Minireview: Aldosterone and Mineralocorticoid Receptors: Past, Present, and Future

John W. Funder

Prince Henry’s Institute of Medical Research, Clayton, Victoria 3168, Australia

Although aldosterone was not isolated and chemically characterized until 1953, the mineralocorticoid action of certain steroids, notably deoxycorticosterone (DOC), had been recognized decades earlier. From 1953 until 1990 saw the establishment of the basic biology and clinical (patho)physiology of aldosterone as an epithelial sodium retaining hormone: its biosynthesis in the adrenal glomerulosa; control of its secretion by ACTH, angiotensin II, and plasma [K\(^+\)]; its action via intracellular mineralocorticoid receptors to promote DNA-directed; RNA-mediated synthesis of proteins responsible for its epithelial effects; and the syndrome of primary aldosteronism, in which secretion of the hormone is relatively autonomous of its normal stimuli. The past 2 decades have been a major extension of our understanding of the pathophysiology of aldosterone and the complexities of mineralocorticoid receptor signaling in particular. This review concludes with a brief consideration of recent findings regarding hormone and receptor, agonists, and antagonists. In 1990 it might reasonably have been argued that we had the overarching framework for understanding the roles of aldosterone and mineralocorticoid receptors, with only the details to be filled in. Two decades later we still do not know the boundaries, and for every answer, two questions are springing up: truly the more we learn, the less we know. (Endocrinology 151: 5098–5102, 2010)

The story of aldosterone begins with deoxycorticosterone (DOC), which we now know to be the immediate biosynthetic precursor of aldosterone in the adrenal cortex. DOC was isolated and crystallized in the 1930s and shown to have preponderantly mineralocorticoid activity in the ingenious rather than exact bioassays then in use. In the early 1940s, Kuizenga (1) showed that the amorphous fraction (what was left after the known steroids had been isolated) contained more residual mineralocorticoid activity than glucocorticoid activity, and recently his best preparations were calculated to contain approximately 20% aldosterone (2).

Ten years passed before aldosterone was finally isolated in 1953, from 500 kg of beef adrenals, and its molecular structure established (3, 4). Three things were necessary for this to happen. First was the development of elegant chromatography separation systems by Bush (5). Second was the use of radioisotopes of sodium and potassium by Simpson and Tait to measure mineralocorticoid activity in vivo (6). The final step required was the steroid chemistry of the Wettstein group in Basel (7), complementing the work in London and the characterization of the novel steroid molecule. The initial designation was electrocortin, from its effects on urinary electrolytes; when its structure was established, it became aldosterone, in recognition of its unique aldehyde (CHO) rather than the usual methyl (CH\(_3\)) group on carbon 18 of the steroid skeleton. It was also recognized that this aldehyde group cyclizes with the neighboring hydroxyl on carbon 11 to form an 11, 18 hemiacetal, or an 11, 18, 20 biacetal; this subsequently proved to be a crucial determinant of the mineralocorticoid specificity of aldosterone action on epithelial tissues.

What followed in short order were a spate of publications from Luetscher et al. in California (8–10) on the clinical pathophysiology of aldosterone and from Conn in Michigan (11, 12) with his description of primary aldosteronism, or Conn’s syndrome. The bioassay by Kagawa

Abbreviations: Cry, Cryptochrome; DOC, deoxycorticosterone; GR, glucocorticoid receptor; 11\(^{\alpha}\)HSD2, 11\(^{\alpha}\) hydroxysteroid dehydrogenase; MR, mineralocorticoid receptor; PR, progesterone receptor; RALES, Randomized Aldactone Evaluation Study.
et al. (13) for mineralocorticoid antagonist activity allowed the development of spironolactone (14). In the 1960s Davis et al. at the National Institutes of Health (15, 16) showed the crucial role of angiotensin in the control of aldosterone secretion, subsequently complemented by the description from the Florey group (17) of the major role for plasma [K⁺].

In terms of aldosterone action, things lagged behind, with the first description of mineralocorticoid receptors in 1973, 20 yr behind that of the hormone (18). The work in the laboratory of Edelman and colleagues (19) showed that rat kidney preparations contain both high-affinity aldosterone binding sites [type 1, or mineralocorticoid receptors (MRs)] and sites with much lower affinity [type 2 or glucocorticoid receptors (GRs)]. Edelman and colleagues (20) also showed that aldosterone action required DNA-directed, RNA-mediated protein synthesis for Na⁺ transport, the first demonstration that a steroid receptor acts as a nuclear transactivating factor.

The subsequent findings about MRs proved challenging. First, they were demonstrated at moderate (heart) or high (hippocampus) levels in a range of nonepithelial tissues (21, 22). Second, MRs were shown to have equivalent high affinity for aldosterone and cortisol and marginally even higher affinity for corticosterone and DOC (22, 23). Given that circulating free glucocorticoid levels are approximately 100-fold those of aldosterone, this raises the obvious question of how aldosterone can ever occupy epithelial MRs to regulate Na⁺ status. Shortly after the human MR was cloned and sequenced in the Evans laboratory, work in Edinburgh (24) and Melbourne (25) showed the pivotal specificity-conferring role of the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD2). This enzyme is expressed at high levels in epithelial aldosterone target cells and converts active glucocorticoids (e.g. cortisol) to receptor-inactive 11-keto analogs (e.g. cortisone). Thus, it was proposed that this process prevented cortisol from occupying epithelial MRs and allowed them to be selectively activated by aldosterone.

In 1990, then, a consensus view on aldosterone and MR might be summarized as follows:

- Angiotensin is the major driver of aldosterone secretion.
- Aldosterone is the physiologic ligand for MR.
- 11βHSD2 acts by excluding cortisol from epithelial MR.
- Aldosterone raises blood pressure via fluid and electrolyte effects.
- Primary aldosteronism is less than 1% of hypertension, benign and involving hypokalemia.
- MR antagonists act by blocking binding of aldosterone to MR.
- Aldosterone acts via MR to regulate target gene transcription.

Twenty years later, as detailed below, the evidence is against propositions 1–6, as bluntly stated, with the last a yes, but, as subsequently detailed.

Since 1990, the evolution of MR has been explored, its role in the pathophysiology of heart failure (and essential hypertension) established (26–28), the pathways whereby aldosterone regulates Na⁺ retention increasingly documented (29), nongenomic effects clearly demonstrated (30) and nonadrenal (ectopic) sites of aldosterone synthesis reported (31–33) and disputed (34–37). Of these the first (evolution), third (pathways), and fourth (nongenomic) are areas of ongoing research and debate. In terms of nongenomic actions, it is still sometimes assumed that all rapid effects involve membrane receptors distinct from the cloned MR, whereas it is now clear that most (but not all) such rapid effects are via the classic intracellular MR, with the physiology in large part awaiting exploration (38). The second (heart failure) has been the occasion of widespread jumping to an unjustified conclusion. The efficacy of spironolactone in the Randomized Aldactone Evaluation Study (RALES) trial has led many people to regard aldosterone as the culprit in heart failure, despite the low normal aldosterone levels in RALES. The remaining advance has been the reporting of ectopic aldosterone secretion in a variety of tissues (heart, vasculature, brain) despite immeasurable plasma levels in patients after bilateral adrenalectomy and formal disproof in the case of the heart (34, 35).

More challenging is the widespread acceptance in 2010 of the 1990 consensus, with the exception of primary aldosteronism. If we put the two lists together, the first three are yes by common consent, but for the remaining seven, major modification or qualification is required. The list looks something like this:

- Primary aldosteronism is higher risk than essential hypertension, its prevalence about 10%, with hypokalemia in only a minority (38).
- Aldosterone induces effector proteins in epithelia: serum- and glucocorticoid-induced kinase-1, CHIP, etc.
- Aldosterone acts genomically and nongenomically.

But

- Angiotensin is a (not the) major driver of aldosterone secretion.
- Aldosterone is a (not the) physiological ligand for MR.
- MR may be the oldest sibling of the MR/GR/androgen receptor/progesterone receptor (PR) subfamily.
- Aldosterone acts homeostatically to restore circulatory volume in hypovolemia but raises blood pressure by actions on the vasculature and central nervous system.
- 11βHSD2 debulks intracellular cortisol but does not exclude it from MR.
• In tissue damage in CHF and essential hypertension cortisol activates MR.
• MR antagonists act as inverse agonists.

Angiotensin is the major driver of the aldosterone secretory response to volume depletion, not sodium deficiency, and physiologically to the volume changes in response to upright posture. Chronic Na\(^+\) depletion causes indistinguishable elevation of plasma aldosterone levels in angiotensinogen knockout and wild-type mice (39); when the diet is low in both Na\(^+\) and K\(^+\), aldosterone secretion falls (from Na\(^+\) depletion alone levels) in wild-type, and even further in knockout mice, evidence for the major role for plasma [K\(^+\)] in regulating aldosterone in response to sodium deficiency.

With regard to the second point of the list of seven, MR appear in evolution well before the enzyme (CYP11B2, or aldosterone synthase), which produces aldosterone from DOC in the adrenal glomerulosa; fish, for example have well-characterized MRs, well before the emergence of aldosterone synthase in terrestrial vertebrates. In terms of MR evolution, MRs and GRs are commonly assumed to share a common ancestral precursor within the MR/GR/PR androgen receptor subfamily, an assumption supported on theoretical grounds but not by sequence alignment or prismatic clinical observations (40).

Received wisdom for more than 5 decades, enshrined in numerous textbooks of physiology, is that blood pressure elevation in response to aldosterone is primarily a renal response. It runs as follows: aldosterone causes Na\(^+\) retention, and with it comes water, which together serve to increase intravascular volume. Increased intravascular volume in turn produces an increase in cardiac output, reflexively normalized by vasoconstriction, which is then reflected in an increase in blood pressure. There is now compelling evidence, over 3 decades, of direct vasoconstrictor effects of MR activation on vascular wall (41–43) and in the central nervous system, i.e. in the circumventricular region of the hypothalamus (44).

More recent evidence against a predominant role for renal MR activation by aldosterone as the prime mover in blood pressure elevation comes from a metaanalysis of two trials in which eplerenone, a second-generation, highly selective MR antagonist, was used as monotherapy in patients with essential hypertension (45). In these dosetitration studies, patients received 50/100/200 mg daily of antagonist, escalating at 4-wk intervals until goal blood pressure (diastolic <90 mm Hg) was reached. Comparison of responders and nonresponders (in terms of achieving goal diastolic blood pressure) at each dose showed absolutely no relationship between the hypotensive and electrolyte effects of MR blockade.

In 1988 the enzyme 11βHSD2 was shown to be expressed in epithelial aldosterone target tissue and to allow aldosterone selectively to activate epithelial MR (24, 25). This was interpreted then, and continues to be interpreted, as evidence that the enzyme metabolizes the physiological glucocorticoids essentially completely, so that they do not occupy substantial levels of epithelial MRs. Although there is no doubt that such cells express 11βHSD2 at high levels, and that monolayers on a permeable support can be shown to metabolize almost all the radioactive glucocorticoid up to a concentration of 2.5 × 10\(^{-7}\) m (46), this system does not take into consideration the constant replenishment of substrate glucocorticoid in vivo, given that the kidneys receive 20–25% of cardiac output. For glucocorticoid levels to represent a noise level of 10% that of aldosterone, 11βHSD2 would need to metabolize 999 of every 1000 incident glucocorticoid molecules to receptor-inactive metabolites.

Almost 15 yr ago, the operation of 11βHSD2 in epithelia was shown to be that of debulking intracellular glucocorticoid levels, reducing them from 100- to 10-fold those of aldosterone, but not that of metabolizing glucocorticoids essentially out of existence (47). Most epithelial MRs are thus occupied by the approximately 10-fold preponderance of cortisol over aldosterone when the enzyme is operating, but are not activated. Indirect support for this is the time-honored Kagawa assay (14): adrenalectomized rats are 10-fold more sensitive to exogenous aldosterone than intact rats maintained on a high salt intake. When the enzyme is blocked, there is a minimal increase in cortisol occupancy of epithelial MRs, but a profound fall in reduction of cosubstrate nicotinamide adenine dinucleotide to reduced nicotinamide adenine dinucleotide. How reduced nicotinamide adenine dinucleotide holds cortisol-occupied MR inactive is not currently clear, and teasing out the mechanisms involved remains a major task for the future.

Under conditions of tissue damage and reactive oxygen species generation, cortisol activates MR (48, 49). In the Langendorf ischemia-reperfusion model, for example, cortisol and aldosterone are equipotent at nanomolar concentrations in increasing the area at risk and apoptosis (48). This action of both steroids is blocked by spironolactone but not the GR, PR antagonist RU486, evidence of an effect via MR. Finally, in the same ischemia-reperfusion model, spironolactone alone is protective (in hearts from intact or adrenalectomized rats) via reducing area at risk and apoptosis. Classically antagonists were thought of as blockers, acting by denying agonists the ability to bind to and activate receptors. An action of its own accord of spironolactone, to increase transcription of key antiapoptotic genes/reduce that of proapoptotic genes, suggests that in addition to blocking at the receptor level, spirono-
lactone acts postreceptor to oppose the effects of receptor activation by classical agonists.

Over the more than 5 decades since its characterization in 1953, aldosterone has been something of an orphan hormone, foster parented by physiologists, endocrinologists, nephrologists, pharmacologists, and most recently cardiologists. The two major drivers for radical reconsideration of its pathophysiological roles, and that of the promiscuous, enigmatic MRs, have been the RALES trial, showing the efficacy of MR antagonists in heart failure and the emergence of primary aldosteronism from a rare benign condition to a common and serious disease. The molecular and cellular physiology has levered off these two salients, but many of the insights from studies over the past 2 decades are yet to be incorporated into the canon. In the words of Machiavelli (30), “There is nothing more difficult and dangerous, or more doubtful of success, than an attempt to introduce a new order of things for the innovation has for enemies all those who derived advantage from the old order, while those who expect to benefit from the new will be but lukewarm defenders.”

One of the most exciting publications relevant to aldosterone, in terms of both physiology and pathophysiology, appeared earlier this year (51). Mice in which the core clock components cryptochrome (Cry)-1 and Cry-2 are absent show abnormally high synthesis of aldosterone [and salt dependent hypertension (no surprise)]. The authors then identified the lesion as the absence of expression of Cry gene products in the zona glomerulosa, compared with wild-type mice, and release from repression of the Cry gene products in the zona glomerulosa, compared with wild-type mice, and release from repression of the Cry gene products in the zona glomerulosa, compared with wild-type mice, and release from repression of the Cry gene products in the zona glomerulosa, compared with wild-type mice, and release from repression of the Cry gene products in the zona glomerulosa. In Cry-null mice Hsd3b6 mRNA and protein levels are constitutively high, leading to an autonomous increase in aldosterone production; in humans the equivalent glomerulosa-specific isoform is encoded by the HSD3B1 gene. In terms of basic biology, this finding challenges our current understanding that the two rate-limiting enzymes in aldosterone biosynthesis are side-chain cleavage and aldosterone synthase. In terms of pathophysiology, it may explain why expression levels of aldosterone synthesis may not be elevated in some adenomas. In terms of the future, it opens for informed investigation the possible mechanisms linking aldosterone secretion to disturbances of sleep patterns and sleep apnea.

There are other questions, basic and clinical, that need to be put, pondered, and addressed. In terms of aldosterone, these include its rapid secretion in response to posture change and the (presumably) rapid nongenomic homeostatic mechanisms set in train; the physiological (as opposed to pathophysiological) roles of aldosterone, if any, in cells such as the cardiomyocyte, which lack the specificity-conferring enzyme 11β-hydroxysteroid dehydrogenase; the mechanisms (vascular, central, renal) that interact to increase blood pressure; and finally the mechanisms whereby aldosterone is homeostatic, at very high circulating levels, in response to chronic sodium depletion, but has deleterious effects in the context of inappropriate sodium status, as seen with modestly elevated levels in primary aldosteronism. In terms of MRs, a similar list of questions can be put, perhaps the most pressing of which are the physiological roles of endogenous glucocorticoids in MRs, and the retained high affinity of MRs for progesterone as well as for the endogenous glucocorticoids. It should be a fascinating journey.

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Address all correspondence and requests for reprints to: John W. Funder, Prince Henry’s Institute of Medical Research, P.O. Box 5152, Clayton 3168, Australia. E-mail: john.funder@princehenrys.org.

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References

22. Krozowski ZS, Funder JW 1983 Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. Proc Natl Acad Sci USA 80:6056–6060
40. Funder J, Myles K 1996 Exclusion of corticosterone from epithelial mineralocorticoid receptors is insufficient for selectivity of aldosterone action: in vivo binding studies. Endocrinology 137:5264–5268
43. Machiavelli N 1515 The prince, translated by Marriott WK. University of Adelaide, Adelaide, South Australia, Australia: eBooks