Molecular and Cellular Endocrinology 408 (2015) 33–37

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Review

# Novel interactions of the mineralocorticoid receptor

Peter J. Fuller *

*MIMR-PHI Institute* (formerly Prince Henry's Institute of Medical Research), *Clayton, Victoria, Australia*

ARTICLE INFO

*Article history:*
Received 12 November 2014
Received in revised form 17 January 2015
Accepted 18 January 2015
Available online 7 February 2015

*Keywords:*
Corticosteroids
Coactivators
N/C-interaction
Aldosterone
Cortisol

ABSTRACT

The mineralocorticoid receptor (MR) differs from the other steroid receptors in that it responds to two physiological ligands, aldosterone and cortisol. In epithelial tissues, aldosterone selectivity is determined by 11β-hydroxysteroid dehydrogenase type II. In other tissues cortisol is the primary ligand; in some tissues cortisol may act as an antagonist. To better target MR, an understanding of the structural determinants of tissue and ligand-specific MR activation is required. Our focus is on interactions of the ligand-binding domain (LBD) with ligand, the N-terminal domain and putative co-regulatory molecules. Molecular modelling has identified a region in the LBD of the MR and indeed other steroid receptors that critically defines ligand-specificity for aldosterone and cortisol, yet is not part of the ligand-binding pocket. An interaction between the N-terminus and LBD observed in the MR is aldosterone-dependent but is unexpectedly antagonised by cortisol. The structural basis of this interaction has been defined. We have identified proteins which interact in the presence of either aldosterone or cortisol but not both. These have been confirmed as coactivators of the full-length hMR. The structural basis of this interaction has been determined for tesmin, a ligand-discriminant coactivator of the MR. The successful identification of the structural basis of antagonism and of ligand-specific interactions of the MR may provide the basis for the development of novel MR ligands with tissue specificity.



**Contents**



## 1. Introduction

Aldosterone and the mineralocorticoid receptor (MR) are classically viewed as mediating the regulation of epithelial sodium transport in the distal nephron and distal colon (Fuller and Young, 2005). The MR is a member of the nuclear receptor superfamily. Its closest homologies are with the other corticosteroid receptor, the glucocorticoid receptor (GR). The MR is unique amongst the steroid receptors in having two physiological ligands, aldosterone and cortisol (corticosterone in rodents); indeed progesterone which is antagonist at the MR may also be viewed as a physiological ligand. Given that cortisol binds the MR with an equivalent affinity to that of aldosterone, yet it circulates at concentrations over 100-fold higher than that of aldosterone, access of aldosterone to the MR under normal physiological conditions would be precluded were it not for the presence of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD2). HSD2 metabolises cortisol to cortisone which in contrast to cortisol is unable to bind or activate the MR (Odermatt and Kratschmar, 2012).

As with other nuclear receptors, the MR consists of 3 principal domains. The first is a relatively unstructured N-terminal domain,

Proceedings of the Adrenal Cortex Conference, Chicago, USA.

* MIMR-PHI Institute, 27-31 Wright Street, Clayton, Victoria 3168, Australia. Tel.: +61 3 9594 4379; fax: +61 3 9594 6125.
*E-mail address:* peter.fuller@princehenrys.org

http://dx.doi.org/10.1016/j.mce.2015.01.027
0303-7207/

which is poorly conserved across the various receptors and whose key structural property is arguably this lack of structure (Fischer et al., 2010). Of all the nuclear receptors, the MR has the longest N-terminal domain. This lack of structure is thought to confer promiscuity of interaction upon the receptor. A central DNA-binding domain (DBD) of 66 or 68 amino acids defines the nuclear receptor superfamily. It is relatively highly conserved both from a functional and structural perspective. The crystal structure of the MR DBD has recently been solved (Hudson et al., 2014). It largely matches the structure of the closely related GR (Luisi et al., 1991) with which it shares considerable sequence homology; however there are subtle differences which may impact signalling (Hudson et al., 2014). At the C-terminal of the receptor is the ligand-binding domain (LBD) which also shares significant homology within the steroid receptor subfamily of the nuclear receptors (androgen receptor (AR), progesterone receptor (PR) and GR). Both the N-terminal domain and the LBD contain activation functions (AF-1 and AF-2 respectively). These activation functions mediate the interaction with the transcriptional apparatus. The MR LBD of 251 amino acids consist of 11α-helices (labeled by convention 1–12; helix 2 is unstructured in the MR) and 4 β-strands (Bledsoe et al., 2005; Fagart et al., 2005; Li et al., 2005). The structural representation of the AF-2 function is formed by helices 3, 4, 5 and 12. This region as with other nuclear receptors is able to bind co-regulatory molecules containing the leucine-x-x-leucine-leucine-motif (LxxLL: where x is another amino acid) characteristic of a number of co-activator molecules (Gronemeyer et al., 2004).

## 2. MR signalling mechanisms

The classical model of MR signalling sees the hydrophobic ligand move into the cytoplasm where it interacts with the unliganded receptor. In the absence of ligand, the receptor is complexed with a heat-shock binding (hsp) complex which holds it in a transcriptionally inactive but high affinity binding state. Binding of ligand sees a significant conformational change with helix 12 moving into position that forms the AF-2 domain. With this confirmational change and a variable degree of dissociation of proteins in the hsp complex, the receptor translocates to the nucleus where it binds to specific response elements, often a palindromic inverted repeat in the regulatory region of target genes. The DNA-bound, activated receptor then recruits the coregulatory complexes which link the receptor to the transcriptional apparatus, resulting in either activation or potentially repression of target gene expression (Glass and Rosenfeld, 2000). Although a number of MR regulated genes have now been identified, a broader whole-of-system picture of MR-regulated genes has not yet been developed in the way it has been for a number of other nuclear receptors, including the GR (Bookout et al., 2006).

For other steroid receptors particularly the GR, signalling may also occur through protein-protein interaction with other transcription factors. This may involve a process of sequestration. It is often termed transrepression or tethering (De Bosscher et al., 2003). The best characterised example is between the GR and the transcription factors AP-1 and NFκB, a key component of the GR-mediated anti-inflammatory effect. Similar mechanisms have also been described for the estrogen receptors (ER) where again the interaction can be with NFκB. In contrast to the GR, transrepression by the MR has not been clearly established. The MR does not interact with AP-1 (Pearce and Yamamoto, 1993) and although an interaction has been reported with the NFκB subunits *in vitro* (Kolla and Litwack, 2000; Liden et al., 1997), the finding somewhat contradicts results from other studies (Leroy et al., 2009; Terada et al., 2012; Wissink et al., 2000). NFκB activation stimulates expression of the adhesion molecule ICAM-1 (Caldenhoven et al., 1995) as does MR activation (Caprio et al., 2008) whereas the GR transrepresses these responses (Caldenhoven et al., 1995). It is also counter-intuitive that the MR might transrepress inflammatory signalling pathways given the large body of work which demonstrates the effects of MR activation to be pro-inflammatory in the cardiovascular system and kidney (Young and Rickard, 2012), including activation of the canonical NFκB signalling pathway (Leroy et al., 2009; Terada et al., 2012). More recently Chantong et al. (2012) have directly contrasted the influence of the MR and the GR on NFκB signalling in microglial cells. They found that MR activated and GR repressed NFκB signalling in this system. This is consistent with the concept that MR activation is pro-inflammatory, occurring at physiological concentrations of cortisol i.e. early in the response whereas higher concentrations of cortisol, acting through the GR, serve to limit and modulate the inflammatory response. Repression of 5HT1A receptor gene expression in the hippocampus by MR activation is however thought to be mediated by transrepression (Meijer et al., 2000).

The third putative mechanism of MR signalling is so-called non-genomic or rapid signalling where the activated MR interacts at the cell membrane to modify the response of second messenger pathways. Interactions with the epidermal growth factor receptor have been well characterised (Grossmann and Gekle, 2012) as have interactions with a range of other signalling pathways (Dooley et al., 2012). The G-protein coupled receptor, GPR30, a member of the seven transmembrane domain family of cell surface receptors, has been reported to be a membrane receptor for aldosterone (Feldman and Gros, 2013); it has also been invoked as a membrane receptor for estrogen where it seems likely to be acting more as a co-receptor for the classical estrogen receptor than the actual receptor per se (Levin, 2009).

## 3. MR tissue distribution

In addition to the aforementioned epithelia, the MR is expressed in an extensive range of tissues, many of which are neither epithelial nor associated with sodium transport. These include high abundance expression in the hippocampus where the receptor appears likely to have a range of effects on memory and affect, as well as in the hypothalamus (Fuller and Young, 2005). Its role in the cardiovascular system has recently been extensively examined and indeed is a major focus given the adverse effects of mineralocorticoid excess on the cardiovascular system (Young and Rickard, 2012). The MR also plays a central role in adipocyte biology (Marzolla et al., 2012) and is expressed in a range of reproductive tissues where its physiological role is yet to be characterised. The MR is expressed in a range of inflammatory cells, particularly the monocyte-macrophage lineage where tissue-specific knockout of the MR in transgenic mice has provided particularly striking insights into the biology of the MR in these tissues (Rickard et al., 2009). In many of these tissues, aside from the distal nephron and colon and the vasculature (Fuller and Young, 2005) and a discreet subpopulation of hypothalamic neurones involved in salt appetite (Geerling and Loewy, 2009), the MR is largely expressed in the absence of HSD2 and is therefore likely to be playing a role as a second receptor for cortisol, particularly in the brain where aldosterone is thought to cross the blood brain barrier poorly, if at all (Fuller and Young, 2005). The occupancy of the MR in these tissues will be determined by the free rather than total levels of circulating cortisol, which in turn will profoundly vary across the diurnal cycle. In disease states such as hyperaldosteronism or disease models such as the DOC/salt model (Rickard et al., 2009), the tonicaly high levels of mineralocorticoid across the day will result in inappropriate activation of the MR with adverse consequences.