
Glucocorticoid Receptor β : View II

Jan Carlstedt-Duke

There is a clear role for mechanisms that modulate glucocorticoid receptor (GR) function. The non-steroid-binding GR β isoform has been proposed to play a role in this modulation but the published data are contradictory. The relative levels of this isoform appear to be low. Alternative mechanisms for the modulation of glucocorticoid action are described and contrasted with the proposed role for GR β .

Glucocorticoids play a key role in protecting the organism from its own defence mechanisms, thereby ensuring that these mechanisms protect without becoming exaggerated and turning against the organism itself. For this reason, there are a wide variety of biological responses induced by glucocorticoids, with a great degree of variation among different target organs. Virtually all tissues in the body are target organs for glucocorticoids and can respond in one way or another. Despite the broad spectrum of biological effects induced by glucocorticoids, there is a single mechanism whereby target cells can respond to the hormone signal, through the action of a receptor protein that is identical in all target cells. There is a single gene for the glucocorticoid receptor (GR) in all higher organisms, including humans. Thus, there must be other mechanisms that modulate the action of this common receptor protein to enable the enormous diversity of glucocorticoid actions.

The principal effects of glucocorticoids can be divided into two groups, metabolic effects and anti-inflammatory effects. In addition, glucocorticoids play a role in brain function (cognitive functions, stress reaction) as well as in development (T-cell development, adrenal chromaffin cell differentiation and lung maturation). The metabolic effects protect the organism from the excessive action of insulin, which would lead to a dangerously low blood glucose level and deprivation of the

central nervous system. Thus, the concerted action of the metabolic effects induced by glucocorticoids raise the level of glucose in the circulation by the induction of gluconeogenesis. The principal action of glucocorticoids involves the induction of genes for key enzymes. This occurs by binding of the GR, after the binding of steroid, to specific DNA sequences – glucocorticoid-response elements (GREs) – thereby stimulating transcriptional activity of the adjacent gene. By contrast, the anti-inflammatory action of glucocorticoids is mainly dependent on protein–protein interactions, resulting in a decrease of transcriptional activity of the relevant genes. After activation of the GR by steroid, the receptor can interact with activating transcriptional factors, such as activating protein 1 (AP-1) and nuclear factor κ B (NF- κ B), thereby blocking their ability to induce transcription of the genes for cytokines and other such signal proteins involved in the inflammatory cascade.

cDNA encoding the GR was the first member of the superfamily of nuclear receptor proteins to be cloned¹. When the full-length cDNA was obtained², two forms of human GR were described: a steroid-binding form of 777 residues (GR α) and a non-steroid-binding truncated form of 742 residues, which differed in the 15 C-terminal residues (GR β). GR β was thought to be either a cloning artefact or a splicing variant, although it was unknown whether it was synthesized as a protein or not. Because GR β did not bind steroid, there was no interest shown in this form of the protein for some time.

A comment should be made here about the nomenclature of the GR isoforms because it differs from that of all other nuclear receptors. In all other cases, greek letters (α , β , γ) are used to denote separate genes for nuclear receptors of the same class, whereas numbers are used to denote isoforms derived from a single gene. On the basis of this principle, GR α and GR β should be called GR α 1 and GR α 2, respectively. However, because the GR was the first member of the family to be cloned, and the names of the two isoforms were already established at that point, it has not been possible to change this. Several attempts to develop a systematic nomenclature for the nuclear receptor superfamily have been unsuccessful.

• The β Isoform of the Glucocorticoid Receptor

During the past few years, attention has been refocused on GR β and its possible biological function. The protein has been found in a variety of glucocorticoid target tissues. However, there are conflicting data in the published results and there is some controversy concerning the relative levels of GR isoforms, as well as the putative function of the GR β isoform, and whether or not it acts as a dominant negative modulator of the GR α isoform.

Relative Levels of GR Isoforms

In three independent studies of mRNA levels for GR isoforms, as measured by quantitative RT-PCR, there is a clear domination of the α isoform³⁻⁵. In the pituitary, in both normal and adenoma tissue, mRNA encoding GR α is expressed in at least 32–37-fold excess over GR β mRNA (Refs 3,4). It was significant that there was no difference in relative levels of the two isoforms in glucocorticoid-resistant tumours compared with glucocorticoid-sensitive tissue. In the first published study of GR β expression, Oakley *et al.*⁵ described a very detailed analysis of the various GR mRNA species as a basis for their quantitative studies. Their conclusions were that mRNA GR β is expressed at 200–500 times lower amounts than GR α mRNA in a variety of tissues and cells, including human

J. Carlstedt-Duke is at the Department of Medical Nutrition, Karolinska Institutet, Huddinge Hospital, Novum, S-141 86 Huddinge, Sweden.

lung and liver and HeLa-S3 and CEM-C7 cells.

In the murine gene encoding GR, the putative splice site in front of exon 9 β does not conform with the required consensus sequence⁶. Analysis by RT-PCR showed no evidence for the existence of a GR β mRNA species in mouse. Thus, there is very strong evidence for the lack of existence of this isoform in the mouse. This, together with the very low relative expression of GR β mRNA in humans, raises serious questions about the biological significance of the GR β isoform

Synthesis of the GR β Protein

The demonstration of mRNA encoding GR β by RT-PCR does not necessarily mean that GR β is synthesized as a protein. Neither do the relative expression levels of GR isoform mRNA necessarily reflect the relative levels of protein produced. Therefore, it was of primary importance to analyse protein synthesis, and several laboratories have developed GR β -specific antibodies to study this question^{7,8}. Because the GR isoforms only differ at the C-terminus, GR β -specific antibodies must be directed towards the 15 C-terminal residues of the protein. In all cases, antibodies were raised by immunizing with a synthetic peptide corresponding to this sequence. However, because 95% of the two GR isoforms is identical, all previously existing antibodies against GR interacted with both isoforms equally well. This is an important detail for the evaluation of the specificity of the GR β -specific antibodies. It is crucial to demonstrate that any band recognized by a GR β -specific antibody can also be recognized by anti-GR antibodies recognizing epitopes common to both isoforms. The antibodies raised by Oakley *et al.*⁷ were extensively characterized in this respect and they clearly demonstrated an absolute specificity for their GR β -specific antibodies. We have also shown this for the antibodies raised in our laboratory⁸. Similar to our findings in HeLa and chronic lymphocytic leukaemia cells, Oakley *et al.* reported that the GR β protein was found in HeLa-S3 and CEM-C7 cells, but at considerably lower concentrations than the GR α protein⁷,

consistent with their previous mRNA data⁵. They reported even lower levels of GR β protein in human tissues, although the protein is detectable. Interestingly, with immunocytochemistry they could show a relatively higher level of GR β in epithelial cells in terminal bronchioles of the lung, the outer layer of Hassall's capsule in the thymus and in the bile ducts in liver⁷. However, the relative levels of the two isoforms of the protein in these epithelial cells are still unknown.

By contrast, de Castro *et al.*⁹ reported completely different levels of the isoforms using the antibodies raised in their laboratory. In this study, they reported that the level of the GR β protein was between being equal to that of GR α and five times higher. However, no comparison with antibodies recognizing common GR epitopes was reported in this study, and the measurements were based on a standard curve using the synthetic peptide fused to bovine serum albumin as standard.

In conclusion, in controlled studies, in comparison with immunoreactivity against antibodies recognizing epitopes common to the two isoforms, the GR β protein appears to be found at much lower levels than the steroid-binding GR isoform.

Function of GR β

There is consensus that GR β does not bind steroid and is in itself transcriptionally functionally inactive. However, there are conflicting data from the various studies about whether or not GR β can exert a specific dominant negative effect on transcriptional activation induced by GR α .

In the first publication in this field, Bamberger *et al.*¹⁰ reported repression of dexamethasone-dependent *trans*-activation by GR α when at least fivefold excess GR β was cotransfected into COS-7 cells. Similar results were reported by Oakley *et al.*⁵ in HeLa-S3 cells. An excess of GR β has also been reported to repress *trans*-activation induced by the mineralocorticoid receptor¹¹. By contrast, both we⁸ and de Lange *et al.* (P. de Lange, unpublished) found no evidence of a specific dominant negative effect on *trans*-activation induced by GR α . In fact, both groups reported only a

non-specific repression of transcriptional activity in general, but only when concentrations of GR β were very high in COS-7 or COS-1 cells. It has also been found that GR β repressed GR α -dependent *trans*-activation if overexpressed at fivefold higher concentrations in COS-7 and HeLa cells but not in HEK-293 embryonic kidney cells (I.J. Brogan, unpublished). Furthermore, cotransfection of hGR β in primary human lymphocytes or Jurkat T lymphoma cells had no effect at all on *trans*-activation by the GR α isoform, even if expressed at more than fivefold higher relative concentration¹². Overproduction of GR β had no effect at all on the GR α -dependent repression of NF- κ B- or AP-1-induced transcriptional activation in HEK-293, COS-7, HeLa (I.J. Brogan, unpublished) or Jurkat¹² cells.

It is unclear why the various groups have obtained conflicting results concerning the effect of GR β on GR α -dependent activation of transcription. In two cases (Ref. 8; P. de Lange, unpublished), it was clearly shown that there was a general squelching of transcriptional activity with increasing amounts of GR β expression vector, although this was not the case in other studies. However, in the former case, general transcription was assayed with the use of vectors that had relatively high levels of constitutive expression, whereas in the latter case, empty reporter vectors with only basal levels of transcription were used. This could explain why squelching was only seen in some cases. Another factor could be tissue-specific effects. Finally, all these experiments were carried out with the use of transient expression, which has its inherent problems. There is a clear need for establishing cell lines that produce various relative levels of GR isoforms to clarify the conflicting data obtained with transient expression. The results obtained with immunohistochemistry, indicating an increased relative level of GR β in epithelial cells⁷, should provide an interesting model for these studies.

• **Modulation of Glucocorticoid Action**

Different target tissues and cells show a marked variation in glucocorticoid

sensitivity and their rate of response under normal physiological conditions. In addition, glucocorticoid insensitivity can develop under clinical conditions; for example, glucocorticoid-insensitive asthma, as seen in a number of patients. To evaluate the potential role of GR β in these cases, it is important to understand the alternative mechanisms that have been described for the modulation of glucocorticoid action, as shown in Fig. 1. The four principal mechanisms are: (1) metabolism of the ligand; (2) export of the ligand out of the target cell; (3) receptor modification; and (4) protein–receptor interaction. In addition, tissue-specific effects of glucocorticoids can be the result of tissue-specific coactivators^{13,14}.

Metabolism of the Ligand

The most striking mechanism for the modulation of steroid hormone action is the mechanism protecting the mineralocorticoid receptor from being activated by the large excess of cortisol over aldosterone that is normally present. In target organs that play a role in fluid and electrolyte balance, such as kidney tubuli or colon epithelium, cortisol is inactivated by 11 β -hydroxysteroid dehydrogenase type II to form cortisone^{15,16}, thereby ensuring that the mineralocorticoid receptor is activated only by mineralocorticoids.

A mechanism for the modulation of glucocorticoid action by receptor crosstalk has recently become apparent after the identification of a family of receptors that recognize a broad variety of ligands, including pregnane X receptor (PXR)¹⁷, a human nuclear receptor (hPAR)¹⁸ and steroid and xenobiotic receptor (SXR)¹⁹. Several different glucocorticoids and glucocorticoid metabolites act as agonists for this family of receptors. The function of these receptors includes the induction of CYP3A enzymes, which inactivate glucocorticoids by 6 β -hydroxylation²⁰.

Export of the Ligand

Attenuation of glucocorticoid signalling can be achieved by the transport of the ligand out of the target cells through the action of membrane transport proteins related to the MDR

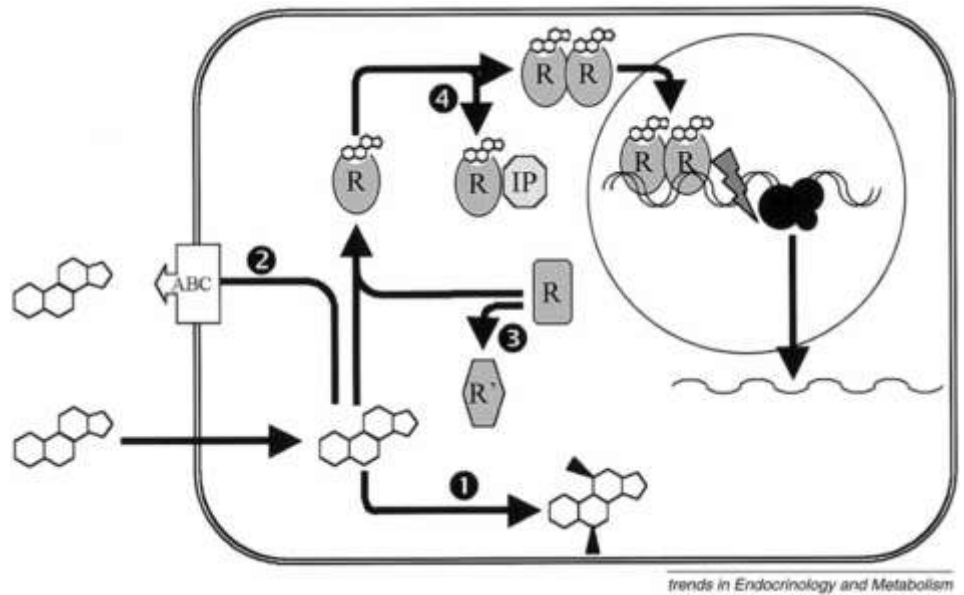


Figure 1. Alternative mechanisms for the modulation of glucocorticoid action. The four principal mechanisms are: (1) metabolism of the ligand; (2) export of the ligand out of the target cell; (3) receptor modification; and (4) protein–receptor interaction. Abbreviations: ABC, ATP-binding cassette protein; IP, interacting protein; R, receptor.

P-glycoprotein and other ATP-binding cassette (ABC) proteins. This mechanism has been described in lung fibroblasts^{21–23} and might be important for the development of the glucocorticoid insensitivity seen in some asthma patients.

Receptor Modification

Covalent modification of nuclear receptors has been shown to have a potential for the modulation of function of these receptors in either a positive or negative fashion in several examples. In the case of the GR, phosphorylation by the action of glycogen synthase kinase-3 (GSK-3) has been shown to reduce the transcriptional activating function of the receptor²⁴. Recently, it has been shown that the GR can be inactivated by nitrosylation through the action of inducible nitric oxide synthase (iNOS)²⁵. This mechanism is of particular interest to the understanding of the difference in sensitivity towards glucocorticoids with regard to the prevention of inflammation compared with the suppression of an established inflammatory reaction.

Receptor–Protein Interaction

The concept of the modulation of glucocorticoid action by the dominant negative action of a GR-interacting

protein as postulated for GR β has been described previously. The negative crosstalk between GR and AP-1 or NF- κ B has been proposed as the mechanism for the anti-inflammatory effect of glucocorticoids. Conversely, RelA specifically represses transcriptional activation by the GR via a glucocorticoid response element, presumably by GR–RelA interaction²⁶.

• Conclusions

Given the broad range and variety of glucocorticoid action, both with respect to dose response and time, under a variety of physiological and clinical conditions, there must be mechanisms that can modulate the action of the common GR found in all target cells. However, logical biological requisites for such mechanisms are that they can be regulated to achieve an appropriate level for the situation in question. All of the putative mechanisms of GR modulation described above (Fig. 1) fulfil this requirement. In many cases, the mechanism of regulation can be correlated to the physiology or pathology of glucocorticoid action. However, the mechanism of regulation for alternative splicing to give GR isoforms is unclear and this alternative appears less attractive from a biological point of view. Most data indicate a

considerably lower degree of production of GR β compared with GR α . The biological significance of GR β has to be questioned because it is not found in the mouse. The functional data are conflicting, which could be the result of the different model systems used or differences in experimental conditions of an unknown nature, related to the use of transient expression systems. This controversy will not be clarified until there are stable cell lines producing varying levels of GR isoforms. In conclusion, there are a number of alternative mechanisms for the modulation of glucocorticoid action described that are more convincing than GR β at this time.

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