**Aldosterone in Australia and Mineralocorticoids in Melbourne: More than Just Physiology**

Peter Fuller* and Morag Young  
Prince Henry’s Institute of Medical Research, Clayton, VIC 3168  
*Corresponding author: peter.fuller@princehenrys.org

The adrenal steroid, aldosterone, was isolated by James and Sylvia Tait in London in 1953, an auspicious time given the work of Watson and Crick in Cambridge that same year. Australia contributed substantially to the subsequent description of the physiology of aldosterone, particularly the group at the Howard Florey Institute: R. Douglas (Pansy) Wright, Derek (Dick) Denton, John Funder (known simply as ‘Funder’), John Coghlan et al. Those who remember their physiology will recall John Funder working at the Baker Institute cloned the gene in patients with a rare autosomal recessive form of mineralocorticoid excess (AME).

Mineralocorticoid Receptors

As for other steroid hormone receptors, the receptor for aldosterone, the mineralocorticoid receptor (MR), is a ligand-dependent transcription factor that is a member of the nuclear receptor superfamily (1). In collaboration with colleagues in France, we characterised the genomic organisation of the MR (2); most significantly, although there is clear use of alternate promoters, the encoded protein is the same at all sites. The MR is, however, unique amongst the steroid receptors in being a receptor for two physiological ligands, aldosterone and cortisol. By contrast, the closely related glucocorticoid receptor (GR) binds only cortisol with high affinity. Given that cortisol circulates at significantly higher levels than aldosterone, this presented a conundrum: how would aldosterone ever get in there?

**11β-Hydroxysteroid Dehydrogenase**

The answer to the puzzle comes from the work of John Funder and Zygmunt Krozowski in Melbourne and Paul Stewart with Chris Edwards in Edinburgh. In the liver, the reductase 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) converts the inactive steroid cortisone to cortisol (hydrocortisone). In epithelial tissues, the vasculature and some regions of the brain, the type 2 isoform, 11βHSD2, mediates the reverse reaction with conversion of cortisol to the receptor-inactive cortisone (Fig. 1). Anthony Albiston, Zygmunt Krozowski and John Funder working at the Baker Institute cloned the type 2 isoform (3) and demonstrated mutations in the gene in patients with a rare autosomal recessive form of early onset hypertension, so-called apparent mineralocorticoid excess (AME).

**Structure-Function Studies in the MR**

Steroid receptors consist of three principal functional domains: a central cysteine-rich DNA binding domain (DBD; this defines the family); an N-terminal domain (poorly conserved across the family); and a C-terminal ligand-binding domain (LBD). The modular nature of the steroid receptors enables one to ‘mix and match’. To understand the molecular basis of the differential binding of aldosterone and cortisol to MR and GR, we created chimeras between their LBDs whilst keeping the GR N-terminal domain and DBD constant. Using 16 LBD chimeras, Fraser Rogerson was able to demonstrate that if the region of the MR (amino acids 804-874) was swapped into the GR, aldosterone binding and transactivation would occur (4). Subchimeras narrowed this to the 25 amino acids, MR 820-844; of these amino acids, 16 differ between the two receptors. We resisted the temptation to use site-directed mutagenesis to create 216 chimeras, but using a series of blocks of mutations, we have narrowed this to four residues. This process has been informed by the crystal structure for the MR LBD (Fig. 2) which became available in 2005. The structural analysis was performed for us by Brian Smith at the Walter and Eliza Hall Institute. Whilst one might predict that the critical residues directly contribute to ligand binding, the residues identified do not contribute to the ligand-binding pocket. This observation emphasises the importance of receptor-interacting molecules, such as the Hsp90-complex, both for defining the binding characteristics of the receptor and for signal transduction.

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**Fig. 1. 11β hydroxysteroid dehydrogenase.**

The type 1 isoform mediates the NADP-dependent reduction of cortisone to cortisol, principally in the liver, but also in a range of other tissues, including abdominal adipose tissue. The type 2 isoform mediates an NAD-dependent conversion of cortisol to the receptor-inactive cortisone in epithelial tissues. In rodents, the principal physiological glucocorticoid is corticosterone, not cortisol.
Interdomain Interactions

Despite the ‘mix and match’ or ‘plug and play’ properties of the receptor domains, interdomain interactions can play a critical role in receptor function. Interactions between the N- and C-terminal domains have been reported in several steroid receptors; the best characterised are in the androgen receptor where mutations in the LBD that specifically target these interactions cause the androgen insensitivity syndrome.

We have characterised a similar interaction in the MR using the mammalian-2-hybrid system (5). This interaction is aldosterone mediated, and blocked by the MR antagonist, spironolactone. Our most striking finding was that cortisol acted as an antagonist, not an agonist in this receptor. The dissociation of these two classic MR agonists is a molecular first, although some physiological differences have been reported.

Coactivation and Corepression

The last decade in steroid receptor biology has been dominated by the coregulatory molecules. Although the MR generally interacts with previously well-characterised steroid-interacting molecules such as the SRC family and PGC-1α, the physiologically relevant MR coregulators have not been identified. Several studies have reported molecules that interact with the N-termini (6). Of more interest is the possibility of ligand-specific interactions which are more likely to be with the LBD. If such differential interactions relate also to tissuespecific differences, then the stage will be set for selective modulation of the MR. In preliminary yeast-2-hybrid screens of kidney and heart cDNA libraries, we have identified interacting proteins that show enriched interactions with either aldosterone or cortisol.

Pathophysiology

It is now clear that mineralocorticoid receptors are involved in regulating many more processes than just epithelial sodium transport. The expression of MR in a range of non-epithelial tissues in itself suggests a broader range of physiological actions. In recent years however, a central role for MR in cardiovascular pathology has been gaining momentum with several lines of clinical and experimental evidence supporting this notion.

The concept of a role for MR in cardiovascular disease followed a study in 1992 by Brilla and Weber, who showed that administration of aldosterone to rats, when drinking saline rather than water, caused diffuse tissue fibrosis along with cardiac hypertrophy and hypertension (10, 11). Further studies from our laboratory (then at the Baker Institute) showed that the tissue remodelling was unrelated to hypertrophy, blood
pressure or renal MR-mediated potassium wasting effects and thereby strongly suggested that this was a direct effect of aldosterone on cardiac MR (Fig. 3). It is now accepted that a critical step in this pathology is the initiation of an inflammatory response and the recruitment of macrophages prior to the onset of collagen deposition (12). It is interesting to note that this was first described over 70 years ago by Hans Selye who investigated the ‘general adaptation syndrome’ in animals given the potent mineralocorticoid deoxycorticosterone. Whilst a potent mineralocorticoid was used, the response was ascribed to glucocorticoid action or a stress response, perhaps reflecting the fact that aldosterone and thus the mineralocorticoid class of hormones was not described until 1953.

While there was increasing experimental evidence during the 1990s for the role of MR in cardiovascular disease and heart failure, it was publication of the RALES and EPHESUS clinical trials that refocused attention on this field (13). These trials collectively showed a substantial survival benefit of low dose MR antagonist in combination with current best practice therapy in congestive heart failure and heart failure after myocardial infarction, respectively. However, it should be noted that patients in these trials did not have elevated plasma aldosterone levels, posing the question as to what was activating the MR.

As mentioned above, plasma glucocorticoids (cortisol and corticosterone) are also ligands for the MR in the absence of the specificity-conferring enzyme 11βHSD2. We therefore hypothesised that it may be the higher plasma levels of glucocorticoids that are somehow activating the MR in these disease states (14). This hypothesis is consistent with studies where 11βHSD2 is inactivated by carbadoxone and an MR-dependent cardiovascular pathology follows that is equivalent to aldosterone-mediated effects. Other clues to the possible mechanisms regulating this pathological MR activation arose from coronary angioplasty studies in pigs at the Baker Institute, where luminal diameter was preserved and the restenosis response was reduced by pre-treatment with the selective MR antagonist eplerenone. These data are interpreted as vascular tissue damage, and by extension, the induction of inflammation, being sufficient to allow endogenous glucocorticoid activation of the MR given that the animals were not treated with aldosterone or salt. This hypothesis has been further refined to question whether the relatively higher levels of NADH, produced by 11βHSD2 from the cofactor NAD+ as it metabolises glucocorticoids (see above), may be involved. NADH may confer another level of ligand specificity to the MR. Patch clamping studies in single cardiomyocytes by Susie Mihailidou at Royal North Shore Hospital have shown that cortisol can mirror aldosterone responses in the presence of glutathione disulphide (GSSG), a reducing agent that serves to lower intracellular NADH levels (15). Whole animal studies investigating the fibrotic response to steroid withdrawal versus MR blockade demonstrated sustained inflammatory responses, whereas MR blockade reversed fibrosis and inflammation. These findings support the single cell patch clamp studies in that ongoing MR activation in the withdrawal model appears to be due to corticosterone activation of MR in the presence of tissue damage and inflammation (16).

Whilst these studies indicate that MR activation in the cardiovascular system, rather than mineralocorticoids levels per se, is responsible for the pathology, the molecular mechanisms of MR action described above suggest that aldosterone and glucocorticoids do not behave identically at the MR. At least at the level of N-terminal/C-terminal interaction this seems to be the case (5). Moreover, gene expression profiles indicate that there may well be differences, in terms of transcriptional responses, at the earliest time points (unpublished data). Whether this relates to cell-specific responses or differential coregulator recruitment remains to be explored. Identification of differential responses may facilitate the development of novel MR blockers that selectively target the pathological events in the cardiovascular system while preserving the physiological epithelial response.

### Conclusion

The study of aldosterone and the MR very accurately reflects the challenges of melding physiology, molecular biology and molecular genetics; each component is essential for the system to be understood. The contrast between the extreme phenotype of the MR knockout mouse developed by Tim Cole and colleagues (17) and the recently described relatively mild phenotype of the aldosterone synthase knockout mouse (18) emphasises the central importance of the MR. With cardiovascular disease, particularly cardiac failure, being the major cause of death in Australia, the importance of developing second and third generation MR antagonists becomes compelling. Such developments critically
depend on identifying the complexity and nuances of MR signalling and identifying the components that may allow tissue-specific ligands to be developed.

References