MECHANISMS OF STEROID ACTION AND RESISTANCE IN INFLAMMATION

Cell- and tissue-specific effects of corticosteroids in relation to glucocorticoid resistance: examples from the brain

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Abstract

The biological mechanisms that determine cell-specific responses to glucocorticoid hormones may overlap with those that are associated with acquired glucocorticoid resistance. Cell and tissue specificity can be brought about in many different ways. Studies on the brain, an important glucocorticoid target tissue, may provide examples of regulatory mechanisms underlying response specificity at

multiple levels. In this commentary a number of such mechanisms are discussed, with emphasis on regulation of glucocorticoid bio-availability by the efflux transporter P-glycoprotein and on the variable presence of nuclear proteins which modulate or interfere with gluco- and mineralocorticoid receptor-mediated transcription.

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Introduction

Glucocorticoid resistance and cell specificity

Glucocorticoid hormones affect probably every organ in the mammalian body, yet many of their effects are specific for certain cell types or tissues. Resistance to the effects of glucocorticoids is a clinical problem, which can be viewed as related to such cell-, tissue- and also state-dependent responses to these hormones as they occur in a healthy organism. Any cell-specific response corresponds with a non-response, or resistance, in all or most of the other cell types present in a particular system. From this perspective, the mechanisms which underlie a cell-specific response, or lack of response, in a 'normal' steady state situation may be acquired by other cell types in the body. Ultimately, this may lead to changes in glucocorticoid responsiveness and steroid resistance in systems that have been challenged physiologically, pathophysiologically or pharmacologically.

The various mechanisms causing cell (or tissue) specificity include, among others, regulation of ligand availability, receptor diversity, and the type of receptor-interacting proteins and of chromatin structure around potential steroid receptor binding sites on the DNA. Studies on the brain, an important glucocorticoid target tissue, may provide examples of regulatory mechanisms underlying response specificity at a number of levels. In this commentary a selected number of these mechanisms are discussed.

Ligand availability

The foremost regulatory mechanism for the availability of endogenous glucocorticoids is the control of adrenal secretion. Circulating glucocorticoid concentrations show an ultradian (Windle *et al.* 1998) and a circadian rhythm, and increase profoundly as a consequence of psychological or physical stressors. The drive in this process is the hormonal cascade comprised by the hypothalamo-pituitary-adrenal (HPA) axis (Dallman *et al.* 2000). The regulation of the activity of the HPA axis is an intriguing and complex area, but is outside the scope of this review. The plasma protein corticosteroid binding globulin binds endogenous glucocorticoids with high affinity but limited capacity, and can modulate the plasma levels of 'free' hormone available for entry into cells and tissues (Hammond *et al.* 1991).

Enzymatic conversion

The bioactivity of the main endogenous glucocorticoids, corticosterone and cortisol, in rodents and humans respectively depends on the presence of a hydroxyl moiety at the 11-position of the steroid. Both endogenous glucocorticoids can be converted *in vivo* into the inactive 11-keto forms, dehydrocorticosterone and cortisone, by the enzyme 11- β -hydroxysteroid (11 β -HSD) type II. This conversion confers aldosterone specificity to

mineralocorticoid receptors (MRs) in a number of tissues, such as the distal nephron (Edwards *et al.* 1988, Funder *et al.* 1988) and brain (Robson *et al.* 1998). The 11 β -HSD type I enzyme mediates the reverse reaction *in vivo* and can regenerate cortisol and corticosterone from the inactive 11-keto forms. The 11 β -HSD type I is expressed in a number of tissues including liver, fat and brain. Variation in expression and activity of the type I enzyme may intracellularly regulate the availability of ligand for the corticosteroid receptors (Seckl & Walker 2001).

P-glycoprotein

During recent years it has become clear that the access of glucocorticoids to certain cells and tissues is hampered by the efflux transporter P-glycoprotein (Pgp), a member of the family of ABC-transporter proteins (Borst & Elferink 2002). The Pgp is encoded by the multi-drug-resistance or *mdr1* genes: *MDR1* in humans, and *mdr1a* and *mdr1b* in rodents. Expression data and substrate specificity suggest that the two rodent proteins together serve the same function as the human *MDR1* gene product. Pgp is a transmembrane protein that actively transports a broad range of substrates from the intracellular compartment to the extracellular space. Pgp is expressed in the capillary endothelial cells of the blood-brain barrier, and limits the access of many compounds to the brain (Schinkel 1999).

The synthetic glucocorticoid, dexamethasone, is a good substrate for Pgp and, accordingly, the accumulation of dexamethasone in the brain of *mdr1a* knockout mice is increased compared with wild-type mice (Schinkel *et al.* 1996), as is binding of dexamethasone to the glucocorticoid receptor in the brain (Meijer *et al.* 1998). The penetration into the rodent brain of the other widely used glucocorticoid, prednisolone, is also hampered by *mdr1a* Pgp (Karssen *et al.* 2002). In addition, studies in cells that have been stably transfected with the human *MDR1* have shown that dexamethasone and prednisolone are also substrates for the human Pgp (Ueda *et al.* 1992, Karssen *et al.* 2002). Thus, it may also be inferred that the human brain is relatively underexposed to synthetic glucocorticoids (and hence relatively glucocorticoid resistant) compared with cells which do not express Pgp, or with tissues which do not have a Pgp-expressing barrier.

For the endogenous steroids corticosterone and cortisol there is a striking difference in Pgp transport. Corticosterone is not a substrate for either the mouse *mdr1a* (Meijer *et al.* 1998, Karssen *et al.* 2001) or the human *MDR1* Pgp (Karssen *et al.* 2001). It is a rather weak substrate for the murine *mdr1b* Pgp (Bourgeois *et al.* 1993) which is not, however, expressed at the blood-brain barrier (Jette *et al.* 1993, Schinkel *et al.* 1997). In contrast, the main endogenous glucocorticoid in humans, cortisol, is a substrate for both mouse *mdr1a* and *mdr1b*, and human *MDR1* (Ueda *et al.* 1992, Bourgeois *et al.* 1993, Karssen *et al.* 2001). The relevance of Pgp transport of cortisol for

glucocorticoid exposure of the human brain is suggested by the comparison of the cortisol:corticosterone ratio between human blood and *post mortem* brain. While in humans plasma corticosterone concentrations are only 5% of the circulating cortisol levels, in the brain corticosterone levels are 30% of those of cortisol (Karssen *et al.* 2001). Although no direct comparison can be made between absolute plasma and brain glucocorticoid levels from the same subjects, these findings do suggest that cortisol levels in the brain are six times lower than those in the blood (making the unproven assumption that there is no selective transport or clearance of corticosterone by some other factor).

Thus, the total exposure to (endogenous or exogenous) glucocorticoids of human brain and other Pgp expressing tissues may be relatively low compared with other tissues. Pgp inhibitors may decrease this relative glucocorticoid resistance by facilitating the uptake of glucocorticoids into the brain, as recently suggested in relation to antidepressants (Pariente *et al.* 2001). Up-regulation of expression of Pgp expression or activity is a theoretical mechanism for the development of further glucocorticoid resistance. Most studies on the variation of Pgp expression relate to selection of cells in tumours, or consequences of allelic variations/polymorphisms, but *mdr1* expression has also been shown to be plastic and respond to e.g. cyclooxygenase 2 (Patel *et al.* 2002) and many other trans-acting factors (Labialle *et al.* 2002).

Receptor diversity

An obvious mechanism underlying cell-specific responses to a single ligand is receptor diversity. Glucocorticoids bind to two types of receptors in the brain, MRs and glucocorticoid receptors (GRs). These ligand-activated transcription factors are related members of the nuclear receptor super-family. Differential distribution and affinities for endogenous and synthetic steroids, as well as different intrinsic output characteristics of MRs and GRs, lead to major differences in cellular sensitivity and (transcriptional) responses in the brain (de Kloet *et al.* 1998). An additional level of complexity comes from the existence of receptor variants as a consequence of alternative splicing (Zennaro *et al.* 2001, Yudit & Cidlowski 2002), and the use of alternative translation start sites at the GR mRNA (Yudit & Cidlowski 2002). Some of these receptor variants have functional characteristics that are clearly different from the classical MR and GR, but information on their physiological or pharmacological importance, e.g. as inferred from their relative abundance in different tissues, is at present scarce.

GRs are expressed in many more cell types than MRs, both in brain and in other tissues. In some cell populations both corticosteroid receptors are expressed and have been shown to mediate very different effects of corticosterone.

This is emphatically the case in the main neuronal cell layers of the hippocampus (de Kloet *et al.* 1998), a forebrain structure critically involved in learning, memory, and (perhaps via cognitive effects) mood. MRs and GRs may form heterodimers on the DNA, which can display characteristics different from the MR or GR homodimers (Trapp & Holsboer 1996). Also, heterodimerizations with other members of the steroid receptor family may occur (Chen *et al.* 1997). Whether interactions of, for example, androgen receptors with GRs lead to diminished glucocorticoid responses in physiological settings is at present unclear.

Cross-talk partners in transrepression

Ligand availability and receptor diversity cannot explain all cell specificity. Down-regulation of the serotonin (or 5-HT) 1A receptor gene *in vivo* is one out of many examples of cell-specific transcriptional effects of glucocorticoids in the brain, as it occurs mainly in the population of granule cells of the hippocampal dentate gyrus (Meijer & de Kloet 1995). In the almost adjacent pyramidal neurons of the CA1 area no regulation is observed, although these cells abundantly express MRs, GRs, and the 5-HT1A receptor. In the case of the corticotrophin releasing hormone gene, activation of the GR leads to regionally opposite effects on mRNA abundance, i.e. a down-regulation in the paraventricular nucleus of the hypothalamus (PVN), but an up-regulation in the amygdala (Makino *et al.* 1994). Such cell-specific effects upon activation of a single receptor type by a single ligand point to the importance of additional, non receptor factors that confer cell-specific responses or resistance.

The transcription factors MR and GR can act in basically two different ways, either by binding, independently or in conjunction with other transcription factors, to DNA motifs (such as the consensus glucocorticoid response element, or GRE), or by interacting with other, non-receptor transcription factors which may or may not be bound to the DNA. The latter mode is mostly referred to as 'transrepression' as it is assumed to lead to mostly repressive effects of glucocorticoids on gene expression. The repression, as observed in test systems, tends to be mutual in that GR- and MR-mediated responses are inhibited by activity of their cross-talk partners. The best characterized cross-talk partners of glucocorticoid signalling are AP-1 and nuclear factor kappaB (NF- κ B) (Gottlicher *et al.* 1998), but a number of other interesting factors exist such as Stat 5 (Stoecklin *et al.* 1999).

The stoichiometry of cross-talk partners is critical to the outcome of interactions with corticosteroid receptors. This was first shown at a composite response element, where GRs could either stimulate or repress AP-1 activity, depending on whether the AP-1 activity was constituted of c-Jun homodimers or c-jun/c-fos heterodimers

(Diamond *et al.* 1990). At the same DNA element, it was shown that GRs but not MRs transrepress AP-1 activity (Pearce & Yamamoto 1993). This finding offered a first transcriptional basis for differential effects mediated by MRs and GRs. However, *in vivo* MRs are also capable of mediating negative effects on transcription, for example in the case of the 5-HT1A receptor gene (Meijer & De Kloet 1995). Accordingly, MRs are able to mediate transrepression at the 5-HT1A receptor promoter, critically depending on the factors which drive transcription (Meijer *et al.* 2000).

The presence of cross-talk partners is highly variable, depending on the cell type and cellular state of activity induced by other signals. c-Fos expression, for example, is a function of neuronal activity, and its abundance in a glucocorticoid target area like the PVN differs dramatically as a function of stress (Ceccatelli *et al.* 1989). Elegant studies have shown that in hypothalamic extracts of stressed rats the binding to AP-1 response elements on the DNA is indeed impaired, suggesting *in vivo* relevance of GR-AP-1 interactions in negative feedback of glucocorticoids on the HPA axis (Kovacs *et al.* 2000). Thus, GRs would repress transcription in activated PVN neurons, but at the same time be less efficient at transactivating from its response elements.

Coregulatory proteins

Binding of the corticosteroid receptors to consensus GREs mostly has stimulatory effects on transcription. These transactivational effects are mediated and modulated by the members of multiple protein families with coactivating and corepressing effects, referred to as coregulators (McKenna *et al.* 1999, Rosenfeld & Glass 2001). Prominent examples of coregulators are the p160 steroid receptor coactivators (SRCs). The SRC family consists of three genes, coding for the structurally related proteins SRC-1 (NCoA-1), SRC-2 (NCoA-2, TIF2, GRIP-1) and the somewhat more distant SRC-3 (NCoA-3, pCIP/ACTR/AIB1/RAC3/TRAM1). The precise type of coregulator stoichiometry is thought to determine the magnitude and nature of steroid responses in a given cell. The SRC family members have different interactions with steroid receptors and unique expression patterns, and therefore are a good example of possible determinants of cellular specificity of glucocorticoid actions *in vivo* (Meijer 2002).

Two splice variants of the SRC-1 gene have consistently been found, SRC-1a and SRC-1e (Kamei *et al.* 1996, Ding *et al.* 1998, Kalkhoven *et al.* 1998). These splice variants have been shown to differentially interact with the (isolated) ligand-binding domain of MRs and GRs (Ding *et al.* 1998), while strong functional differences were found for estrogen receptor (ER)-mediated transcription (Kalkhoven *et al.* 1998). Our own results suggest

Figure 1 Diagram depicting a number of cellular levels at which modulation of the glucocorticoid signal may occur. See text for details. Modified from Meijer, 2002 (with permission).

that MRs and GRs are also differentially affected by these SRC-1 splice variants (O C Meijer & E Kalkhoven, unpublished observations). These findings prompted us to investigate whether the functionally distinct splice variants are also differentially expressed in certain brain areas, and whether, in that way, they are involved in cell-specific responses. We observed that mRNA of both SRC-1 splice variants is expressed in most brain areas, but that, particularly in the hypothalamic nuclei (including PVN), the relative SRC-1a mRNA levels are much higher than those of SRC-1e mRNA (Meijer *et al.* 2000b). This suggests, *a priori*, a specific steroid responsiveness in these nuclei compared with other brain areas. In the brain, SRC-1 is the most abundant p160 coactivator (Xu *et al.* 1998), but SRC-3 is expressed quite specifically in some subfields of the hippocampus – albeit at very low levels (Xu *et al.* 2000). The highly variable stoichiometry of the SRCs and other coregulators in different brain regions under basal conditions likely affects the precise nature and magnitude of steroid responses.

Regulation of the expression or activity levels of coactivators is expected to change steroid responses. In fact, SRC-3 was cloned by one group of researchers as a gene possibly involved in the development of steroid sensitive cancer (amplified in breast cancer-1 or AIB-1; Anzick *et al.* 1997). SRC activity can be changed at the mRNA level, but can also be modulated post-translationally (Rowan *et al.* 2000, Kotaja *et al.* 2002). The expression of SRC-1 can be subject to hormonal regulation in the pituitary (Misiti *et al.* 1998), and also shows

variation in the brain (Bousios *et al.* 2001). Thus, SRCs may well be factors involved in physiological modulation of steroid responsiveness. As a last fascinating aspect of SRCs, it may be mentioned that their interactions with steroid receptors can be ligand dependent. For the vitamin D receptor, a synthetic ligand was shown to preferentially interact with SRC-2, suggesting relative resistance to that particular ligand in tissues with low expression of this coactivator (Takeyama *et al.* 1999).

Many criteria for determinants of inherent or acquired differences in glucocorticoid sensitivity apply to the SRC family members: there is proof of principle for specific interactions with steroid receptors, there are specific expression patterns in the brain and other tissues, and their activity and abundance can be regulated. However, the precise roles of the SRCs and other relevant coregulators such as the corepressors NCoR and SMRT, for glucocorticoid sensitivity of different organ systems *in vivo*, remains to be resolved.

When is the brain 'glucocorticoid resistant'?

Use of the term glucocorticoid resistance may be based on one functional output characteristic of a complex organ. For example, an elevation of cortisol levels with age may be viewed as indicative of functional 'glucocorticoid resistance' within the HPA axis. Impaired GR function may play a role in such cases, e.g. as a consequence of receptor down-regulation in the PVN, which is the main

driving force of the HPA axis (Makino *et al.* 1995). However, such states do not necessarily reflect impaired functioning of steroid receptors, but may reflect increased activity of neurons which have stimulatory inputs on the HPA axis, and which do not have an inherent negative feedback glucocorticoid sensitivity. Thus, 'feedback resistance' as defined at the level of adrenocorticotrophin and corticosteroid plasma levels may coexist with unchanged or even increased glucocorticoid responses in neurons outside the core of the HPA axis.

Conclusions

The nature and magnitude of the transcriptional responses to glucocorticoids can depend on many factors. Here, we described some mechanisms that bear relevance to glucocorticoid signalling in the brain: regulation of ligand availability and cellular differences in the levels of receptor and of numerous nuclear proteins involved in transcription (see Fig. 1).

A number of other cellular factors have not been discussed, such as the role of the cytoplasmatic factors (e.g. heat shock proteins), or many aspects of chromatin remodelling (e.g. DNA methylation). In theory, all of these mechanisms could contribute to an acquired glucocorticoid resistance, e.g. an up-regulation of the P-glycoprotein or a down-regulation of coactivator levels.

Insights into which of these mechanisms can be relevant should be obtained from direct measurements in resistant cells, in combination with more comprehensive studies on the types of regulation that actually occur in *in vivo* situations.

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