Steroids 113 (2016) 38–45

Contents lists available at ScienceDirect

Steroids

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

Evolution of corticosteroid specificity for human, chicken, alligator and frog glucocorticoid receptors

Yoshinao Katsu [a,b,*], Satomi Kohno [c], Kaori Oka [a], Michael E. Baker [d,*]

[a] Graduate School of Life Science, Hokkaido University, Sapporo, Japan
[b] Department of Biological Sciences, Hokkaido University, Sapporo, Japan
[c] Department of Obstetrics and Gynecology, Medical University of South Carolina, and Marine Biomedicine and Environmental Science Center, Hollings Marine Laboratory, Charleston, SC, USA
[d] Department of Medicine, University of California, San Diego, CA, USA

ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 25 May 2016
Accepted 12 June 2016
Available online 16 June 2016

Keywords:
Glucocorticoid receptor
Glucocorticoid evolution
Allosteric regulation
Alligator
Xenopus
Chicken

ABSTRACT

We investigated the evolution of the response of human, chicken, alligator and frog glucocorticoid receptors (GRs) to dexamethasone, cortisol, cortisone, corticosterone, 11-deoxycorticosterone, 11-deoxycortisol and aldosterone. We find significant differences among these vertebrates in the transcriptional activation of their full length GRs by these steroids, indicating that there were changes in the specificity of the GR for steroids during the evolution of terrestrial vertebrates. To begin to study the role of interactions between different domains on the GR in steroid sensitivity and specificity for terrestrial GRs, we investigated transcriptional activation of truncated GRs containing their hinge domain and ligand binding domain (LBD) fused to a GAL4 DNA binding domain (GAL4-DBD). Compared to corresponding full length GRs, transcriptional activation of GAL4-DBD_GR-hinge/LBD constructs required higher steroid concentrations and displayed altered steroid specificity, indicating that interactions between the hinge/LBD and other domains are important in glucocorticoid activation of these terrestrial GRs.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Glucocorticoids (Fig. 1) regulate a variety of physiological functions including carbohydrate and protein metabolism, blood pressure, immune function and the body's anti-inflammatory processes via transcriptional activation of the glucocorticoid receptor (GR) [1–5]. The GR and other steroid receptors belong to the nuclear receptor family, a large family of transcription factors, which includes receptors for thyroid hormone, retinoids and other small lipophilic molecules [6–10]. The GR and other steroid receptors have a characteristic modular structure consisting of an N-terminal domain (NTD) (domains A and B), a central DNA-binding domain (DBD) (domain C), a hinge domain (D) and a C-terminal ligand-binding domain (LBD) (domain E) [9,11–13] (Fig. 2). The E domain alone is competent to bind steroids [11,12,14–17].

The NTD contains an activation function 1 [AF1] domain, which is a strong transcriptional activator of the GR [18–20]. Interestingly, AF1 is intrinsically disordered, unlike the DBD and LBD [20–22]. Allosteric interactions between AF1 and other domains on the GR and coactivators lead to a conformational rearrangement of AF1 that is important in transcriptional activation of the GR [22–25]. In rat GR, there is evidence that allosteric interactions between DBD and other domains regulate gene transcription [26,27]. Recent crystal structures of the DBD-Hinge-LBD domains of other nuclear receptors [13,21] identified allosteric signaling between the DBD and LBD domains.

Although dexamethasone (DEX) and cortisol (F) activation of rodent [28] and human [19,29–32] GRs has been investigated, there has been no systematic assessment of corticosteroid specificity among phylogenetically diverse terrestrial vertebrate GRs, such as amphibians, reptiles, birds and mammals. This is important because more than one corticosteroid may be a physiological glucocorticoid in terrestrial vertebrates [33–35]. Reports of transcriptional activation by corticosteroids of the GR for other terrestrial vertebrates: amphibians, reptiles and birds, are limited [36,37]. Oka et al. [36] reported half-maximal response (EC50) values for transcriptional activation of full length alligator GR by F, corticosterone (B), 11-deoxycorticosterone (DOC) and aldosterone (Aldo). The EC50s for F and B were 0.29 nM and 0.16 nM, respectively, which is consistent with the known role of these two steroids as

* Corresponding authors at: Graduate School of Life Science, Hokkaido University, Sapporo, Japan (Y. Katsu). Department of Medicine, University of California, San Diego, CA, USA (M.E. Baker).
E-mail addresses: ykatsu@sci.hokudai.ac.jp (Y. Katsu), mbaker@ucsd.edu (M.E. Baker).

http://dx.doi.org/10.1016/j.steroids.2016.06.005
0039-128X/© 2016 Elsevier Inc. All rights reserved.

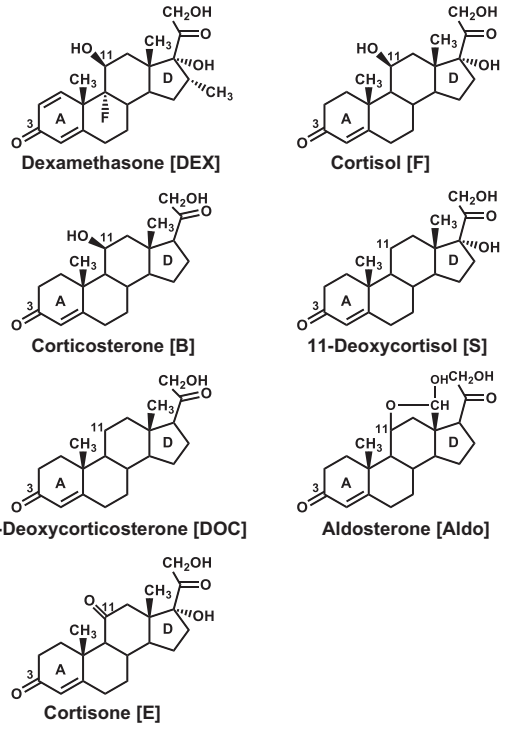

Fig. 1. Structures of various corticosteroids. Cortisol and corticosterone are physiological glucocorticoids in terrestrial vertebrates and ray-finned fish [12,67,74]. Cortisone is a metabolite of cortisol, in which the C11-alcohol is metabolized to a ketone. Aldosterone, 11-deoxycorticosterone and 11-deoxycortisol are physiological mineralocorticoids [12,41,61,70]. Aldo and DOC are weak transcriptional activators of human GR [29,31,38]. 11-deoxycortisol is both a mineralocorticoid and a glucocorticoid in lamprey [59].

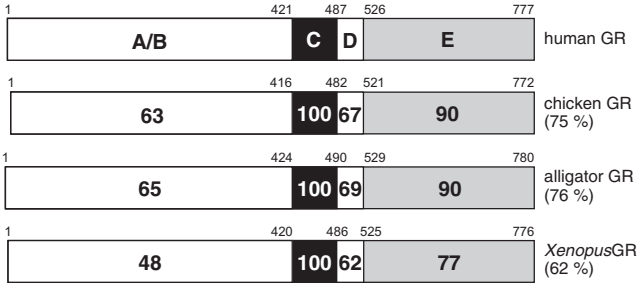

Fig. 2. Comparison of domains in some terrestrial vertebrate GRs. GRs from human, chicken, alligator and *X. laevis* are compared. The functional A/B domain to E domains are schematically represented with the numbers of amino acid residues and the percentage of amino acid identity between the domain in the human GR and the corresponding domain in the other vertebrate GRs. For example, the entire human GR sequence is 75% identical to that of chicken GR, while domain E (LBD) on human GR is 90% identical to that of chicken GR. GenBank accession numbers: human GR (NM_000176), chicken GR (NM_001037826), alligator GR (AB701407), *X. laevis* GR (NM_001088062).

glucocorticoids in mammals. However, the EC50s for Aldo and DOC were 2.9 nM and 2.8 nM, which is unexpected because both steroids have a lower binding affinity for human GR [29,31] and are weak transcriptional activators of human GR [29,38,39]. Similar intriguing findings for Aldo were reported for chicken GR by Proszkowiec-Weglarz and Porter [37], who found that the EC50s for transcriptional activation of chicken GR by Aldo and B were 0.8 nM and 1.8 nM, respectively, with the level of transcription due to B being about 30% higher than to Aldo. The EC50s of DOC and other corticosteroids for chicken GR and of DEX for alligator GR were not determined.

These unexpected responses of alligator and chicken GRs to Aldo and our interest in the evolution of specificity for corticosteroids in the GR in vertebrates [12,36,40–42] motivated us to investigate the response to a panel corticosteroids, DEX, F, cortisone (E), B, DOC, 11-deoxycortisol (S) and Aldo of the GR from chicken and the amphibian [*Xenopus laevis*] for comparison to human and alligator GR with the goal of clarifying the evolution of corticosteroid specificity in terrestrial vertebrates. In addition, we were interested in investigating the role of domains A-C and domains D-E [13,20–22,42–45] in the response of GRs to steroids. The influence of domains A-C on steroid responses for the GR has not been studied previously in non-mammalian terrestrial vertebrates. For these studies we constructed a plasmid containing the GAL4 DBD fused to the D domain and E domain of the GR (GR-LBD).

We found significant differences in the EC50s of these full length GRs to corticosteroids indicating that during the evolution of these terrestrial vertebrates there were changes in their response to various corticosteroids. Moreover, in the presence of corticosteroids, truncated GRs containing a GR LBD fused to a GAL4 DBD had a higher EC50 value (weaker activation) than their corresponding full length GRs, indicating altered steroid specificity among these terrestrial vertebrate GRs and that the evolution of the response of terrestrial vertebrate GRs to different steroids was complex. The effect of interactions between the domains D-E and other GR domains [21,42,43] on transcriptional activation may involve post-translational modification of domains A, B or C [46–48], alterations in the binding of co-regulator proteins [23,46,49] or a combination of these mechanisms.

2. Materials and methods

2.1. Chemical reagents

DEX, F, E, B, Aldo, DOC and S were purchased from Sigma-Aldrich. For the reporter gene assays, all hormones were dissolved in dimethylsulfoxide (DMSO) and the final concentration of DMSO in the culture medium did not exceed 0.1%.

2.2. Construction of plasmid vectors

The full-coding regions and D/E domains of the GR from *X. laevis*, alligator, chicken and human were amplified by PCR with KOD DNA polymerase (TOYOBO Biochemicals, Osaka, Japan). The PCR products were gel-purified and ligated into pcDNA3.1 vector (KpnI-NotI site for human, chicken and alligator GRs, and HindIII-NotI site for *X. laevis* GR) (Invitrogen) for the full-coding region or pBIND vector (MluI-NotI site) (Promega) for D-E domains. As shown in Fig. 2, the D domain begins at human GR (487), chicken GR (482), alligator GR (490) and *X. laevis* GR (486) [36].

2.3. Transactivation assay and statistical methods

CHO-K1 cells (Chinese hamster ovary cell) were used in the reporter gene assay. Transfection and reporter assays were carried out as described previously [36,50]. The use of CHO-K1 cells and an assay temperature of 37 °C does not replicate the physiological environment of *X. laevis*, alligator and chicken. Nevertheless, studies with mammalian cell lines at 37 °C have proven useful for other studies of transcriptional activation by corticosteroids of teleost fish GRs [51–54] and other non-mammalian GRs [37,55,56]. Levels of expression of the different non-mammalian GRs and their truncated counterparts may differ in CHO-K1 cells. However, comparisons of the EC50 of different corticosteroids for each GR would be valid, which is the goal of our study. All transfections were performed at least three times, employing triplicate sample points in

Download English Version:

https://daneshyari.com/en/article/2027670

Download Persian Version:

https://daneshyari.com/article/2027670

Daneshyari.com