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Commentary

Biased signalling from the glucocorticoid receptor: Renewed opportunity for tailoring glucocorticoid activity

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ABSTRACT

Recent landmark studies applying analytical pharmacology approaches to the glucocorticoid receptor (GR) have demonstrated that different ligands can cause differential activation of distinct GR-regulated genes. Drawing on concepts of signalling bias from the field of G protein-coupled receptor (GPCR) biology, we speculate that ligand-dependent differences in GR signalling can be considered analogous to GPCR biased signalling, and thus can be quantitatively analysed in a similar way. This type of approach opens up the possibility of using rational structure-based drug optimisation strategies to improve the therapeutic selectivity of glucocorticoid drugs to maximise their efficacy and minimise adverse effects.

1. The quest for safer and/or more effective corticosteroids

Glucocorticoid steroids (also known as corticosteroids) are widely used and effective anti-inflammatory drugs. As a critical component in the hypothalamic–pituitary–adrenal axis, the endogenous glucocorticoid, cortisol plays an important physiological role in most tissues, mediating stress responses and influencing cardiovascular tone, fluid volume, immunity, metabolism, neural function and reproduction [1]. Some endocrine actions of synthetic anti-inflammatory glucocorticoids are avoided by selective agonism of the glucocorticoid receptor (GRα, herein referred to as GR) over the mineralocorticoid receptor (MR) [2]. However, the pharmacological utility of glucocorticoids, particularly with chronic administration and high doses, as anti-inflammatory agents remains limited by adverse effects including a diabetic-like state, peptic ulcer, gastrointestinal bleeding, iatrogenic Cushing's syndrome, osteoporosis, skin atrophy, delayed wound healing, glaucoma, cataract, and others [3,4]. These adverse effects can be reduced by localised administration, for example to the skin through topical ointments or to the lungs through inhalation. However, in some situations the burden of disease is increased because therapeutically sub-optimal doses have to be used. Furthermore, the fear of adverse effects decreases patient adherence to therapy [5]. For many of their indications, no other anti-inflammatory drugs even approach the breadth of effectiveness of glucocorticoid agents. Thus, the quest for safer and/or more effective glucocorticoids continues.

2. Glucocorticoid receptor mechanisms

Synthetic glucocorticoids act through the GR to produce anti-inflammatory actions predominantly through modulating gene expression: increasing anti-inflammatory gene expression and

Abbreviations: AP-1, activator protein 1; AR, androgen receptor; cAMP, cyclic adenosine monophosphate; p57, Kip2, cyclin-dependent kinase inhibitor 1C; COX-2, cyclooxygenase-2; DC, deisobutyrylciclesonide; Dex, dexamethasone; K_A, dissociation constant; ER, oestrogen receptor; ERK, extracellular signal-regulated kinase; FF, fluticasone furoate; GILZ, glucocorticoid-induced leucine zipper; GPCR, G protein coupled receptor; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GRIP-1, glucocorticoid receptor-interacting protein 1; Gas5, growth arrest specific-5; GW, GW870086X; HC, hydrocortisone; IκBα, inhibitor of nuclear factor kappa B, alpha; IL, interleukin; ICAM-1, intracellular adhesion molecule 1; LBD, ligand-binding domain; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MKP-1, MAP kinase phosphatase 1; Mif, mifepristone; MR, mineralocorticoid receptor; NF-κB, nuclear factor-kappaB; NCoR, nuclear receptor co-repressor 1; Org, Org34517; PI3K, phosphoinositide 3-kinase; SERM, selective oestrogen response modifier; STAT, signal transducer and activator of transcription; SMRT, silencing mediator of retinoid and thyroid hormone; SRC, steroid receptor coactivator; TSLP, thymic stromal lymphopoietin; τ, transduction coefficient; TNF-α, tumour necrosis factor-α.

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suppressing pro-inflammatory gene expression. In the absence of ligand, the GR is predominantly located in the cytoplasm in a multi-protein complex with chaperone proteins (HSP90, HSP70, p23) and immunophillins (FKBP51, FKBP52) [6]. Upon ligand binding, the GR undergoes a conformational change whereby the complexed proteins dissociate and nuclear localisation signals are exposed, promoting rapid nuclear translocation. The ligand-bound GR can then bind directly to DNA (as a homodimer) at glucocorticoid response elements (GREs) leading to recruitment of coactivator proteins such as p300/CBP, glucocorticoid receptor interacting protein (GRIP-1), and steroid receptor coactivator-1 (SRC-1). These co-activators have intrinsic histone acetyltransferase (HAT) activity and also recruit the SWI/SNF complex which leads to local chromatin remodelling. The subsequent recruitment of transcriptional enzymes, such as RNA polymerase II (RNA pol II) leads to gene transcription, comprising a process known as 'transactivation' [7]. Key anti-inflammatory genes induced by GR activation include glucocorticoid-induced leucine zipper (GILZ), inhibitor of NF-κB (IκBα), interleukin 10 (IL-10) and MAP kinase phosphatase 1 (MKP-1) (reviewed in [8,9]).

The ligand-bound GR can also bind to negative GREs (nGREs) [10] leading to suppression of gene transcription through recruitment of co-repressors such as nuclear receptor corepressor 1 (NCoR1) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) [11]. Pro-inflammatory genes negatively regulated by GR through nGREs include thymic stromal lymphopoietin (TSLP), interleukin-8 receptor A (IL8RA) and interleukin-17 receptor A (IL17RA). As well as interacting with DNA and transcriptional co-activators and co-repressors, the ligand-bound GR can directly interact with pro-inflammatory transcription factors such as activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB) antagonising their transcriptional activity in a process called 'transrepression'. A suite of pro-inflammatory genes are repressed in this manner, including cyclooxygenase-2 (COX-2), intracellular adhesion molecule 1 (ICAM-1), IL-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor-α (TNF-α), RANTES and Eotaxin.

The anti-inflammatory actions of GR are predominantly thought to be mediated through genomic mechanisms. However, there are increasing numbers of reports that GR can also signal through non-genomic mechanisms (recently reviewed in [12]). These effects seem to be attributable to rapid modulation of the activity of several kinases by ligand-bound GR such as phosphoinositide-3 kinase (PI3K) [13], cytosolic phospholipase A2 (cPLA2) [14] and c-Jun N-terminal kinase (JNK) [15]. The vasopressor effects of glucocorticoids used to treat cardiovascular shock are evident within minutes and therefore too soon to be attributable to genomic actions [16]. However, the relative importance of non-genomic actions for anti-inflammatory actions of glucocorticoids has been difficult to establish.

3. Recent advances: the discovery of ligand-dependent differences in GR action

Our understanding of GR mechanisms is ever increasing as new mechanisms are identified and more complexity is uncovered (reviewed in [17,18]). It has been known for some time that GR can interact directly with some transcription factors to enhance their transcriptional activity [19,20] but this type of interaction was thought to be limited to members of the signal transducer and activator of transcription (STAT) family. However, recent observations suggest that in some circumstances GR can enhance the activity of AP-1 [21] and NF-κB [22]. Genome-wide analyses suggest that GR bound to canonical pGREs can also lead to negative regulation of some genes [23]. Moreover, GR-binding sites are most often not in the promoter region of target genes and may be far removed from the transcription start site [24]. It has also been discovered that there are multiple isoforms of GR from alternative translation initiation [25,26], as well as the classical splice variant isoform GRβ that is now known to have some transcriptional activity distinct from that of GRα [27,28]. Importantly, the activation of different GR mechanisms has generally been ascribed to contextual differences. Thus, differences in cell type, circadian rhythm, or the health or disease state of the tissue in question [18,29,30] influence glucocorticoid responses. Adding to this complexity, two recent landmark studies suggest that different ligands activate GR differently [31,32]. These new findings raise the prospect that structure-based drug optimisation strategies may be applied to glucocorticoids to rationally improve the safety and/or effectiveness of glucocorticoid agents.

In these studies, Joshi et al. compare the pharmacodynamics of seven different GR ligands in activating gene expression in the BEAS-2B human bronchial epithelial cell line: the clinically used fluticasone furoate (FF), dexamethasone (Dex) and deisobutyrylciclesonide (DC), each of which is assumed to be a full agonist; the partial agonist GW870086X (GW); the endogenous agonist hydrocortisone (HC); and, the antagonists mifepristone (Mif, RU486) and Org34517 (Org). Importantly, the authors demonstrate that these ligands elicit differential activation of distinct genes. They measured the induction of the gene encoding $p57^{kip2}$ (CDKN1C) and showed the rank order of activation (measured by E_{max}) of the agonists resembled activation of a GRE reporter plasmid, a classic tool for measuring transcriptional activity: (agonist rank order of induction of $p57^{kip2}$: FF ⩾ Dex > DC ⩾ GW ⩾ HC versus GRE reporter: FF > Dex > DC ⩾ HC > GW). However, the induction of the gene encoding PDK4 showed the different rank order of agonism FF ≫ Dex > HC > DC > GW. In contrast, all agonists were equally effective at inducing the genes encoding GILZ (TSC22D3) and CRISPLD2 [31]. Further, they showed that Dex and DC actually act as partial agonists compared to FF in a GRE reporter assay [31].

In the second study, Joshi et al. examined the effect of indacaterol, a long acting β_2-adrenoceptor agonist on the pharmacodynamics of the GR ligands [32]. They showed that indacaterol had no effect on the GRE reporter itself, but it increased the maximum activation by GR ligands in a ligand-dependent manner. This increase in maximum activation occurred without any effect of indacaterol on the affinity (K_A) or operational efficacy (τ) of the ligand for the GR. The authors then examined the effect of indacaterol on GR-dependent gene expression and found that the effect of indacaterol was both gene- and agonist-dependent. For example, indacaterol significantly enhanced the expression of PDK4, $p57^{kip2}$ and CRISPDL2 gene expression, but remarkably showed no effect on GILZ gene expression induced by any of the GR agonists examined. The extent of enhancement by indacaterol was dependent on the intrinsic activity of the GR ligand and was shown to be saturable with a different maximal enhancement of different genes (4-fold for PDK4 and 2.7-fold for $p57^{kip2}$) [32].

Differences between GRE reporter assays and induction of gene expression are well documented due to differences in complexity of regulation between a plasmid and the native promoters of a gene in its orthotopic genomic context [33,34]. However, these latest studies by Joshi et al. demonstrate systematically and for the first time that different GR ligands induce differential gene expression profiles. Such a demonstration is highly significant, as it suggests a type of biased signalling from the GR that has the potential to be rationally harnessed in a way that contextual differences in GR action may not be. The application of classical analytical pharmacology approaches by Joshi et al. to generate quantitative information about the pharmacodynamic properties of the ligand-bound GR is an important and novel contribution to steroid receptor pharmacology.