Journal of Steroid Biochemistry & Molecular Biology xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

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

Evolution of hormone selectivity in glucocorticoid and mineralocorticoid receptors

Michael E. Baker[a,*], John W. Funder[b,*], Stephanie R. Kattoula[c]

[a] Department of Medicine, 0693, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0693, United States
[b] Prince Henry's Institute of Medical Research, P.O. Box 5152, 248 Clayton Road, Clayton, Victoria 3168, Australia
[c] Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0693, United States

ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form 2 July 2013
Accepted 22 July 2013

Keywords:
Corticosteroid receptor evolution
Mineralocorticoid receptor
Glucocorticoid receptor
Lamprey
Sharks
Coelacanths
Helix 3–helix 5 contact

ABSTRACT

Mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) are descended from an ancestral corticoid receptor (CR). To date, the earliest CR have been found in lamprey and hagfish, two jawless fish (cyclostomes) that evolved at the base of the vertebrate line. Lamprey CR has both MR and GR activity. Distinct orthologs of the GR and MR first appear in skates and sharks, which are cartilaginous fishes (Chondrichthyes). Aldosterone, the physiological mineralocorticoid in terrestrial vertebrates, first appears in lobe-finned fish, such as lungfish and coelacanth, forerunners of terrestrial vertebrates, but not in sharks, skates or ray-finned fish. Skate MR are transcriptionally activated by glucocorticoids, such as corticosterone and cortisol, as well as by mineralocorticoids such as deoxycorticosterone and (experimentally) aldosterone; skate GR have low affinity for all human corticosteroids and 1α-OH-corticosterone, which has been proposed to be biologically active glucocorticoid. In fish, cortisol is both physiological mineralocorticoid and glucocorticoid; in terrestrial vertebrates, cortisol or corticosterone are the physiological glucocorticoids acting through GR, and aldosterone via MR as the physiologic mineralocorticoid. MR have equally high affinity for cortisol, corticosterone and progesterone. We review this evolutionary process through an analysis of changes in sequence and structure of vertebrate GR and MR, identifying changes in these receptors in skates and lobe-fined fish important in allowing aldosterone to act as an agonist at epithelial MR and glucocorticoid specificity for GR. hMR and hGR have lost a key contact between helix 3 and helix 5 that was present in their common ancestor. A serine that is diagnostic for vertebrate MR, and absent in terrestrial and fish GR, is present in lamprey CR, skate MR and GR, but not in coelacanth GR, marking the transition of the GR from MR ancestor. Based on the response of the CR and skate MR and GR to corticosteroids, we conclude that the mechanism(s) for selectivity of GR for cortisol and corticosterone and the specificity of aldosterone for MR are incompletely understood.

This article is part of a Special Issue entitled 'CSR 2013'.

1. Introduction

The terms mineralocorticoid and glucocorticoid emerged in the mid-twentieth century as descriptors of the predominant action of various steroids. Deoxycorticosterone (DOC) was the signature mineralocorticoid hormone, causing sodium retention and elevating blood pressure, and cortisol (F) or corticosterone (B) the predominant glucocorticoid, reflecting their action on hepatic glycogen deposition [1–3]. The distinction was ultimately validated by the characterization in 1953 of aldosterone [Aldo], and its subsequent demonstration to be the physiological mineralocorticoid in terrestrial vertebrates. Whereas Aldo appears to have a single physiologic role – regulation of epithelial sodium transport – roles for glucocorticoids are diverse and multiple – in development, differentiation, metabolism, body clocks and response to stress, inter alia. Linking this variety of actions into a coherent physiology is a daunting task, and one not yet accomplished.

The mineralocorticoid actions of Aldo are mediated through the mineralocorticoid receptor (MR). MR, GR, progesterone (PR) and androgen (AR) receptors belong to the closely related subfamily of nuclear receptor super-family, a diverse group of transcription factors that arose in multicellular animals [4–9]. Sequence analyses indicate that MR and GR are descended from a common ancestor [10–15], the corticoid receptor (CR) [12,14,16], found in lampreys and hagfish; lampreys also contain a second member of the subfamily termed a progesterone receptor (PR). Lampreys and hagfish are cyclostomes (jawless fish), and evolved at the base of the vertebrate line (Fig. 1) [17,18]. Neither the identity of the corticosteroids that may regulate CR responses in cyclostomes, nor the evolution

* Corresponding authors.
E-mail addresses: mbaker@ucsd.edu (M.E. Baker), john.funder@princehenrys.org (J.W. Funder).

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http://dx.doi.org/10.1016/j.jsbmb.2013.07.009

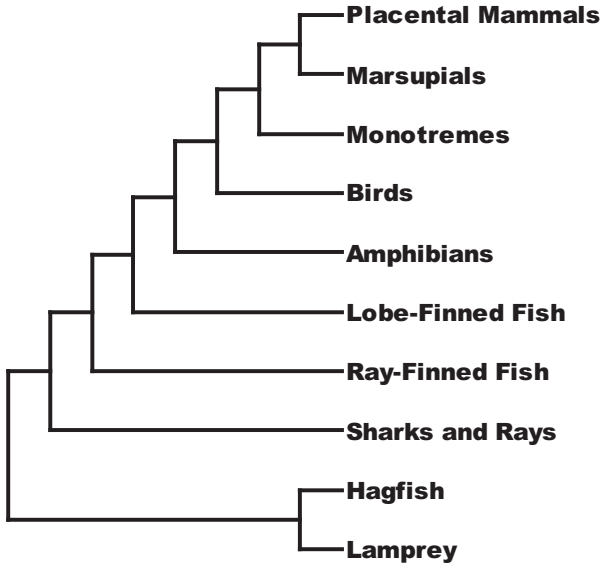

Fig. 1. Vertebrate phylogeny. Lampreys and hagfish are jawless fish, belonging to cyclostome taxon, which evolved at the base of the vertebrate line. Skates, rays and sharks are cartilaginous fishes, belonging to the elasmobranch subclass, and are basal jawed vertebrates. Land vertebrates are descended from lobe-finned fishes (coelacanths and lungfishes).

of specificity for corticosterone (B) nor cortisol (F) (Fig. 2) by the GR, and of the selectivity of Aldo for MR are fully understood.

Like other steroid receptors, MR and GR consist of a steroid-binding domain at the C-terminus, a DNA-binding domain near the center, an activation function 1 (AF1) domain at the N-terminus and an AF2 domain in the steroid binding domain [9,19–22]. Each of these domains in MR and GR is important for transcriptional responses [23,24]. The crystal structures of the steroid hormone binding domain on human MR (hMR) [25–27] and hGR [28] and of several mutant MR [26,27,29] have been solved, and provide important insights into the similarities and differences in the binding of corticosteroids to the MR and GR.

Whereas Aldo appears a simple effector and the actions of glucocorticoids complex, the inverse is true for the MR and GR. MR is, in fact, a misnomer in that the overwhelming majority of MR in the body are occupied (and activated or not) by physiological glucocorticoids. MR may be activated by Aldo in the kidney, colon, sweat gland and salivary gland, but elsewhere they are overwhelmingly high-affinity glucocorticoid (and perhaps even progesterone) receptors [1,2,30].

The MR has equivalent high affinity for progesterone, DOC, B, F, 11-deoxycortisol (S) and Aldo (Fig. 2) – and thus able to accommodate both 17α-hydroxylation (F) and the 11, 18, or 11, 18, 20 cyclized structures characteristic of Aldo in solution [10,31,32]. In terms of activation, in the mineralocorticoid sense, DOC and Aldo are agonist, progesterone an antagonist, and depending upon the context F and B, is MR or GR in terms of action [10,19,32]. Aldo may be a relatively simple hormone in that it binds to no other nuclear receptor, and is thus selective for MR. In contrast, its receptor is curiously complex, in that MR binds a wide range of steroid ligands equally well!

In contrast, GR appear relatively simple. Their affinity for physiologic glucocorticoids is an order of magnitude lower than that for MR, and with the possible exception of progesterone in pregnancy, they have no other potential physiologic ligand, agonist or antagonist. Under normal conditions GR are largely occupied by peak plasma free concentrations of glucocorticoids, and essentially empty at the nadir of diurnal levels, suggesting a major peripheral role in resetting the biological clock, given that all nucleated cells (except, curiously, the intermediate lobe of the pituitary) express GR. In situations of stress glucocorticoid levels remain elevated, consistent with their demonstrated roles in mediating and/or modulating systemic responses [33,34].

In cell culture, various reports rate B and F anywhere from equivalent to Aldo to two orders of magnitude less potent as a transcriptional activator of hMR [19,32,35], despite the same affinity of these steroids for the MR. Unusually, in the syndrome of apparent mineralocorticoid excess [36,37], F acts as a full mineralocorticoid agonist, consistent with equal potency in the absence of 11β-hydroxysteroid dehydrogenase. In contrast, GR are specific for B and F [10,32]. Although the basis for these differences is still not fully understood, it is clear that structural as well as sequence differences between MR and GR may play important roles [12,25,28].

Another salient difference between physiologic mineralocorticoids and glucocorticoids is their circulating concentrations. Both are secreted over a diurnal range, but total concentrations of F are ~1000 time higher than those of Aldo, and plasma-free concentrations ~100 fold higher [1,2]. Aldo is not transcortin-bound, but ~50% loosely bound to albumin: in organs with relatively long transit times such as the liver, allowing it to dissociate from albumin. Aldo is totally cleared, so that its metabolic clearance rate is equal to hepatic blood flow – i.e. 1100 l/day in humans. Given that MR evolved well before aldosterone synthase, and in the absence of appreciable affinity of Aldo for other NR, the low secretion rate/plasma levels and first-pass hepatic clearance point to higher levels having potentially untoward effects via MR normally overwhelmingly occupied by F.

Implicit in our nomenclature is an arbitrary designation of an agonist as having a measurable effect, with designation (in terms of the renal effects of Aldo) then sliding into definition, clearly ludicrous given the millions of years of separating the skate (which expresses MR) and the lungfish, which secretes Aldo. We refer to spironolactone as an MR antagonist, in that it opposes the effects of Aldo; unsurprisingly, however, given the very low doses proving clinically effective in the RALES trial [1,30], it is in fact an inverse agonist, being cardioprotective at nanomolar concentrations in experimental studies in the absence of any other steroid.

In an attempt to provide a unified perspective of this complex area – steroids, enzymes, receptors; agonists, inverse agonists/bivalent ligands/antagonists – three potentially valuable sources of insight are available. These are (a) structural studies on ligand–receptor complexes, (b) experiments of nature (e.g. the Ser810Leu mutant MR described by Geller et al. [38]) and exploring receptor evolution, in this particular instance that of MR and GR.

Here we use these structures [25,26,28,29] and analyses from several excellent reviews of the evolution of sequence and structure of corticosteroid receptors in land vertebrates and fish [22,23,39–43] to review and further explore the evolution of structures on the steroid-binding domain of the MR and GR that influence their specificity for steroids.

2. Structure and function of motifs in the human MR and GR

Previous analyses of the crystal structures of the steroid binding domain on MR [25,26,29] and GR [28] identified motifs in helices 3, 5, 6, 7 and 12 and the loop connecting helix 11 and helix 12 [14] that are important in the response to corticosteroids (Figs. 3 and 4). Thus, we first review the sequence (Fig. 3) and structure (Fig. 4) of motifs in helices 3, 5, 6, 7 and the connecting loop, as found in the crystal structures of wild-type hMR [25] and hGR [26] and mutant hMR [26,29]; then we examine the evolution of these motifs in vertebrate CR, MR and GR.

Please cite this article in press as: M.E. Baker, et al., Evolution of hormone selectivity in glucocorticoid and mineralocorticoid receptors, J. Steroid Biochem. Mol. Biol. (2013), http://dx.doi.org/10.1016/j.jsbmb.2013.07.009