Glucocorticoid Receptor Modulators

Les modulateurs du récepteur aux glucocorticoïdes

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ABSTRACT

The glucocorticoid hormone cortisol acts throughout the body to support circadian processes and adaptation to stress. The glucocorticoid receptor is the target of cortisol and of synthetic glucocorticoids, which are used widely in the clinic. Both agonism and antagonism of the glucocorticoid receptor may be beneficial in disease, but given the wide expression of the receptor and involvement in various processes, beneficial effects are often accompanied by unwanted side effects. Selective glucocorticoid receptor modulators are ligands that induce a receptor conformation that allows activation of only a subset of downstream signalling pathways. Such molecules thereby combine agonistic and antagonistic properties. Here we discuss the mechanisms underlying selective receptor modulation and their promise in treating diseases in several organ systems where cortisol signalling plays a role.

Keywords

Glucocorticoids, Cortisol, Coactivators, Stress, Disease, Side Effects, brain, metabolism, cancer

Cortisol is our main glucocorticoid hormone and acts throughout the body to support circadian synchronization of the organs, and – notably – to support coordinated adaptation to stressors. Many, if not all, organs may be either the cause of a stress response (e.g. bone fracture) or involved in the adaptive response to stressors (e.g. immune system, metabolic tissues etc). Therefore most cell types of the body are also responsive to cortisol. While cortisol as a physiological factor has many beneficial actions, chronic exposure to elevated concentrations of endogenous cortisol, disturbed rhythmicity of cortisol, and exposure to exogenous glucocorticoids can have extremely deleterious effects. Both chronic stress and the medical use of glucocorticoids are the cause of many unwanted glucocorticoid (side) effects.

Cortisol acts via two types of receptors: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). MR also acts as the receptor for aldosterone, in cell types that enzymatically degrade cortisol. The MR has a very high affinity (K_d = 0.5 nM) for cortisol , whereas GR affinity is lower (K_d = 5 nM). The dynamic range of GR occupancy is such that it is very low during the circadian trough (late evening for humans). GRs get substantially occupied during normal circadian peak levels, and stress-induced steroid levels lead to further occupancy, towards saturation of the receptors (1). While there are likely more processes that are affected by cortisol via MR than is often appreciated, we will focus here on GR-dependent signalling. GR is expressed in most cells of the body, and is held responsible for most of the classical side effects of glucocorticoid overexposure. For example, the therapeutic goal of GR-mediated immune suppression goes

hand in hand with GR-mediated hyperglycemia and – in chronic conditions - osteoporosis.

The predominant (or: best understood) mode of action of MR and GR is their role as transcription factors, next to non-genomic actions that also exist (2). The unliganded receptors are intracellular and upon binding of cortisol they translocate to the nucleus to bind to the DNA and stimulate (e.g. gluconeogenic liver enzymes) or represses (e.g. pro-inflammatory cytokines) gene expression. There are two dominant mechanism of gene expression regulation via GR (3). The first, most classic, mechanism is that GRs bind as a dimer to specific Glucocorticoid Response Elements (GREs) on the DNA. Gene transcription tends to be stimulated via GRE-bound GRs, for example in case of gluconeogenic genes in liver, the PER1/2 clock genes, and the molecular chaperone FKBP5 (4-7). Also 'negative GREs' exist where direct GR binding is associated with gene repression (8). The second mechanism of cortisol signalling via GRs that bind to other – non-receptor – transcription factors to modulate their activity. This mode of signalling can be either stimulatory or repressive, and is exemplified by the suppression of the proinflammatory transcription factor NF-kB (9). While the second mode of action transrepression seems to be a very important in suppressing an activated immune system, whole genome analysis of GR (and MR) DNA binding points to GRE-dependent transcription as being dominant in (rodent) brain (10,11).

Given the side effects that can occur after immune suppressive GC treatment, and the role of trans-repressive protein-protein interactions of GR in this process, a

logical approach has been to generate so called 'dissociated GR ligands' as 'prednisolone without the side effects', based on effective protein-protein based transrepression of NF-kB, without efficacy at GREs (12), figure 1. Since then it has turned out that full anti-inflammatory efficacy also depends on induction of GRE-driven GR target genes. So far there have been no clinical breakthroughs of this kind of molecules (13), but developments are still on-going (14). However, the notion of gaining specificity of cortisol effects by dissociating GR signalling pathways still bears substantial promise – if only because ligands for the related estrogen receptor (ER) show proof of principle. So called selective estrogen receptor modulators (SERMs) like tamoxifen act as ER antagonists in breast cancer cells, but as agonist in bone and endometrium. Thus, it has been clear for a long time that tissue specific efficacy of steroid receptor ligands can exist (15).

Selective targeting of GR dependent effects may be achieved by targeting specific tissues, but this is often not an option. The tissue-specific action of selective receptor modulators is linked to what happens after steroid receptors bind to the DNA (figure 1). The stimulation of gene transcription entails the physical recruitment of downstream signalling partners. The interacting partners act in complexes to either open up chromatin or help assembly of transcription machinery. These signalling factors that form the bridge between steroid receptors and actual transcription are called nuclear receptor coregulators, which may either act as coactivator or as corepressor (16). An operational definition of a coactivator is a protein that enhances transcriptional activity of a steroid receptor but does itself not bind directly to the DNA. There a tens or hundreds of proteins that may act as coactivators via one of the two activation

function domains that GR harbours (17). Importantly, expression of these coregulators is highly cell type specific (18), and GREs in different genes depend on particular (sets of) coactivators (19,20). The tissue specific (ant)agonism of the SERM tamoxifen is in fact attributed to the induction of an ER conformation that allows ER-coactivator interactions within the endometrium, but not with coactivators that are necessary for ER-induced mitosis in breast cancer cells (21).

Already in 2003, it appeared that a GR ligand that could suppress inflammation with a degree of specificity over metabolic side effects did so by selective recruitment of GR coactivators (22). Recently is has become possible to predict at a medium throughput scale whether or not a compound will have selective modulator type effects, using a peptide array. The basis for this prediction is that the interaction domains on coactivators are known and are characterized by the presence of an LxxLL 'NR-box' amino acid motif (23). Agonistic activity of a ligand may be defined as induction of GR-coactivator interactions (24). Our own work showed that the specific GR ligand CORT108297 induces a pattern of coactivator interactions that is intermediate to that of the full agonist dexamethasone and the antagonist mifepristone (25). Such interaction profiles immediately suggest efficacy (that is: agonism) in processes that depend on those interactions that do occur and antagonism for the processes that simply cannot be induced for lack of interaction. Although such assays typically involve reduced (in vitro) systems, the prediction of selective GR modulation has indeed been accurate for a number of compounds that we have tested (25,26).

We have evaluated the effects of two such compounds in some detail in rodent models. Of interest, the two compounds CORT108297 and CORT118335 differ subtly in the interactions they induce between the GR AF-2 and coregulator NR-boxes *in vitro* (26). Thus, they are both expected to act as selective GR modulators, but with a different molecular profile. This prediction held true when we tested the two compounds in well-known behaviour paradigm that is sensitive to GR activation (25,26).

It is known that glucocorticoids facilitate the formation of memories, by strengthening the consolidation process that takes place after a learning experience (27). This effect is apparent is the 'passive avoidance' learning paradigm. In this setup rats are placed in an exposed bright light, which makes them enter a – presumed safe – dark chamber, typically within 10 second or so. Upon entry the rat receives a mild but unpleasant electric shock. Because of this learning experience, the rats will linger in the exposed outside of the chamber for two to three minutes, when placed back the next day. In this paradigm, C108297 treatment led to 5 to 6 minute delay before entering the next day. This suggests strong agonism on the GR, and blockade with the antagonist mifepristone (a.k.a. RU486) confirmed this (25). In contrast, the SGRM CORT118335, which has only a subtly different coregulator interaction profile, displayed very strong *ant*agonism in the same memory task (26). At the same time, CORT108297 also has functional antagonism on some process, and both compounds show agonism on the suppression of the hypothalamus-pituitary-adrenal axis (25,28).

Thus, differential coregulator interactions can be a means to identify selective GR modulators. At present, we do not know whether the differences from the protein arrays lead to an over- or underestimation of the actual differences between ligands *in vivo.* Moreover, the compounds may also differ in other signalling modes. C108297 has substantial transrepressive anti-inflammatory efficacy (29), while C118335 is much less potent in this respect (unpublished observations). Moreover, the SGRMs that act via GREs may also differ in their ability to induce interactions with other transcription factors that bind the DNA in the vicinity of the GREs. Such interactions are suggested by genome-wide analysis of GR target genes and binding sites. Only a subset of GRE binding events leads to actual transcriptional regulation (30). It turns out that functional GREs are enriched in binding sites for other transcription factors (31). Such associated transcription factors can indeed modulate the transcriptional activity of the GRE-bound GR (10). It is presently unknown how different GR ligands affect such interactions. Lastly, we have no idea how these compounds behave in terms of non-genomic GR-mediated effects that also occur (2). And so: much remains to be determined in terms of full molecular characterization of SGRMs.

A more pragmatic question is: what can be the use of SGRMs in treating disease? GR agonism is a very common goal in inflammation, and separation of anti-inflammatory action from all other GR-dependent effects would still be a 'golden bullet' in medicine (32). At the other side of the spectrum, there is full (or predominant) antagonism of GR using mifepristone, as currently used in a subset of patients with Cushing's Disease (33). However, attenuating glucocorticoid signalling may also be of use in other conditions in absence of clear

hypercortisolemia, such as metabolic disease (34,35), brain disorders (36,37) and certain types of cancer (38,39). Of note, in many disease models SGRMs like CORT108297 and CORT118335 are also effective, sometimes more so than the classical antagonist mifepristone (25,29,40-44). Because the GR is expressed in so many tissues, the potential for the use of SGRMs seems substantial. An additional advantage of new compounds – whether they are SGRMs of full GR antagonists - is that they lack affinity for androgen and progesterone receptors that is a characteristic of mifepristone (45-48). Of note, the compound C118335 does act as an antagonist for the mineralocorticoid receptor, at a lower affinity than for GR (26), and this may be responsible for some it its effects (49).

While there is promise for the use of SGRMs in treatment of disease, it is a big challenge to predict which tissues and processes will be affected by new GR modulating compounds. For example, even if we know the coactivators that will be recruited by a SGRM-GR complex, in most cases it is unknown which signalling pathways are involved in which transcriptional process. Given the large number of coactivators (17) and their highly gene and tissue specific regulation, such analyses are very time consuming, if informative (20,50). One way to better predict efficacy of specific SGRMs is to have a comprehensive overview of which coactivators are co-expressed with GR in specific tissues. As an example of this approach, we made use of the mouse Allen Brain Atlas, in which expression of 20,000 genes in about 900 distinct brain regions is described (51). This repository allows for analysis of co-expression of genes, and it has been possible to in this way describe co-expression of GR with its potential coregulators (18). Such an analysis revealed that dopaminergic

regions in the brain are strongly enriched in the coactivator Pak-6 (52). These descriptions predict (correctly (53)) that Pak6 affects dopaminergic function, but also that GR can affect dopamine neurons via GR-Pak6 dependent pathway (untested). In the end, molecular characterization of receptor interactions as induced by SGRMs, insight in cellular distribution and activation status of coactivators, and gene-specific need for coactivators should lead to better predictions of potential indications for SGRM use.

In conclusion, the wide expression of GR defines it as a target in many diseases, but immediately predicts many potential side effects of global activation or inhibition. The use of selective modulators that with a degree of specificity separate signalling pathways is an attractive approach to separate wanted from unwanted effects. Different SGRMs vary substantially in their biological activities. This reflects the complexity of GR signalling, but it emphasizes the potential of these compounds to help understand which signalling modes are active in particular processes, *and* it emphasizes that very different pathological processes may be modulated with a fair degree of selectivity.

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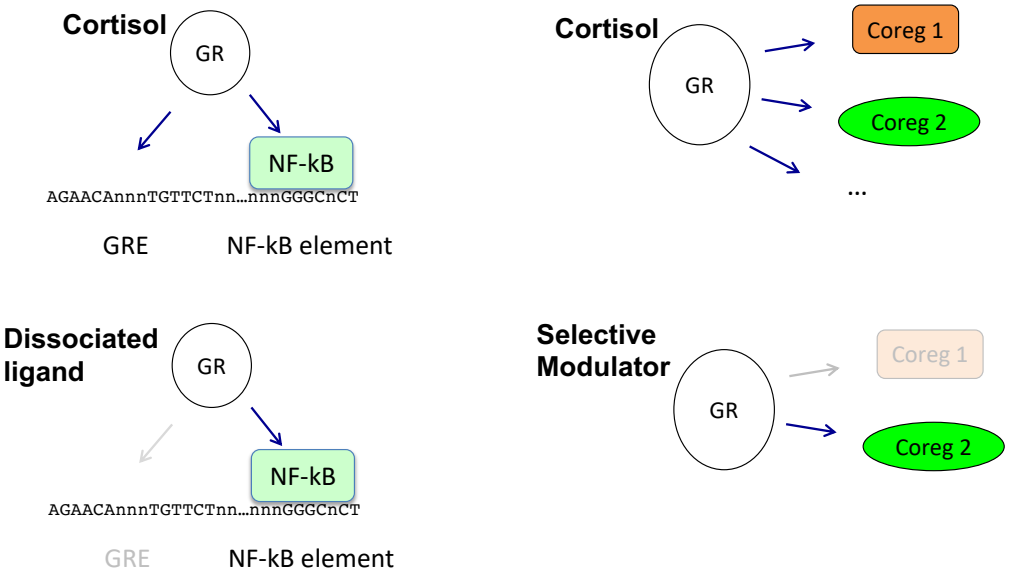

Figure 1. Left hand side: GR may bind to the DNA either directly, via GREs, or via protein-protein interactions with transcription factors like NF-kB. 'Dissociated ligands' favor the latter interaction, which results in fewer side effects, but also loss of antiinflammatory efficacy.