriminant. Over the past decade, the relatively small numbers of afflicted patients, and their families, have been subjected to genetic analysis for the enzyme responsible for cortisol to cortisone conversion (11β hydroxysteroid dehydrogenase Type II), and a series of mutations causing deficient or absent enzyme activity charted. In normal subjects, or in experimental animals, the enzyme can be blocked by carbenoxolone, the hemisuccinate of glycyrrhetinic acid, leading to sodium retention and blood pressure elevation [23]; overindulgence in licorice, which contains glycyrrhizin, can produce a similar clinical picture.

On the basis of these clinical studies it was established that the physiologic role of 11β hydroxysteroid dehydrogenase in the kidney was to convert cortisol to receptor-inactive cortisone (corticosterone to 11-dehydrocorticosterone in rats and mice) thus protecting mineralocorticoid receptors from occupancy and activation by glucocorticoids [24,25]. Aldosterone, in place of an inert CH_3 (methyl) group at carbon 18 has a very reactive CHO (aldehyde group), which cyclizes with the 11β hydroxyl group to form an 18,20 hemiacetal. This cyclized form constitutes $\geq$99% of circulating aldosterone, and is not a substrate for the enzyme, so that it can occupy and activate the protected receptor in response to homeostatic physiologic stimuli.

As previously noted in another context [26] all really great lies are half true: and while incomplete understanding may be more accurate in the present case, it would appear that the scenario shown in cartoon form in Figure 1, is half true, in that the action of the enzyme is indeed to prevent mineralocorticoid receptor activation, *but not receptor occupancy,* by cortisol. Though 11β hydroxysteroid is expressed at very high abundance in aldosterone target cells—for example, 3.5–4 $\times 10^6$ copies per renal principal cell—and the enzyme has a low K_m (high affinity) for the physiologic glucocorticoids, and is operationally unidirectional *in vivo*, it seems prima facie unlikely that the ratio of plasma free cortisol to aldosterone (normally of the order of 100:1) could be sufficiently altered in terms of intracellular concentrations to reduce the

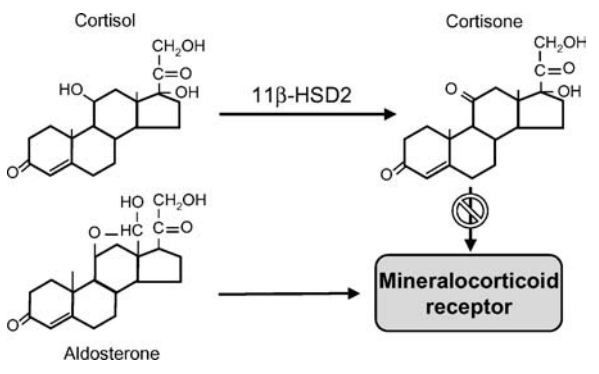

Fig. 1. *Cartoon of exclusion of cortisol from epithelial mineralocorticoid receptors by its conversion to receptor inactive metabolite cortisone.*

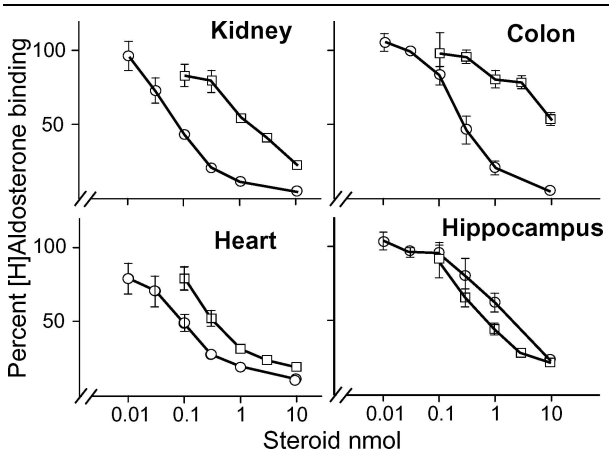

Fig. 2. *Binding of tracer [^{3}H]aldosterone in kidney, colon, heart and hippocampal extracts 15 min after injection of tracer alone, or with half-logarithmic increasing doses of nonradioactive aldosterone (•) or corticosterone (□). Reproduced, with permission from [28].*

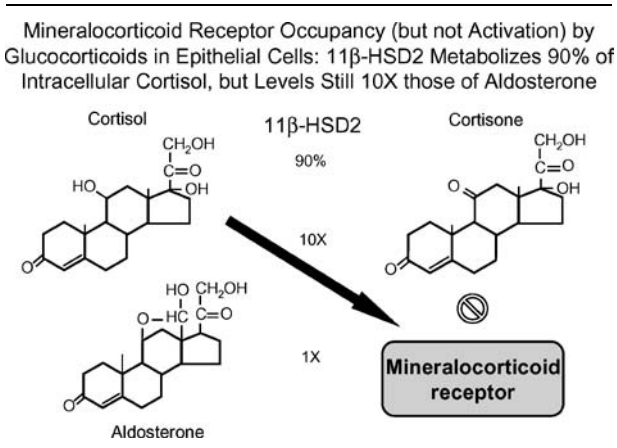

Fig. 3. *Cartoon of debulking of cortisol in epithelial cells expressing 11βhydroxysteroid dehydrogenase, based on data shown in Figure 2.*

noise to signal ratio from 100:1 to an acceptable level, say 1:10, or better 1:100.

What the calculations [27] and the appropriate experiments [28] showed was that 11β hydroxysteroid dehydrogenase confers an order of magnitude increase in aldosterone selectivity on epithelial mineralocorticoid receptors, as shown in Figure 2. In this study adrenalectomized rats were injected with [^{3}H]aldosterone, alone (for the 100% binding level) or with half-logarithmic increasing doses of nonradioactive aldosterone or corticosterone, with the animals killed 15 min later and tissues harvested. For the heart, aldosterone is a 3-fold better binder to mineralocorticoid receptors *in vivo*, as might be predicted from the 3-fold higher affinity of corticosterone for the receptor, but its 10-fold higher binding in plasma. In the hippocampus, the aldosterone displacement curve is markedly shifted to the right, and corticosterone has higher *in vivo* binding activity, as might be predicted from the much higher reflection coefficient of aldosterone at the blood brain barrier. In the classic epithelial aldosterone target tissues (kidney, colon), which unlike heart and hippocampus express 11β hydroxysteroid dehydrogenase Type II at high levels, the enzyme does not operate to exclude glucocorticoids: what it does is to reduce their ability to bid for receptor occupancy by approximately an order of magnitude. In such circumstances there are still of the order of tenfold higher intracellular glucocorticoid than aldosterone levels, even with the enzyme working normally (Fig. 3)—so the receptors are largely glucocorticoid occupied, but somehow not activated. When the enzyme is blocked intracellular glucocorticoid levels rise—but from a very high baseline, in terms of receptor occupancy, and thus unlikely to be the crucial factor in activating occupied but somehow silence mineralocorticoid receptors.

What is often overlooked is that NAD, normally present at ~600 fold intracellular abundance over NADH, is the obligate cosubstrate for the dehydrogenation of cortisol to cortisone. 11β hydroxysteroid dehydrogenase is expressed at high abundance in renal principal cells, as noted previously: and even though such cells constitute perhaps only ~5% of the renal cell mass, and renal blood flow accounts for 20–25% of cardiac output, there is a significant decrease in plasma cortisol to cortisone ratios across the renal vascular bed. Under such circumstances NADH levels can rise sharply, with minimal effects on substrate NAD levels, potentially allowing it to act as an intracellular signal over a very wide dynamic range. When the enzyme is blocked substrate cortisol levels rise, but from a very high baseline in terms of receptor occupancy. In contrast, when the enzyme is blocked NADH levels fall precipitously, allowing cortisol to activate mineralocorticoid receptors by mechanisms yet to be established. An unremarked example of the rapidity of this time course is illustrated by studies on Na^+/H^+ exchanger activity in human vascular smooth muscle cells in response to mineralocorticoid receptor activation [29]. Aldosterone raises intracellular pH, with a time course of 5 min to plateau effect; cortisol is without agonist effect until carbenoxolone is added to block 11β hydroxysteroid dehydrogenase activity. When this is done cortisol immediately becomes a mineralocorticoid receptor agonist (Fig. 4), elevating intracellular pH to an extent indistinguishable from aldosterone. In other systems [30,31] NADH has been shown to regulate transcription factors by activating corepressors, which may thus play a role in abrogating the genomic actions of glucocorticoid occupied mineralocorticoid receptors. That

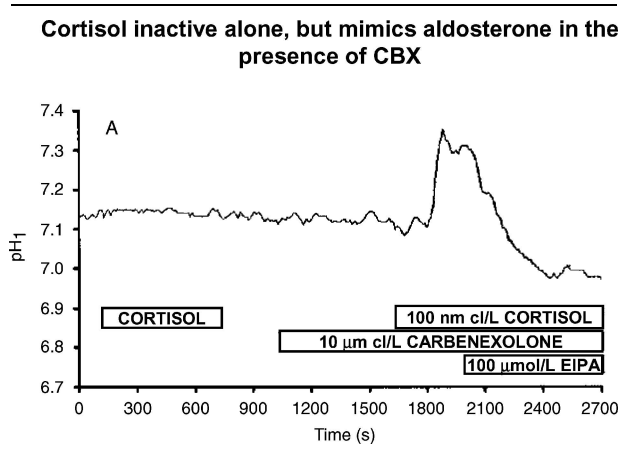

Fig. 4. *Cortisol does not activate vascular smooth muscle cells at a concentration of 100 nM when given alone, but does in the presence of carbenoxolone via MR activation of EIPA-inhibitable Na^+/H^+ exchange. Redrawn with permission from [29].*

this is not the only mechanism of its action is suggested by the rapid nongenomic studies shown in Figure 4, and by the rabbit cardiomyocyte studies to be briefly discussed below.

Apparent mineralocorticoid excess is rare, carbenoxolone has been superseded by at least three generations of peptic ulcer treatment, and licorice overindulgence should be excluded by appropriate history-taking: these factors apart, what is the pathophysiologic import of mineralocorticoid receptor activation determined by NADH/NAD ratio, by intracellular (or intracompartment) redox state? A clue to the wider significance of redox state as a pivotal control mechanism in terms of whether or not glucocorticoid occupied mineralocorticoid receptors are activated from studies on experimental coronary angioplasty in pigs [32]. When animals were treated for five days before and 28 days after vigorous balloon angioplasty with the selective mineralocorticoid receptor antagonist eplerenone their coronary vascular lumen was preserved, at levels ~160% those of control animals, with a trend to lower levels of neointima formation but significantly less fibrosis and constriction. These animals were on normal pig rations without sodium supplementation or exogenous mineralocorticoids: what, then, was the eplerenone blocking in terms of mineralocorticoid receptor activation, more commonly found in situations of inappropriate mineralocorticoid for salt status, as seen in rats given aldosterone or corticosterone plus 0.9% NaCl solution to drink [33–37]? The answer would appear to be normal physiologic levels of endogenous cortisol, occupying but not activating vascular wall mineralocorticoid receptors in normal vessels, but occupying and activating vascular mineralocorticoid receptors in the context of post-angioplasty tissue damage, generation of reactive oxygen species and the consequential effects on intracellular redox state.

That cortisol can indeed be an agonist or antagonist in mineralocorticoid receptors is also suggested by preliminary studies in rabbit cardiomyocytes, published to date only in abstract [38]. Unlike vascular smooth muscle cells, cardiomyocytes do not express 11β hydroxysteroid dehydrogenase, and their mineralocorticoid receptors are therefore unprotected and overwhelmingly occupied by glucocorticoids, previously shown not to mimic the effects of aldosterone via mineralocorticoid receptors in neonatal rat cardiomyocytes [39]. When rabbit cardiomyocytes are patch clamped and treated with 10 nM aldosterone, a ten-fold increase in ion flux across the plasma membrane is seen. Cortisol alone at 100 nM has no effect, but when given with 10 nM aldosterone stoichometrically antagonizes the aldosterone effect down to ~10% of maximum. When oxidised glutathione (GSSG: 5 mM) is instilled intracellularly via the broad-tipped pipet, to mimic the change in redox state seen in tissue damage, no change in current is seen with GSSG alone: when, however cortisol is added in the presence of GSSG it becomes a mineralocorticoid receptor agonist, mimicking the effect of aldosterone rather than antagonizing it.

Preclinical Studies and Clinical Implications

Whereas cardiac hypertrophy and fibrosis were the focus of experimental studies on the deleterious effects of inappropriate aldosterone for salt status a decade ago [33,34], more recent studies have shown that the initial effects are on the coronary vascular and perivascular compartments, where inflammatory changes can be clearly seen before any increase in collagen deposition [35–37], recapitulating the hypertension and vascular changes described almost 60 years ago following deoxycorticosterone and salt administration to rats [40]. In several recent studies the different pathophysiologic importance of targeted mineralocorticoid receptor activation has been explored, in an attempt to tease out possible differences between vascular and cardiomyocyte contributions. When uninephrectomized rats on 0.9% NaCl solution to drink are given carbenoxolone in their drinking solution they show coronary vascular and perivascular responses indistinguishable from those seen after administration of deoxycorticosterone [41]. These carbenoxolone induced changes are completely abolished by inclusion of eplerenone in the chow, evidence that the carbenoxolone effect is mediated via mineralocorticoid receptors, and that these receptors are

being activated by normal levels of endogenous glucocorticoids in the context of 11B hydroxysteroid dehydrogenase blockade in vascular smooth muscle cells.

In subsequent longer term studies [42] indices of vascular and perivascular inflammation, and cardiac fibrosis, were not different in rats killed after four weeks deoxycorticosterone plus salt, or killed four weeks later (i.e. after a second four week, withdrawal period). In contrast, eplerenone given for weeks 5–8 returned inflammatory markers—and significantly, fibrosis—to control levels despite the continued administration of deoxycorticosterone. These studies show that the effects of mineralocorticoid/salt excess can not only be blocked by concurrent mineralocorticoid receptor blockade, as previously shown [33–35], but also that eplerenone can reverse established fibrosis. Secondly, they show that in the absence of antagonist—and of initiating steroid—the inflammation and fibrosis does not resolve, interpreted as further evidence for continued activation of vascular wall mineralocorticoid receptors by normal levels of circulating glucocorticoids in the context of established tissue damage.

A third study [43] addressed the cardiomyocyte as a potential site of cardiovascular damage induced by inappropriate mineralocorticoid receptor activation. When transgenic mice express 11β hydroxysteroid dehydrogenase ectopically in cardiomyocytes, at levels $\sim \frac{1}{10}$ those in renal principal cells, they develop dilated cardiomyopathy, cardiac fibrosis and cardiac failure, interpreted as reflecting activation of a minority of but sufficient cardiomyocytes by endogenous aldosterone. The symptoms were improved by eplerenone administration [43], and abolished by cardiomyocyte-selective mineralocorticoid receptor knockout in the transgenic mice [44]. Of interest is that the single transgenic mice overexpressing 11β hydroxysteroid dehydrogenase Type II appeared to have normal coronary vessels and an unremarkable perivascular compartment, in contrast with the more global mineralocorticoid/salt imbalance models [34–36,40,41].

Similarly, clinical studies have established the benefits of mineralocorticoid receptor blockade in both hypertension [45,46] and heart failure [47,48]. In the 4E trial [45] essential hypertensives showed equivalent blood pressure responses to eplerenone and enalapril, and similar benefits in terms of left ventricular hypertrophy on magnetic resonance imaging and of renal function, as measured by proteinuria [46]: in combination, eplerenone and enalapril showed a marked synergistic effect on both parameters. In the RALES trial spironolactone, at a modest average dose of 26 mg/day, produced a 30% increase in survival when added to standard of care for patients with progressive heart failure, and a 35% improvement in hospitalization [47]. In the EPHESUS trial [48], eplerenone at an average dose of 43 mg/day in addition to standard of care similarly showed significant positive effects in patients with heart failure post-myocardial infarct. Importantly, in all of these studies aldosterone levels were normal, and sodium status unremarkable. In hypertension it is unclear whether the locus of the antihypertensive effect of mineralocorticoid receptor blockade is central (e.g. the A3V3 region) or peripheral (e.g. vessel wall); meta-analysis of several studies on hypertensive patients clearly distinguishes the antihypertensive and electrolyte effects [49], suggesting a lesser role for the kidney in mineralocorticoid receptor mediated blood pressure control than previously thought.

In the clinical context of heart failure, where cardiomyocytes are known to show high levels of reactive oxygen species, it appears probable that antagonists are blocking the effects of normal levels of cortisol activating mineralocorticoid receptors in the context of tissue damage. In hypertension there is clear evidence that central MR need to be activated for systemic mineralocorticoid excess for salt status to result in hypertension [50]; their role in clinical physiology, and the possibility of activation by glucocorticoids as well as aldosterone under particular circumstances, is not clear. To the extent that vessel walls are damaged in hypertension, at least some of the vasculoprotective effect of mineralocorticoid receptor blockade may reflect exclusion of normal levels of glucocorticoids from activating the receptors in the context of tissue damage.

Speculation

If mineralocorticoid receptors in non-epithelial tissues, in particular, are overwhelmingly always occupied by glucocorticoids it seems unlikely that they will respond to changes in circulating glucocorticoid levels. If such receptors are occupied by glucocorticoids in tonic inhibitory mode under normal circumstances, but can be activated—ligand constant—by changes in the redox state of a cell then they may function as a sensor, and presumably in some way a modulator, of cellular stress. The therapeutic implications of this for cardiovascular, and perhaps other, disease are profound, in that they provide a rationale for the proven therapeutic utility of mineralocorticoid receptor blockade even when aldosterone levels are normal. The biologic implications and physiologic roles of an always glucocorticoid occupied mineralocorticoid receptor, in cardiomyocytes and neurons inter alia, remain to be explored.

References

1. Simpson S, Tait J, Wettstein A, Neher R, von Euw J, Reichstein T. Isolierung eines neuen kristallisierten Hormons aus Nebennerien mit besonders hoher Wirksamkeit auf den Mineralsoffwechsel. *Experientia* 1953;9:333–335.
2. Rousseau G, Baxter J, Funder J, Edelman I, Tomkins G. Glucocorticoid and mineralocorticoid receptors for aldosterone. *J Steroid Biochem* 1972;3:219–227.
3. Funder JW, Feldman D, Edelman IS. Specific aldosterone binding in rat kidney and parotid. *J Steroid Biochemistry* 1972;3:209–218.
4. Funder JW, Feldman D, Edelman IS. The roles of plasma binding and receptor specificity in the mineralocorticoid action of aldosterone. Endocrinology 1973;92:992–1004.
5. Marver D, Stewart J, Funder JW, Feldman D, Edelman IS. Renal aldosterone receptors: Studies with (3H) aldosterone and the anti-mineralocorticoid (3H) spirolactone (SC26304). *Proc Natl Acad Sci USA* 1974;71:1431–1435.
6. McEwen BS, De Kloet ER, Rostene W. Adrenal steroid receptors and actions in the nervous system. *Physiol Rev* 1986;66:1121–1188.
7. Krozowski ZS, Funder JW. Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. *Proc Natl Acad Sci USA* 1983;80:6056–6060.
8. Patel PD, Sherman TG, Goldman DJ, Watson SJ. Molecular cloning of a mineralocorticoid (type 1) receptor complementary DNA from rat hippocampus. *Mol Endocrinol* 1989;3:1877–1885.
9. Doyle D, Krozowski Z, Morgan FJ, Funder JW. Analysis of renal and hippocampal Type I and Type II receptors by Fast Protein Liquid Chromatography. *J Steroid Biochem* 1988;29:415–421.
10. Berecek KH, Barron KW, Webb RL, Brody MJ. Vasopressin-central nervous system interactions in the development of DOCA-hypertension. *Hypertension* 1982;4:131–137.
11. Pearce P, Funder JW. High affinity aldosterone binding sites (Type 1 receptors) in rat heart. *Clin Exp Pharmacol Physiol* 1987;14:859–866.
12. Funder JW, Pearce PT, Smith R, Campbell J. Vascular type I aldosterone binding sites are physiological mineralocorticoid receptors. *Endocrinology* 1989;125:2224–2226.
13. Arriza JL, Weinberger C, Cerelli G, et al. Cloning of human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. *Science* 1987;237:268–275.
14. Myles K, Funder JW. Progesterone binding to mineralocorticoid receptors: *in vitro* and *in vivo* studies. *Amer J Physiol* 1996;270:E601–E607.
15. Lifton RP, Dluhy RG, Powers M, et al. A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature* 1992;355:262–265.
16. Dunlop F, Crock P, Montalto J, Funder JW, Curnow K. A compound heterozygote case of type II aldosterone synthase deficiency. *J Clin Endocrinol Metab* 2003;88:2518–2526.
17. Thornton JW. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. *Proc Natl Acad Sci USA* 2001;98:5671–5676.
18. Greenwood A, Butler P, White R, DeMarco U, Pearce D, Fernald R. Multiple corticosteroid receptors in a teleost fish: Distinct sequences. *Endocrinology* 2003;144:4226–4236.
19. Baker ME. Evolution of glucocorticoid and mineralocorticoid responses: Go fish. *Endocrinology* 2003;144:4223–4225.
20. Sturm A, Bury N, Dengreville L, et al. 11-deoxycorticosterone is a potent agonist of the rainbow trout (Oncorhynchus mykiss) mineralocorticoid receptor. *Endocrinology* 2004:in press.
21. Geller D, Farhi A, Pinkerton N, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science* 2000;289:119–123.
22. New MI, Levine LS, Biglieri EG, Pareira J, Ulick S. Evidence for an unidentified steroid in a child with apparent mineralocorticoid hypertension. *J Clin Endocrinol Metab* 1977;44:924–933.
23. Stewart PM, Wallace AM, Valentino R, Burt D, Shackleton CH, Edwards CR. Mineralocorticoid activity of liquorice: 11-beta-hydroxysteroid dehydrogenase deficiency comes of age. *Lancet* 1987;2:821–824.
24. Funder JW, Pearce P, Smith R, Smith AI. Mineralocorticoid action: Target-tissue specificity is enzyme, not receptor, mediated. *Science* 1988;242:583–585.
25. Edwards CR, Stewart PM, Burt D, et al. Localisation of 11 beta-hydroxysteroid dehydrogenase—tissue specific protector of the mineralocorticoid receptor. *Lancet* 1988;2:986–989.
26. Funder JW. All really great lies are half true. *Science* 1979;206:1139.
27. Funder J. Enzymes and receptors: Challenges and future directions. *Steroids* 1994;59:164–169.
28. Funder JW, Myles K. Exclusion of corticosterone from epithelial mineralocorticoid receptors is insufficient for selectivity of aldosterone action: *In vivo* binding studies. *Endocrinology* 1996;137:5264–5268.
29. Alzamora R, Michea L, Marusic ET. Role of 11beta-hydroxysteroid dehydrogenase on nongenomic aldosterone effects in human arteries. *Hypertension* 2000;35:1099–1104.
30. Zhang Q, Piston D, Goodman R. Regulation of corepressor function by nuclear NADH. *Science* 2002;295:1895–1897.
31. Fjeld C, Birdsong W, Goodman R. Differential binding of NAD^+ and NADH allows the transcriptional corepressor carboxyl-terminal binding protein to serve as a metabolic sensor. *Proc Natl Acad Sci USA* 2003;100:9202–9207.
32. Ward MR, Kanellakis P, Ramsey D, Funder JW, Bobik A. Eplerenone suppresses constrictive remodeling and collagen accumulation after angioplasty in porcine coronary arteries. *Circulation* 2001;104:467–472.
33. Brilla CG, Weber KT. Mineralocorticoid excess, dietary sodium, and myocardial fibrosis. *J Lab Clin Med* 1992;120:893–901.
34. Young M, Fullerton M, Dilley R, Funder J. Mineralocorticoids, hypertension, and cardiac fibrosis. *J Clin Invest* 1994;93:2578–2583.
35. Rocha R, Martin-Berger C, Yang P, Scherrar R, Delyani J, McMahon E. Selective aldosterone blockade prevents angiotensin II/salt-induced vascular inflammation in the rat heart. *Endocrinology* 2002;143:4828–4836.
36. Rocha R, Rudolph A, Frierdich G, et al. Aldosterone induces a vascular inflammatory phenotype in the rat heart. *Amer J Physiol* 2002;283:H1802–H1810.
37. Blasi ER, Rocha R, Rudolph AE, Blomme EA, Polly ML, McMahon EG. Aldosterone/salt induces renal

inflammation and fibrosis in hypertensive rats. *Kidney International* 2003;63:1791–1800.
38. Mihailidou AS, Mardini M, Fraser T, Knights D, Funder JW. Agonist/antagonist activity of cortisol in cardiomyocyte mineralocorticoid receptors is determined by redox state. 30th Annual Meeting of the International Aldosterone Conference, New Orleans, 2004.
39. Sato A, Funder JW. High glucose stimulates aldosterone-induced hypertrophy via Type I mineralocorticoid receptors in neonatal rat cardiomyocytes. *Endocrinology* 1996;137:4145–4153.
40. Selye H. The general adaptation syndrome and the disease of adaptation. *J Clin Endocrinol* 1946;6:117–230.
41. Young M, Moussa L, Dilley R, Funder JW. Early inflammatory responses in experimental cardiac hypertrophy and fibrosis: Effects of 11 beta-hydroxysteroid dehydrogenase inactivation. *Endocrinology* 2003;144:1121–1125.
42. Young MJ, Funder JW. Eplerenone, but not steroid withdrawal, reverses cardiac fibrosis in DOC/salt rats. *Endocrinology* 2004;145:3153–3157.
43. Qin W, Rudolph A, Bond B, et al. A transgenic model of aldosterone-driven cardiac hypertrophy and heart failure. *Circ Res* 2003;93:69–76.
44. Qin W, Bond BR, Donoho GP, et al. Mineralocorticoid receptor mediates heart failure in the 11beta-hydroxysteroid dehydrogenase type 2 transgenic mouse model. American Heart Association Scientific Sessions, New Orleans, 2004.
45. Pitt B, Reichek N, Willenbrock R, et al. Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: The 4E-left ventricular hypertrophy study. *Circulation* 2003;108:1831–1838.
46. Epstein M, Buckalew V, Martinez F, et al. Antiproteinuric efficacy of eplerenone, enalapril and eplerenone/enalapril combination in diabetic hypertensives with microalbuminuria. *Am J Hypertens* 2002;15:24A (abstract).
47. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *New Eng J Med* 1999;341:709–717.
48. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *New Engl J Med* 2003;348:1309–1321.
49. Levy D, Rocha R, Funder JW. Distinguishing the antihypertensive and electrolyte effects of eplerenone. *J Clin Endocrinol Metab* 2004;89:2736–2740.
50. Gomez-Sanchez EP, Fort CM, Gomez-Sanchez CE. Intracerebroventricular infusion of RU28318 blocks aldosterone-salt hypertension. *Am J Physiol* 1990;258:E482–E484.