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Regulation of Glucocorticoid Receptor Signaling and the Diabetogenic Effects of Glucocorticoid Excess

Henrik Ortsäter, Åke Sjöholm and Alex Rafacho

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1. Introduction

Glucocorticoids (GCs), such as cortisol, are key hormones to regulate carbohydrate metabolism (Wajchenberg et al., 1984). Furthermore, CG-based drugs are effective in providing anti-inflammatory and immunosuppressive effects (Stahn & Buttgereit, 2008). Their clinical desired effects are generally associated with adverse effects that include muscle atrophy, hypertension, osteoporosis, increased central fat deposition, and metabolic disturbance such as induction of peripheral insulin resistance (IR) and glucose intolerance (Schacke et al., 2002). In this context, we aim, in this chapter, to present and discuss the different factors that control tissue sensitivity towards GCs. These factors include expression levels of the GC receptor (GR), GR interacting proteins, GR phosphorylation and pre receptor regulation of GC availability. In this chapter we will also present some clinical manifestations of endogenous or exogenous CG excess on glucose homeostasis. Latter, in the coming chapter a special focus will be placed on GC effects on the endocrine pancreas.

1.1. General aspects of the glucocorticoid

GCs, like cortisol and dehydroepiandrosterone (DHEA), are produced and released from the *zona fasciculata* of the adrenal gland cortex. Especially cortisol secretion has an inherent rhythm over a 24 hours sleeping-wake period. The most pronounced feature of the diurnal cortisol cycle is a burst of secretory activity following awakening with a diurnal decline thereafter. Like other steroid hormones, GCs are derived from cholesterol via pregnenolone by a series of enzymatic reactions. Moreover, with the exception of vitamin D, they all contain the same cyclopentanophenanthrene ring and atomic numbering system as cholesterol. Common names of the steroid hormones are widely recognized, but systematic

nomenclature is gaining acceptance and familiarity with both nomenclatures is increasingly important. Steroids with 21 carbon atoms are known systematically as pregnanes, whereas those containing 19 and 18 carbon atoms are known as androstanes (male sex hormones, *e.g.* testosterone) and estranes (female sex hormones, *e.g.* estrogen), respectively. Figure 1 depicts the pathways for biosynthesis of pregnanes, androstanes and estranes.

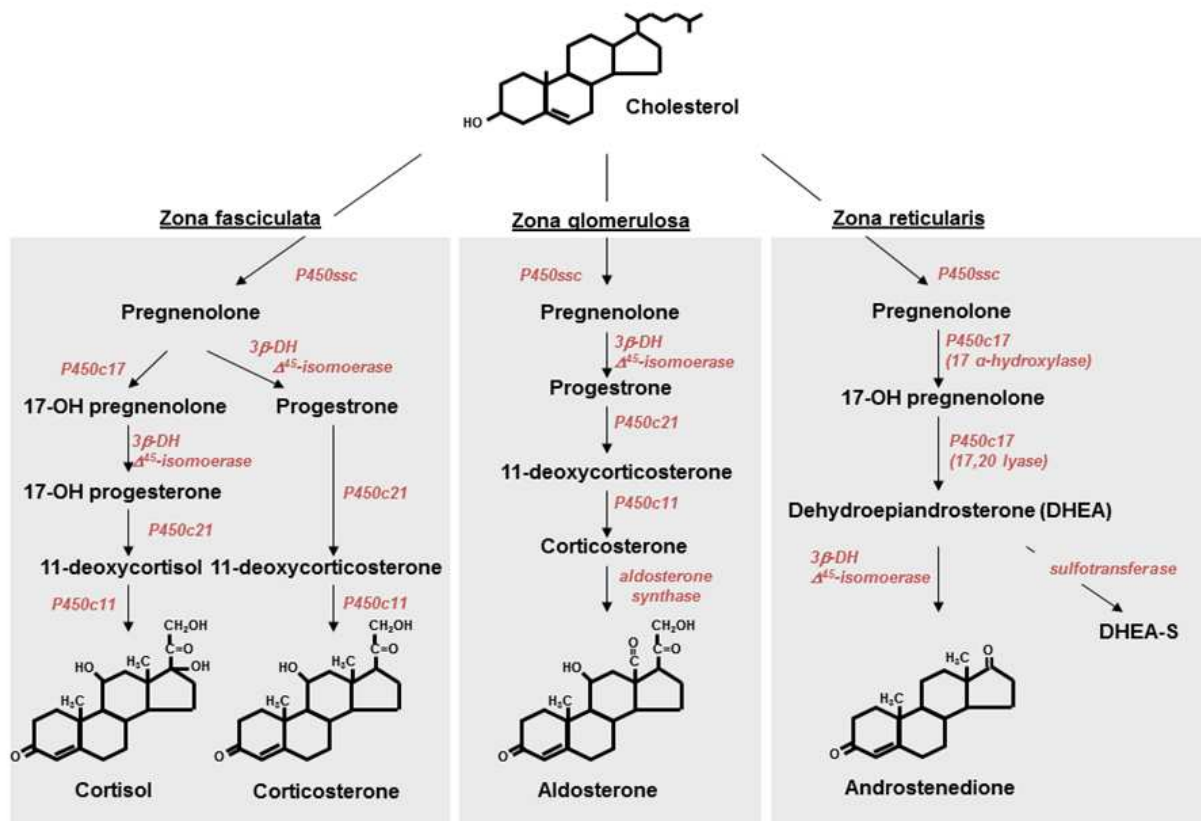


Figure 1. Synthesis of steroid hormones in the adrenal cortex. Synthesis of the adrenal steroid hormones from cholesterol. Steroid synthesis originates from cholesterol. In *zona fasciculata*, *zona glomerulosa* and *zona reticularis*, a series of enzymatic reactions give rise to glucocorticoids, mineralocorticoids and androgens, respectively. P450ssc enzyme (also called 20,22-desmolase or cholesterol desmolase) is identified as CYP11A1. 3 β -DH and $\Delta^{4,5}$ -isomerase are the two activities of 3 β -hydroxysteroid dehydrogenase type 1 (gene symbol HSD3B2), P450c11 is 11 β -hydroxylase (CYP11B1), P450c17 is CYP17A1. CYP17A1 is a single microsomal enzyme that has two steroid biosynthetic activities: 17 α -hydroxylase which converts pregnenolone to 17-hydroxypregnenolone (17-OH pregnenolone) and 17,20-lyase which converts 17-OH pregnenolone to DHEA. P450c21 is 21-hydroxylase (CYP21A2, also identified as CYP21 or CYP21B). Aldosterone synthase is also known as 18 α -hydroxylase (CYP11B2). The gene symbol for sulfotransferase is SULT2A1.

Secretion of cortisol and other GCs by the adrenal cortex are under the control of a prototypic neuroendocrine feedback system, the hypothalamic-pituitary-adrenal (HPA) axis. GCs are secreted in response to a single stimulator, adrenocorticotrophic hormone (ACTH) from the anterior pituitary (Feek et al., 1983). ACTH is itself secreted mainly under control of the hypothalamic peptide corticotropin-releasing hormone (CRH). Secreted GC has a negative influence on both CRH and ACTH release; hence, the steroid regulates its

own release in a negative feedback loop. The central nervous system (CNS) is thus the commander and chief of GC responses, providing an excellent example of close integration between the nervous and endocrine systems (Vegiopoulos & Herzig, 2007).

The GCs are a class of hormones that is primarily responsible for modulating carbohydrate metabolism (Wajchenberg et al., 1984). In principle, GCs mobilize glucose to the systemic circulation. In the liver cortisol induces gluconeogenesis, potentiates the action of other hyperglycemic hormones (*e.g.* glucagon, catecholamines and growth hormone) on glycogen breakdown, which culminates in release of glucose from hepatocytes. Cortisol inhibits uptake and utilization of glucose in skeletal muscle and adipose tissue by interfering with insulin signaling. The hormone also promotes muscle wasting via reduction of protein synthesis and degradation of protein and release of amino acids. The effect of cortisol on blood glucose levels is further enhanced through the increased breakdown of triglycerides (TG) in adipose tissues, which provide energy and substrates for gluconeogenesis. The increased rate of protein metabolism leads to increased urinary nitrogen excretion and the induction of urea cycle enzymes.

In addition to its metabolic effects, GCs have strong immunomodulatory properties for which they now are used as standard therapy for reducing inflammation and immune activation. At supraphysiological concentrations (greater than normally present in the body), GCs display strong clinical applications. In this regard, the relevant properties are the immunosuppressive, anti-inflammatory and anti-allergic effects that GCs exert on primary and secondary immune cells, tissues and organs (Stahn & Buttgereit, 2008), and the alleviation of the emesis associated with chemotherapy (Maranzano et al., 2005). GCs are used in virtually all medical specialties for both systemic and topical therapy. To have an idea of GCs relevance on clinical therapies, approximately 10 million new prescriptions for oral corticosteroids are issued in the United States annually (Schacke et al., 2002). The most prescribed synthetic GCs (*e.g.*, prednisolone, methylprednisolone, dexamethasone [DEX] and betamethasone – agents with high GC potency and low mineralocorticoid activities) are relatively inexpensive drugs, but due to the large volume prescribed they achieve a market size of about 10 billion US\$ per year (Schacke et al., 2002). In dermatology, GCs are the most widely used therapy to, for example, treat atopic eczema. Inhalation of GCs is used to treat allergic reactions in airways and to dampen bronchial hyperreactivity in asthma. Systemically, GCs are used to combat inflammations in connective tissue, rheumatoid arthritis, bowel diseases as well as in allotransplantation. The anti-inflammatory activity of the GCs is exerted, in part, through inhibition of phospholipase A2 (PLA2) activity with a consequent reduction in the release of arachidonic acid from membrane phospholipids. Arachidonic acid serves as the precursor for the synthesis of various eicosanoids. GCs also affect circulation and migration of leukocytes. For more detailed information on the immunomodulatory properties of GCs the reader is kindly referred to reference (Löwenberg et al., 2008). Despite their excellent effects for the treatment of inflammatory and allergic diseases, GCs use is limited by their side effects on several systems, organs and/or tissues (Table 1), which are dependent on the dose and duration of the GC treatment. Among these adverse effects are the endocrine derangements that include increase in central fat

deposition (Rockall et al., 2003; Asensio et al., 2004), hyperphagia (Debons et al., 1986), hepatic steatosis (Rockall et al., 2003), dyslipidemia characterized by increased TG and nonesterified fatty acid (NEFA) levels (Taskinen et al., 1983; Rafacho et al., 2008), muscle atrophy (Prelovsek et al., 2006), IR and/or glucose intolerance (Stojanovska et al., 1990; Binnert et al., 2004; Rafacho et al., 2008), as well as overt diabetes in susceptible individuals (Schacke et al., 2002).

ORGANS AND/OR TISSUES AND THE RESPECTIVE ALTERATIONS
Skin: atrophy, delayed wound healing
Skeleton and muscle: osteoporosis, muscle atrophy/myopathy
Eye: glaucoma, cataract
Central nervous system: disturbance in mood, behavior, memory, and cognition
Endocrine system/metabolism: dyslipidemia, insulin resistance and/or glucose intolerance, β -cell dysfunction (susceptible individuals)
Cardiovascular system: hypertension
Immune system: increased risk of infection, re-activation of viruses
Gastrointestinal system: peptic ulcer, pancreatitis

Table 1. Some typical side effects in GC-treated patients ordered by the affected organs. Modified from Schäcke et al., 2002 (Schacke et al., 2002).

2. Factors controlling tissue sensitivity towards glucocorticoids

2.1. The Glucocorticoid Receptor

The GC receptor (GR), a ligand-regulated transcription factor that belongs to the superfamily of nuclear receptors, binds GCs and regulates transcription of target genes after binding specific DNA sequences in their promoters or enhancers regions (Mangelsdorf et al., 1995).

The human GR ([NCBI Reference Sequence: NM_000176, Uniprot identifier P04150]) cDNA was isolated by expression cloning in 1985 (Hollenberg et al., 1985). The hGR gene consists of 9 exons and is located on chromosome 5. The mouse GR gene (NCBI Reference Sequence: NM_008173, Uniprot identifier P06537) maps to chromosome 18 and the rat GR gene (NCBI Reference Sequence: NM_012576, Uniprot identifier P06536) to chromosome 18.

Alternative splicing of the human GR gene in exon 9 generates two highly homologous receptor isoforms, termed α and β . These are identical through amino acid 727 but then diverge, with the α isoform having an additional 50 amino acids and the β isoform having an additional, nonhomologous, 15 amino acids. In addition, different translation initiation sites increase the number of possible isoforms of the GR to 16 (8 α isoforms + 8 β isoforms) (Duma et al., 2006). All these variants have different transcriptional activity in response to DEX, varies in the subcellular distribution, and display distinct transactivation or transrepression patterns on gene expression as judged by cDNA microarray analyses (Lu &

Cidlowski, 2005). The relative expression of different GRs isoforms in pancreatic β -cells is not known but it is conceivable that differences in the expression pattern might predispose certain individuals to develop glucose intolerance upon GC exposure. The molecular weights of the canonical α and β receptor isoforms are 97 and 94 kilo-Dalton, respectively. The α isoform of human GR resides primarily in the cytoplasm of cells and represents the classic GR that functions as a ligand-dependent transcription factor. The β isoform of the human GR, on the other hand, does not bind GC agonists, may or may not bind the synthetic GC antagonist RU38486 (mifepristone), has intrinsic, α isoform-independent, gene-specific transcriptional activity, and exerts a dominant negative effect upon the transcriptional activity of the α isoform (Oakley et al., 1999; Zhou & Cidlowski, 2005; Kino et al., 2009).

The human GR is a modular protein composed of distinct regions, as illustrated in Figure 2. In the N-terminal part of the receptor, is the A/B domain that contains transcription activation function-1 (AF-1) that in many cases acts synergistically with ligand-dependent AF-2 located in the ligand binding domain (LBD) of the receptor (Ma et al., 1999). In addition, this domain harbours several phosphorylation sites and is the target of various signaling kinases, such as mitogen-activated protein kinases (MAPK) and cyclin-dependent kinases (Cdk) (Ismaili & Garabedian, 2004). Thereafter follows the DNA binding domain (DBD) and a hinge region (HR). In the C-terminal part is the LBD that starts with the important site for interaction with heat-shock proteins (Hsp) and ends with a second transcription activation function (AF-2).

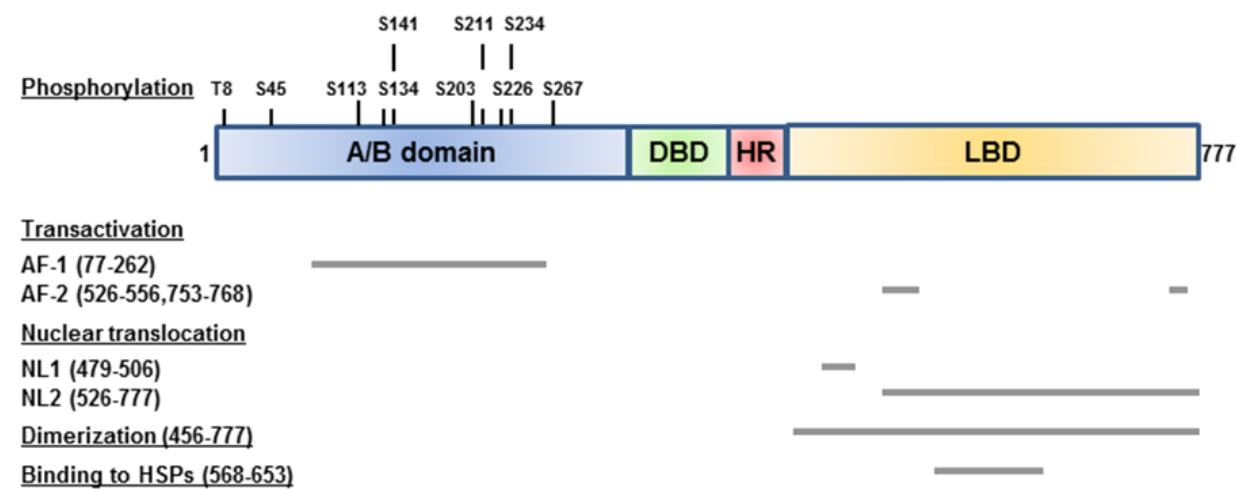


Figure 2. Structure and domains in the human GR α isoform. The human GR (Uniprot identifier P04150) isoform α is considered to be the canonical version. This isoform is made up of 777 amino acids. The β isoform is similar to α variant up amino acid 727 but then contain only 15 more amino acids that are non-homologous to those in the α isoform. The human GR is a modular protein composed of distinct regions. In the N-terminal part of the receptor is the A/B domain that contains transcription activation function-1 (AF-1) that in many cases acts synergistically with ligand-dependent AF-2 located in the ligand binding domain (LBD) of the receptor. In addition, this domain harbours several phosphorylation sites. Thereafter follows the DNA binding domain (DBD) and a hinge region (HR). In the C-terminal part is the LBD that starts with the important site for interaction with heat-shock proteins (Hsp) and ends with a second transcription activation function AF-2. This last domain also contains amino acid sequences responsible for receptor dimerization and nuclear translocation.

Ligand-activated GR exerts its classic transcriptional activity by binding via zinc finger motifs in the DBD to the promotor region, a GC-responsive element (GRE), of GC-responsive genes. To initiate transcription, the GR uses its transcriptional activation domains (AF-1 and AF-2) to interact with various transcriptional coactivators that bridges to RNA polymerase II (McKenna et al., 1999; Auboeuf et al., 2002; McKenna & O'Malley, 2002).

2.2. GR interacting proteins

The GR is expressed in virtually all tissues, yet it has the capacity to regulate genes in a cell-specific manner, indicating that the response to GCs is regulated by factors beyond receptor expression. Steroid hormones, such as cortisol, act as the primary signal in activating the receptor's transcriptional regulatory functions. But, GRs do not proceed through their signaling pathway alone. They are guided from the moment of their synthesis, through signal transduction and until they decay by a variety of molecular chaperones, which facilitate their encounter with various fates (Grad & Picard, 2007; Sanchez, 2012). In addition, GR-mediated transcriptional activation is modulated both positively and negatively by phosphorylation (Ismaili & Garabedian, 2004) exerted by kinases and phosphatases. These interacting proteins have profound implications for GC action as they regulate folding, maturation, phosphorylation, trafficking and degradation of the GR. An overview of these different proteins is given in Table 2.

Protein name	Uniprot ID (Human)	Function	Phenotypic effects in genetic mouse knock out models	References
Hsp90	P07900 (α)	Molecular chaperone involved in GR maturation and trafficking. Binds to cochaperones.	Hsp90 α knock mice are viable and healthy but male have defective spermatogenesis resulting in male sterility. Hsp90 β knock out generates embryonic lethality at E9 in the mouse due to defective placental development.	(Brehmer et al., 2001; Pearl & Prodromou, 2006)
	P08238 (β)			
Hsp70	P08107	Molecular chaperone involved in GR folding. Binds to cochaperones.	Male infertility. At the cellular level, mice homozygous for a knock out allele exhibit impaired thermotolerance and increased sensitivity to heat stress-induced apoptosis.	(Brehmer et al., 2001; Pearl & Prodromou, 2006)
Hsp40	P25685	Cochaperone of Hsp70, activate Hsp70 ATPase activity.	Mice homozygous for a knock out allele are viable, fertile, and overtly normal; however, homozygous null peritoneal macrophages display impaired thermotolerance in the early (but not in the late) phase after mild heat treatment.	(Laufen et al., 1999)
Hip	P50502	Cochaperone of Hsp70, catalyzes folding.	Not described	(Höhfeld et al., 1995)
Bag-1	Q99933	Cochaperone of Hsp70, promotes	Homozygous null mice display embryonic lethality and liver hypoplasia.	(Ballinger et al., 1999;

Protein name	Uniprot ID (Human)	Function	Phenotypic effects in genetic mouse knock out models	References
		GR degradation.		Kanelakis et al., 2000)
Hop	P31948	Cochaperone of both Hsp70 and Hsp90, contains three TPR domains, transfers GR from Hsp70 to Hsp90.	Not described	(Chen & Smith, 1998; Odunuga et al., 2004)
CHIP	Q9UNE7	Cochaperone of Hsp70, promotes GR degradation.	Homozygous null mice develop normally but are susceptible to stress-induced apoptosis of multiple organs. Increased peri- and postnatal lethality.	(Ballinger et al., 1999; Kanelakis et al., 2000)
p23	Q15185	Cochaperone of Hsp90, stabilizes Hsp90 to catalyze ligand binding,	Disruption of gene function results in neonatal lethality, respiratory system abnormalities, as well as skin morphological and physiological defects.	(Grad et al., 2006; Lovgren et al., 2007; Nakatani et al., 2007)
FKBP5 1	Q13451	Cochaperone of Hsp90, contains TPR domain.	Mice homozygous for a null allele are normal and fertile. Mice homozygous for another knock out allele exhibit decreased depression-related behavior and increased anxiety-related behavior.	(O'Leary et al., 2011)
FKBP5 2	Q02790	Cochaperone of Hsp90, contains TPR domain, interacts with dynein to support nuclear translocation via microtubuli.	<i>Fkb52^{-/-}</i> mice display a high rate of embryonic mortality. <i>Fkb52^{+/-}</i> mice placed on a high-fat diet demonstrate a susceptibility to hyperglycemia and hyperinsulinemia that correlate with reduced insulin clearance.	(Warrier et al., 2010)
PP5	P53041	Cochaperone of Hsp90, contains TPR domain, protein phosphatase.	Reduced body weight, and improved glucose clearance. Mice homozygous for a null allele exhibit a decrease in cell cycle check-point arrest following treatment with ionizing radiation.	(Hinds et al., 2011; Grankvist et al., 2012)
Uniprot ID (http://www.uniprot.org/) are given for the human version. Information on phenotypes are partly taken from the Mouse Genome Informatics (MGI) database http://www.informatics.jax.org/ .				

Table 2. Proteins interacting with the GR.

2.2.1. Heat shock proteins

Over its entire lifespan, the GR is tightly associated with Hsp, mostly notably Hsp70 and Hsp90. Hsp70 and Hsp90 are ATP-dependent and their interaction with either ATP or ADP controls the binding and release of client proteins. Their activities are regulated via interaction with cochaperones that can act as modulators of the ATPase activity or as nucleotide exchange factors (Brehmer et al., 2001; Pearl & Prodromou, 2006). Several of these regulators are proteins containing so called tetratricopeptide repeat (TPR) domains. Via their TPR domains they bind to conserved C-terminal parts of Hsp70 (EEVD) and Hsp90 (MEEVD), respectively (Liu et al., 1999; Scheufler et al., 2000). Notably, only one species of these TPR containing protein can bind to one Hsp at any given time, leading to a competition among the TPR proteins for Hsp binding. This means that in any given cell at given time the actual levels of TPR proteins will determine the cellular response to GCs. This is an open arena for new research, both in pancreatic β -cells as well as in other types of cells.

The nascent translation product of GR mRNA is brought to Hsp70 by Hsp40 that at the same time accelerates ATP hydrolysis (Laufen et al., 1999). ADP-bound Hsp70 forms a tight complex with unfolded GR. In this configuration the receptor undergoes conformational changes, which results in a tertiary structure with low hormone affinity. A cochaperone, Hip, then binds to the ATPase domain of Hsp70 and catalyzes the folding process by keeping ADP bound to Hsp70 (Höhfeld et al., 1995). During the folding process, Hsp70 undergoes cycles of client binding and client release in conjunction with ADP and ATP interaction, respectively. Once the GR is correctly folded, Hip is exchanged for yet another cochaperone, Hop, which binds Hsp70 via one of its TPR domains (Odunuga et al., 2004). In fact, Hop contains three TPR domains that allows for simultaneous binding of Hsp70 and Hsp90 and can therefore transfer the newly folded GR from Hsp70 to Hsp90 (Chen & Smith, 1998). Before moving on to the function of the GR-Hsp90 complex, we need to acknowledge that Hsp70 also plays a role for proteosomal degradation of the GR. The two proteins Bag-1 and CHIP compete with Hip and Hop in their binding to Hsp70 (Ballinger et al., 1999; Kanelakis et al., 2000). In a configuration with either Bag-1 or CHIP, Hsp70 interaction with client protein does not promote folding but rather facilitates ubiquitination and subsequent proteosomal degradation of unfolded GR. Thus, increased cellular levels of Bag-1 and CHIP would negatively impact cellular responses to GC exposure by enhancing GR degradation.

While Hsp70 is the molecular chaperone that is essential for folding in nascent GR polypeptide chains, it is Hsp90 that is required for obtaining a mature GR with high affinity for GCs. Hsp90 interact with GR as a homodimer. The receptor's affinity for ligands is 100-fold lowered in the absence of Hsp90 as was investigated in cell-free steroid binding assays (Nemoto et al., 1990). Transfer of GR from the Hsp70 to the Hsp90 complex is facilitated by Hop (Chen & Smith, 1998). Hsp90 binds to Hop in an ADP state but, once Hsp70 is released, ADP is exchanged for ATP. The Hsp90 – GR complex, in its ATP-bound form, recruits the protein p23 that stabilizes this configuration (Figure 3). According to the model proposed by Pratt et al. (Pratt et al., 2006), unligated GR constantly undergoes cycles of rapid opening and closing of the ligand binding site. When stabilized by p23, the opening time is

prolonged and therefore p23 expression can augment GC action by facilitating ligand binding to GR. Different versions of p23-deficient mice have been generated (Grad et al., 2006; Lovgren et al., 2007; Nakatani et al., 2007) and these mice display pathologies similar to those seen in GR knock out mice (Cole et al., 1995; Bayo et al., 2008), including atelectatic lungs and skin defects, indicating that p23 is essential for GC signaling.

The exchange of ADP for ATP in the Hsp90–GR complex also decreases Hsp90's affinity for Hop. As a result, Hop is released and the binding site for other TPR domain containing protein is made available. The net result of this folding and maturation processes, orchestrated by first Hsp70 and then Hsp90, is a receptor that has high affinity for steroid hormones. In addition, the Hsp90 protein is ready to interact with a new set of cochaperones that will regulate future GC action.

2.2.2. *Immunophilin-related cochaperones*

Immunophilins are members of a highly conserved family of proteins, all of which are cis-trans peptidyl-prolyl isomerases (PPI) (Marks, 1996). The prototypic members of the immunophilin family, cyclophilin A and FKBP12, were discovered on the basis of their ability to bind and mediate the immunosuppressive effects of the drugs cyclosporin, FK506, and rapamycin. However, the prolyl isomerase activity of these proteins is not involved in any of the immunosuppressive effects. Two other members of this family, FKBP51 and FKBP52, play a fundamental role during cytosol to nucleus translocation of activated GR (Figure 3). Before stimulation with a GC hormone, the majority of Hsp90–GR complexes coprecipitates with FKBP51 but upon ligand binding there is a rapid shift of FKBP51 in favour of FKBP52 (Davies et al., 2002). In contrast to FKBP51, the PPI domain of FKBP52 interacts with the microtubule motor protein dynein via protein-protein binding. This is a function of the PPI domain that is independent of its enzymatic activity and is not affected by FK506 (Galigniana et al., 2004). Interestingly, swapping of the PPI domains between FKBP51 and FKBP52 reverses their respective function, indicating that the true function of FKBP52 is, via dynein, to make a bridge between the Hsp90–GR complex and the microtubuli system. FKBP52 immunoprecipitates with dynein and the FKBP52 co-localizes to the microtubule system in the cytosol (Czar et al., 1994; Galigniana et al., 2002). In accordance with these observations, ligand-activated GR rapidly accumulates in the nucleus (half time = 4-5 minutes) and this rate is slowed by injection of FKBP52 neutralizing antibodies (Czar et al., 1995). In addition, overexpression of a FKBP52 fragment that contained the dynein-interacting PPI domain, but not the TPR domain, disrupted the interaction of full length FKBP52 protein with dynein and delayed nuclear translocation of GR (Wohnik et al., 2005). The same type of reduced speed for GR translocation (half time = 40-60 minutes) is seen after treatment with the Hsp90 inhibitor geldanamycin (Czar et al., 1997) or after disruption of the microtubule network (Czar et al., 1995). However, nuclear GR translocation is not completely inhibited during these conditions. Thus, there is a possibility for GR translocation and, hence, signaling that is independent of Hsp90, FKBP52 and a functional microtubuli system (Figure 3); however, it is not clear if this pathway is of any physiological relevance.

From the information presented above, we can conclude that in the absence of steroid ligand the GR resides in the cytosol in a complex with Hsp90 and FKBP51 but, upon ligand binding, FKBP51 is replaced by FKBP52 that will interact with dynein and promote nuclear translocation via the microtubuli system (Figure 3). It is not clear whether Hsp90 is released from the complex during transportation or if Hsp90 sticks on to the receptor during nuclear translocation. There are also some indications that the GR can translocate to the nucleus independent of Hsp90, FKBP52 and a functional microtubuli system.

This model would imply that the binding of FKBP51 to Hsp90 has a suppressive impact on GC signaling, whereas FKBP52 serves as an enhancer. Indeed, FKBP52 selectively potentiated hormone-dependent gene activation in *Saccharomyces cerevisiae* by as much as 20-fold at limiting concentrations and this potentiation was blocked when FKBP51 was co-expressed (Riggs et al., 2003). Another striking example on how GR-interacting proteins can regulate GC signaling comes from studies of new world primates, like the Squirrel monkeys (*Genus Saimiri*). Many new world primates have high circulating levels of cortisol to compensate for GC resistance. A role for changes in immunophilins, causing GC resistance in neotropical primates, is supported by enhanced protein levels of FKBP51 and reduced levels FKBP52 in these neotropical primates with GC resistance (Reynolds et al., 1999; Scammell et al., 2001).

Mice lacking FKBP52 display a high, but not total, rate of embryonic lethality, especially when backcrossed on the C57BL/6 background (Cheung-Flynn et al., 2005; Yang et al., 2006; Warriar et al., 2010). The exact cause for mortality in FKBP52 null mice has not been established but notably FKBP52 null mice do not appear to die from the atelectasis that is typical for GR knock out mice. However, surviving mice of both sexes grow into healthy adults except for reduced fertility due to defective penis development in males and sterility due to failure of uterus to support oocyte implantation in females.

FKBP52 does seem to have a role in GC control of metabolism (Warriar et al., 2010). *Fkb52*^{+/-} mice placed on a high-fat diet demonstrated a propensity to hyperglycemia and hyperinsulinemia. Livers of high-fat diet fed mutant mice were steatotic and showed elevated expression of lipogenic genes and pro-inflammatory markers. Interestingly, mutant mice on high-fat diet showed elevated serum corticosterone but their steatotic livers had reduced expression of gluconeogenic genes, whereas muscle and adipose tissue expressed normal to elevated levels of GC markers. These findings suggest a state of GC resistance mainly affecting hepatocytes.

To this date, no metabolic studies have been performed in mice lacking FKBP51. However, with respect to metabolism FKBP51 null mice would be expected to have elevated GR activity, presumably leading to increased susceptibility towards GC-induced glucose intolerance and diabetes. But, such a *a priori* assumption should be made with caution. GCs affect virtually every type of mammalian cell and therefore the overall effect of a global knock out is difficult to anticipate. Indeed, tissue-specific deletion of both FKBP51 and FKBP52 would be vital tools to dissect the role played by these GR-interacting proteins.

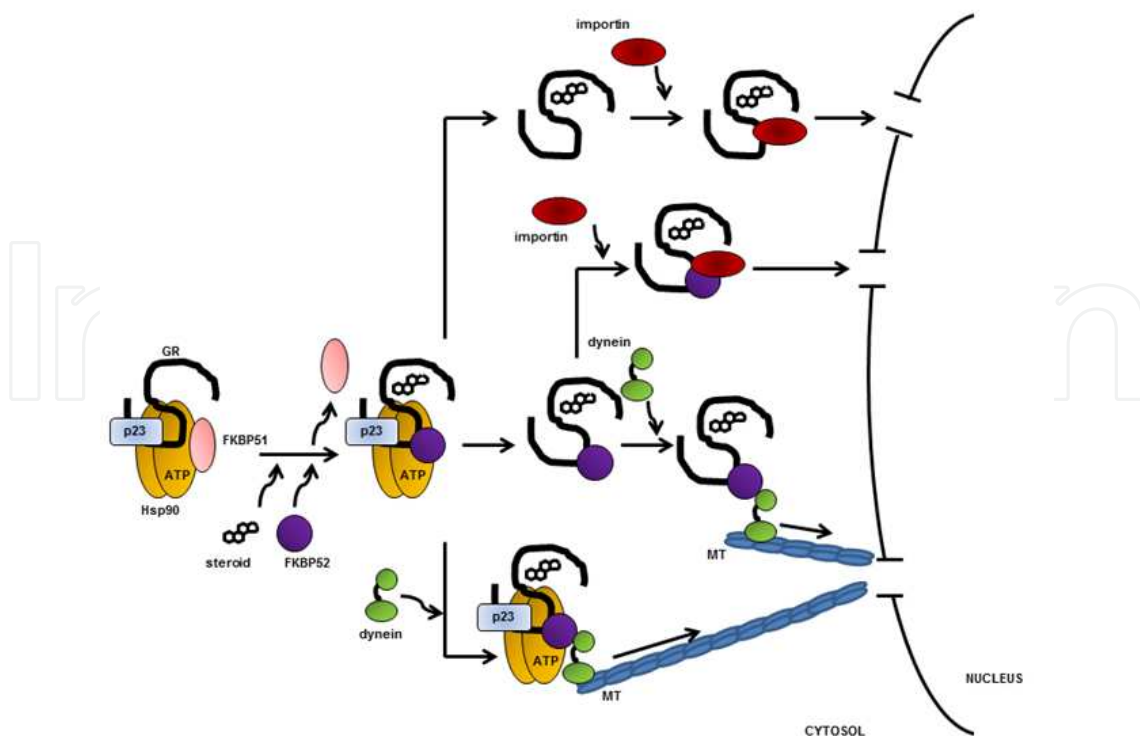


Figure 3. Cytosol to nucleus transportation of activated GR. Glucocorticoids exert their effects after binding to an intracellular receptor that, upon ligand binding, is translocated to the nucleus. Before ligand binding the GR is held in a complex with Hsp90, p23 and FKBP51. After ligand binding, FKBP51 is replaced by FKBP52 and Hsp90 and p23 detach or can be kept bound to the GR. FKBP52 stimulates interaction with dynein and translocation to the nucleus via the microtubuli (MT) system. However, some experimental evidence suggests that GR can be translocated to the nucleus independent on the MT system. Figure adopted from Grad and Picard 2007 (Grad & Picard, 2007).

2.3. GR phosphorylation

As depicted in Figure 2, the GR contains several phosphorylation sites within the C-terminal A/B-domain. The receptor is phosphorylated in the absence of hormone and additional phosphorylation events occur in conjunction with agonist, but not antagonist, binding (Almlof et al., 1995; Webster et al., 1997; Wang et al., 2002). The hormone-dependent increase in receptor phosphorylation has led to the hypothesis that phosphorylation may modulate GR transcriptional regulatory functions.

Consistent with this notion is the finding that GR is phosphorylated at three serine residues, S203, S211 and S226, which are particularly associated with activation of the GR (Wang et al., 2002). Serine to alanine mutations of S203 and S211, individually or in combination, decrease transcriptional activation in mammalian cells, indicating that phosphorylation of these residues are required for full GR activity (Almlof et al., 1995; Webster et al., 1997; Miller et al., 2005). In contrast, an alanine substitution for S226 increases GR transcriptional activity relative to the wild-type receptor, suggesting that phosphorylation of S226 is inhibitory to GR function (Rogatsky et al., 1998; Itoh et al., 2002). Thus, phosphorylation appears to provide both positive and negative regulatory inputs with respect to GR

transcriptional activation. In an analysis of the relative contribution of the different phosphorylation sites within the GR, it was found that GR-mediated transcriptional activation was greatest when the relative phosphorylation of S211 exceeded that of S226 (Krstic et al., 1997).

Two different Cdks have been identified as responsible for phosphorylation of S203 and S211; cyclin E/Cdk2 and cyclin A/Cdk2 phosphorylate S203 and, S203 and S211, respectively (Krstic et al., 1997). Mammalian cells lacking p27^{KIP1} demonstrate a concomitant rise in cyclin/Cdk2 activity and increased GR phosphorylation at S203 and S211, as well as enhanced receptor transcriptional activity, further strengthening the role for Cdks in GR phosphorylation and activity (Wang & Garabedian, 2003). In addition, it was recently shown that the phosphorylation site S211 is a substrate for p38 MAPK (Miller et al., 2005), an observation that provides a mechanistic link as to why inhibitors of p38 MAPK protect pancreatic β -cells against cytotoxic effects of DEX (Reich et al., 2012).

The c-Jun N-terminal kinase (JNK) is the kinase primarily responsible for phosphorylation of S226 (Rogatsky et al., 1998). Thus, inhibitors of JNK can be expected to have a negative impact on GR activity. JNK phosphorylation of GR has also been reported to increase receptor nuclear export under conditions of hormone withdrawal (Itoh et al., 2002). Thus, JNK phosphorylation inhibits receptor activity by at least two distinct mechanisms; in the presence of hormone GR phosphorylation by JNK affects receptor interaction with factors involved in transcriptional activation, whereas in the absence of hormone it enhances receptor nuclear export.

Perturbations in protein phosphatase activity have also been shown to affect GR function. Treatment of cells with okadaic acid, a general serine/threonine protein phosphatase inhibitor, results in receptor hyperphosphorylation, retaining of the receptor in the cytosol but also a transcriptional activation in mammalian cells (DeFranco et al., 1991; Somers & DeFranco, 1992). Endogenous phosphatase activity of the GR is catalyzed by serine/threonine protein phosphatase 5 (PP5). Like FKBP51 and FKBP52, the PP5 protein contains TPR domains (Chen et al., 1994), is a major component of the Hsp90-GR complex and has also been shown to be associated with ligand-free receptor in the nucleus (Silverstein et al., 1997).

In vitro experiments with A549 cells showed that suppression of PP5 expression through antisense oligonucleotides increased GR transcriptional activity both in the absence and presence of hormone (Zuo et al., 1999). Embryonic fibroblasts generated from a line of PP5 knock out mice were used to study the balance between lipolysis and lipogenesis. In these studies, embryonic fibroblasts from mice lacking PP5 demonstrated resistance to lipid accumulation in response to adipogenic stimuli, which was due to elevated GR phosphorylation and reduced peroxisome proliferator activated receptor (PPAR) γ activity on genes controlling lipid metabolism (Hinds et al., 2011). In line with these observation, PP5 null mice have reduced body weight (Amable et al., 2011). Interestingly, PP5 null mice have improved glucose tolerance when subjected to a glucose tolerance test, despite having normal insulin sensitivity, indicating enhanced insulin secretion capacity (Grankvist et al., 2012).

2.4. 11 β -hydroxysteroid dehydrogenase

In humans, circulating GCs exist in two forms. The plasma levels of the inactive form, cortisone, are around 50-100 nM and the hormone is largely unbound to plasma proteins (Walker et al., 1992). In contrast, approximately 95% of the active form, cortisol, is bound to corticosteroid-binding globulin. In the rat and mouse, the plasma concentration of 11-dehydrocorticosterone (DHC), which is the rodent equivalent to cortisone, is also around 50 nM (Kotelevtsev et al., 1997). As has been discussed above, tissue response to GCs is regulated both by the expression level of the GR, cochaperones interacting with the receptor and by the intracellular concentration of the active form of the hormone. But for GCs there is a possibility for an additional level of control that involves intracellular pre-receptor regulation of inactive and active forms of GCs. Conversion between the inactive and active forms of GCs is performed by the enzyme 11 β -hydroxysteroid dehydrogenase (11 β -HSD; EC 1.1.1.146). In rodents, 11 β -HSD type 1 (11 β -HSD1, Uniprot identifier for the human form P28845) works as a NADPH-dependent reductase converting inactive DHC to active corticosterone (Low et al., 1994; Voice et al., 1996; Davani et al., 2000). The type 2 (11 β -HSD2, Uniprot identifier for the human form P80365) isoform works as a NAD⁺-dependent dehydrogenase catalyzing the opposite reaction (Brown et al., 1993). 11 β -HSD2 expression is particularly abundant in kidney and placenta where the enzyme modulates intracellular GC levels, thus protecting the non-selective mineralocorticoid receptor from occupancy by GCs (Albiston et al., 1994). Thus, cellular activities of 11 β -HSD1 and 11 β -HSD2 function as pre-receptor regulators of GC action. The former enzyme is widely expressed, most notably in liver, lung, adipose tissue, vascular tissue, ovary and the CNS (Stewart & Krozowski, 1999). Sequence analysis of the cloned 11 β -HSD1 gene revealed a putative GC-responsive element in the promoter region (Tannin et al., 1991), suggesting that corticosterone or cortisol can regulate the transcription of 11 β -HSD1. Evidence for such a mechanism was obtained in human skeletal muscle biopsy, where cortisol induced elevated levels of 11 β -HSD1 mRNA (Whorwood et al., 2001). Also in rat and human hepatocytes, it was demonstrated that carbenoxolone (CBX), an inhibitor of both type 1 and type 2 11 β -HSD, reduced 11 β -HSD1 reductase activity (Ricketts et al., 1998).

It has been proposed that local variations in tissue cortisol levels can occur in the absence of any discernible changes in circulating cortisol (Walker & Andrew, 2006). Although cortisol plasma levels are slightly elevated in patients with the metabolic syndrome or in obese subjects of cortisol (Phillips et al., 1998; Duclos et al., 2005; Misra et al., 2008; Sen et al., 2008; Weigensberg et al., 2008) they are within the normal range (Walker, 2006). This would imply that tissue-specific expression levels 11 β -HSD1 and 11 β -HSD2 are determinants of the local cellular concentration of active steroid that can influence the metabolic effects of GC. In agreement with this concept, in a study of 101 obese patients (BMI 34.4 ± 4.3 kg/m²) of both sexes, impaired glucose tolerance and IR was associated with increased adipose 11 β -HSD1 expression (Tomlinson et al., 2008). Furthermore, transgenic mice over-expressing 11 β -HSD1 selectively in adipose tissue faithfully recapitulate the phenotype of the metabolic syndrome (Masuzaki et al., 2001; Masuzaki et al., 2003). These mice had increased adipose levels of corticosterone and developed visceral obesity that was

exaggerated by a high-fat diet. The transgenic mice also exhibited profound insulin-resistant diabetes and hyperlipidemia. As these studies suggest that local GC excess perturbs glucose homeostasis via 11β -HSD1, attempts have been made to pharmacologically inhibit 11β -HSD1 (Tomlinson & Stewart, 2007). In this respect, carbenoxolone, an inhibitor of both 11β -HSD1 and 11β -HSD2, increases hepatic insulin sensitivity in man (Walker et al., 1995). Selective inhibition of 11β -HSD1 decreases glycemia and improves hepatic insulin sensitivity in hyperglycemic mouse strains (Alberts et al., 2002; Alberts et al., 2003). Clinical studies in humans with selective 11β -HSD1 inhibitors are ongoing, for a review see reference (Pereira et al., 2012).

Finally, 11β -HSD1 mRNA and enzyme activity has been detected in both human and mouse islets (Davani et al., 2000), indicating that 11β -HSD1 might regulate GC action in the endocrine pancreas. Indeed, islets treated with DHC had a suppressed glucose-stimulated insulin secretion (GSIS) (Davani et al., 2000) and this aspect will be further discussed in the coming chapter.

3. Diabetogenic effects of glucocorticoid excess

Hyperglycemia and diabetes mellitus are important causes of mortality and morbidity worldwide. The number of people with impaired glucose tolerance or type 2 diabetes (T2DM) is rising in all regions of the world. A systemic analysis of health examination surveys and epidemiological studies showed that between 1980 and 2008 there were nearly 194 million new cases of diabetes (Danaei et al., 2011). Of these, 70% could be attributed to population growth and ageing but the cause for the remaining 30% must be found among environmental changes that support the increased disease prevalence. Indeed, lifestyle changes including a higher caloric intake and decreased energy expenditure play a large part to explain increased prevalence of T2DM. However, as will be discussed in this chapter, the impact of GCs shall not be neglected.

GC-induced diabetes is a special form of glucose intolerance that can occur when endogenous GC activity is enhanced or during treatment with GC-based drugs (Raul Ariza-Andraca et al., 1998; Vegiopoulos & Herzig, 2007; van Raalte et al., 2009). Perhaps the most clear cut case is endogenous over production of GCs by adrenal cortex as it occurs in Cushing's syndrome. In 80-85 % of the cases the syndrome is caused by a pituitary tumour (referred as Cushing's disease) and is ACTH-dependent. Symptoms include rapid weight gain, particularly of the trunk and face with sparing of the limbs (central obesity). Other signs include persistent hypertension (due to activation of the mineralocorticoid receptor leading to increased sodium retention and expanded plasma volume) and IR (due to insulin signaling defects), which in turn may lead to hyperglycemia. In patients with hypercortisolism due to Cushing's syndrome, the incidence of T2DM is 30-40% (Biering et al., 2000). The similar phenotypes in patients with Cushing's syndrome and in patients with the metabolic syndrome has led to the hypothesis that cortisol can play a pathological role in the metabolic syndrome (Anagnostis et al., 2009).

Subclinical Cushing's syndrome is also observed and it is defined as alterations of the HPA axis that result in elevated circulating cortisol levels without those gross adverse metabolic effects of GC excess as mentioned above. In a study including patients of both sexes (aged 18-87 years), diagnosed with adrenal incidentaloma via imaging techniques, participants were classified according to levels of cortisolemia after administration of 1 mg DEX (Di Dalmazi et al., 2012). DEX is a synthetic GC analogue that will suppress endogenous cortisol production via the existing negative feedback loop. Patients with cortisol levels above 138 nM on the morning after administration of 1 mg DEX were classified as subclinical Cushing's syndrome that can be compared with those diagnosed with non-secreting adenoma, whose cortisol levels were below 50 nM. Among these patients, T2DM was over represented as were coronary heart disease and osteoporosis.

Yet, a third example of GC excess, and a much more common one, is during pharmacological treatment. Low-dose GC is considered when the daily dose is less than 7.5 mg prednisolone or equivalent (van der Goes et al., 2010). When such a dose is administered orally, plasma prednisolone levels peaks 2-4 hours after intake at about 400-500 nM (~150-200 ng/ml) and returns to baseline within 12 hours after steroid administration (Wilson et al., 1977; Tauber et al., 1984). These values are in the same range as normal endogenous cortisol values, reference values for samples taken between 4:00 am and 8:00 am are 250-750 nM and for samples taken between 8:00 pm and 12:00 pm are 50-300 nM. This indicates that the absolute cortisol values are not as important for developing adverse effects during low-dose GC therapy as is the diurnal variation. Current knowledge gives at hand that developing diabetes after starting low-dose GC treatment seems rare but progression of already impaired glucose tolerance to overt diabetes is possible (van der Goes et al., 2010). Therefore, clinical recommendation states that baseline fasting glucose should be monitored before initiating therapy and during following up according to standard patient care.

Certainly, the adverse effects are more pronounced during high-dose GC therapies (>30 mg prednisolone or equivalent daily). In a retrospective study of hemoglobin A1c (HbA1c) levels in patients with rheumatic diseases subjected to prednisolone treatment, it was found that around 82% had HbA1c levels higher than 48 mmol/mol (given in IFCC standard, corresponding to 6.7% in DCCT standard). Serum HbA1c levels higher than 52 mmol/mol (7.1%), were seen in 46% of the patients and 23% of the patients had HbA1c levels as high as 57 mmol/mol (7.6%) which should be considered as a high risk factor for diabetes. Taken together, it was found that the cumulative prednisolone dose was the only factor significantly associated with the development of steroid induced diabetes among rheumatic patients (Origuchi et al., 2011).

Inhaled GCs are the mainstay of therapy in asthma, but their use raises certain safety concerns. In a study of 21,645 elderly subjects using inhaled beclomethasone, an increased risk of developing diabetes was found (Dendukuri et al., 2002). However, when adjusting for the simultaneous use of oral GCs no evidence was found for an increased risk of diabetes among users of inhaled GCs. In contrast, a more recent study of 388,584 patients treated for respiratory disease identified that inhaled GC use is associated with modest increases in the

risks of diabetes onset and diabetes progression (Suisa et al., 2010). The risks are more pronounced at the higher doses (equivalent to 1.0 g or more per day of fluticasone) currently prescribed for the treatment of chronic obstructive pulmonary disease. Therefore, diabetes should be considered as a risk factor during treatment with inhaled GCs and especially in those cases when higher doses are used or when GCs are taken orally at the same time.

Diabetogenic effects of GCs include the induction or aggravation of preexisting IR in peripheral tissues (Grill et al., 1990; Larsson & Ahren, 1999; Nicod et al., 2003; Besse et al., 2005). The molecular base for IR was studied in rodent models or in primary cells subjected to GC treatment (Olefsky et al., 1975; Caro & Amatruda, 1982; Saad et al., 1993; Ishizuka et al., 1997; Sakoda et al., 2000; Burén et al., 2002; Ruzzin et al., 2005; Burén et al., 2008). DEX-treated rats (1.5 mg/kg *b.w.* for 6 consecutive days) exhibit around 50-70% and 40-50% reduction of insulin binding to its receptors in hepatocytes and adipocytes, respectively (Olefsky et al., 1975). Significant reduction in insulin receptor density was also observed in hepatocytes from rats chronically treated with DEX (1.0 mg/kg *b.w.*) (Caro & Amatruda, 1982). Previous studies demonstrated that especially post-receptor events are involved on the reduction of peripheral insulin action after GC treatment *in vivo*. Diminished tyrosine phosphorylation in either insulin receptor and insulin receptor substrate (IRS)-1 was observed in liver from rats treated with DEX for 5 consecutive days (1.0 mg/kg *b.w.*) (Saad et al., 1993). Decreased insulin-stimulated association of IRS-1/phosphatidylinositol 3-kinase (PI3K) in skeletal muscle tissue was also observed. These *in vivo* data are in accordance with *in vitro* findings. Adipocytes and myocytes cultured in the presence of DEX show reduction of insulin-stimulated glucose uptake, which is associated with impairment of post-insulin receptor signaling transduction and/or reduction of glucose transporter protein content (Ishizuka et al., 1997; Sakoda et al., 2000; Burén et al., 2002). Rats treated with DEX for 11 consecutive days (1.0 mg/kg *b.w.*) have around 40% and 70% reduction in insulin-induced glucose uptake in adipose and muscle tissues, respectively (Burén et al., 2008). The authors also observed increased lipolysis in response to 8-bromo-AMP and reduced antilipolytic insulin effects. These alterations are associated with diminished total protein kinase B (PKB) content and insulin-stimulated PKB serine/threonine phosphorylation in muscle and white adipose tissue (Burén et al., 2008). It is interesting to note that DEX-induced IR may occur through GR independent mechanisms. It was demonstrated that DEX induces reduction in insulin action in adipocytes even in the presence of the GR antagonist RU38486 or even in the presence of a protein synthesis inhibitor (cycloheximide) (Ishizuka et al., 1997; Kawai et al., 2002). It was also demonstrated that inhibition of protein kinase C (PKC) isoform β improves glucose uptake into adipocytes cultured in the presence of DEX (Kawai et al., 2002). Subsequent studies have demonstrated this GR- and/or transcription factor-independent mechanisms of GC action on insulin signaling, which leads to impairment of insulin action (Löwenberg et al., 2006).

Finally, when considering situations of GC excess, it is important to keep in mind that cortisol is a stress hormone with a diurnal secretion pattern that peaks at the time of waking. Various stressful situations, including low socioeconomic status, chronic work stress (Eller et al., 2006; Maier et al., 2006), anxiety and depression (Chrousos, 2000; Kinder et al., 2004) may stimulate neuroendocrine responses. These conditions are all associated with disturbed

sleeping patterns that often result in interrupted sleeping sessions and hence, wakening. In this latter condition, not only increased circulating cortisol levels are found, but also enhanced sympathetic nervous drive. Sleep deprivation alters hormonal glucose regulation and is especially affecting pancreatic insulin secretion (Schmid et al., 2007). Activation of the HPA axis works together with increased sympathetic nervous tone to mediate the effects of stress on various organ systems and may disturb glucose homeostasis (Buren & Eriksson, 2005).

4. Conclusions

Prolonged therapies based on moderate or high GC doses are clearly diabetogenic for healthy individuals. In susceptible subjects (obese, low-insulin responders, first-degree relatives of patients with T2DM, pregnant, etc) even low doses GC treatment may disrupt glucose homeostasis. These adverse effects of the CGs vary according to the specific tissue responses to the hormone. Tissue sensitivity towards GCs is regulated at several points from 11 β -HSD1 activity to GR phosphorylation. The impact of various cochaperones that regulate GR function on the effects of GCs is an open field for coming research. Tissue specific knock-out models for the different GR interacting proteins would provide valuable tools to elucidate the roles played by these proteins in various tissues. Development of novel drugs with desirable GC activity (gene transrepression) without undesirable side effects (gene transactivation) are in progress and hold promise as good pharmacological options for GC-based therapies (Stahn et al., 2007).

Author details

Henrik Ortsäter and Åke Sjöholm

Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Sweden

Alex Rafacho

Department of Physiological Sciences, Centre of Biological Sciences, Universidade Federal de Santa Catarina, Brazil

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