Glucocorticoid-stimulated gene expression knocked out by knock-in mutation

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Glucocorticoid receptors (GRs) are ligand-dependent transcription modulators. They belong to a superfamily of nuclear hormone receptors including receptors for steroid and thyroid hormones, retinoids, vitamin D and peroxisomal activators among others (1). The receptor can be divided into three functional domains: (1) an N-terminal activation domain (AF1 or τ1); (2) a central DNA-binding domain; and (3) a ligand-dependent activation domain (AF2 or τ2), which is a part of the ligand-binding domain in the C-terminus. The GR is essential for survival. 'Knock-out' of the receptor in mice resulted in impaired lung development, and the newborn mice died shortly after birth because of respiratory failure (2). Several other abnormalities associated with a loss of glucocorticoid stimulation were observed in these mice including a reduced level of gluconeogenic enzymes in the liver, increased serum levels of corticotrophin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and corticosterone, hyperplasia of the adrenal cortex, loss of glucocorticoid-dependent thymocyte apoptosis, and impaired proliferation of erythroid progenitor cells.

Activation of GRs may stimulate or repress gene transcription (3). The glucocorticoid-induced transcription of target genes is mediated by glucocorticoid-responsive elements (GREs), which are palindromic sequences in the regulatory region of these genes. A GRE is a binding site for the activated GR, and transactivation depends on the dimerization of two GRs on the GRE. There are probably three ways in which GRs can repress gene transcription. The pro-opiomelanocortin (POMC) gene is negatively regulated by a direct interaction of GR with its promoter (4). Secondly, it may interact with the DNA-binding domain together with another transcription factor, and, finally, glucocorticoid-induced repression of gene transcription can be independent of direct binding of GR to DNA. As examples, the activated receptor has been shown to interfere with signalling via the cAMP-responsive transcription factor (CREB) or transcription factors using AP-1 elements by protein–protein interactions (5–7). Furthermore, some of the immunosuppressive effects of glucocorticoids have been associated with a repression of the activity of the transcription factor NF-κB by a direct interaction between GRs and NF-κB, even though this regulation may be even more complex (8). In cell culture, the consequences of a defect in the dimerization of GRs have been studied by introducing a point mutation in the region of the GR gene coding for the D loop of the receptor protein (9, 10). This loop consists of five amino acids critical for receptor dimerization. Changing the amino acid alanine in position 458 in the mouse GR to threonine (A458T) abolished the formation of receptor dimers and activation of gene transcription via GREs, whereas effects on transcriptional repression were almost unaffected.

The consequences of this dimerization defect have now been studied in vivo. Reichardt et al. (11) (reviewed by Karin (12)) created a strain of mice with alanine 458 replaced by threonine in the GR protein. In contrast with GR knock-out mice, there were no signs of impaired lung development and both sexes were fertile. An unexpected almost Mendelian ratio of the three genotypes with 21% homozygous mutants (denoted GRdim/dim) was found among the offspring in contrast with the lethal effect of homozygous GR knock-out in mice.

As expected a series of experiments demonstrated that the mutation specifically affected DNA-binding-dependent transcriptional activation by the GR. Immortalized embryonic fibroblasts from GRdim/dim and control mice were incubated in the absence or presence of dexamethasone and two reporter genes with different GREs. Only minimal activation of the reporter genes was observed in GRdim/dim cells in contrast with the activation seen in control cells. Dexamethasone-induced transactivation of gluconeogenic enzymes in the liver was also lost in the A458T mutants, indicating the need for dimerization and GRE binding for these effects. However, the protein–protein interactions were preserved, as the expression of collagenase 3 and gelatinase B, which is transcriptionally activated by phorbol esters via an AP-1 element, was almost completely abolished by dexamethasone in control but also in GRdim/dim cells.

Glucocorticoid effects have typically been demonstrated in the autoregulatory circuit consisting of the hypothalamus, pituitary and adrenal glands. Negative feedback via the GR has been shown on both the expression and secretion of CRH and ACTH. In GRdim/dim mice, CRH immunoreactivity in the hypothalamic median eminence was unaltered in contrast with the increase observed in the GR knock-out mice. However, the expression of POMC and ACTH in the anterior pituitary was increased in mice with the A458T GR mutation. This indicated that GR uses different mechanisms to regulate CRH and POMC/ACTH. The lack of CRH up-regulation in GRdim/dim mice indicated that GR repressed CRH transcription without binding to DNA.
whereas repression of POMC expression seemed to require GR–DNA binding activity. The increase in ACTH immunoreactivity in the anterior pituitary of GRdim/dim mice did not result in increased serum levels of ACTH, which indicated that ACTH secretion was regulated by a mechanism independent of DNA binding. However, serum levels of corticosterone, the major glucocorticoid in mice, increased. The adrenal medullas of GR knock-out mice were disorganized, and the expression of phenylethanolamine-N-methyltransferase (PNMT), an enzyme involved in adrenaline synthesis, was impaired. Although the PNMT gene has a classical GRE in its promoter, the expression of the gene and the development of the adrenal medulla were unaltered in GRdim/dim mice. Reichardt et al. (11) speculate that basal expression of the gene is independent of the DNA-binding activity of the GR. Additional experiments are needed to exclude the possibility that GR A458T mutants transactivate target genes by binding to other not yet defined responsive elements on DNA. It will be particularly interesting to know how PNMT expression and the production and secretion of CRH, ACTH and corticosterone change during acute stress.

The mouse model developed by Reichardt et al. (11) may become a tool that can be used to indicate mechanisms by which GRs induce their effects, i.e. whether they are mediated by activation or repression of gene expression. In mice, T-lymphocyte development and differentiation are controlled by glucocorticoid-induced apoptosis, but there has been controversy about the mechanism. T-cells from GRdim/dim mice were found to be refractive to apoptosis induced by dexamethasone, which indicated a mechanism requiring binding of GR to GRE and transcriptional activation.

GRdim/dim mice survive in the absence of glucocorticoid-activated GR binding to GRE and the subsequent direct stimulation of gene transcription under standard laboratory conditions. The most important effects of glucocorticoids and GRs seem to be via cross-talk with other transcription factors. This opens a pharmacological window of opportunity. New GR ligands that activate the mechanisms used for transcriptional repression more than those for activation have already been tested (13). They bring hope of finding glucocorticoid analogues with potent anti-inflammatory properties and limited side effects on glucose, lipid and calcium metabolism.

References