Neuroscience 242 (2013) 97–109

NEUROSCIENCE FOREFRONT REVIEW

UNDERSTANDING STRESS-EFFECTS IN THE BRAIN VIA TRANSCRIPTIONAL SIGNAL TRANSDUCTION PATHWAYS

I. ZALACHORAS, [a]* **R. HOUTMAN** [b] **AND O. C. MEIJER** [a]

[a] *Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands*

[b] *Pamgene International, Den Bosch, The Netherlands*

Abstract—Glucocorticoid hormones exert crucial effects on the brain in relation to physiology, endocrine regulation, mood and cognition. Their two receptor types, glucocorticoid and mineralocorticoid receptors (GR and MR), are members of the nuclear receptor superfamily and act in large measure as transcription factors. The outcome of MR/GR action on the genome depends on interaction with members from different protein families, which are of crucial importance for cross-talk with other neuronal and hormonal signals that impinge on the glucocorticoid sensitive circuitry. Relevant interacting proteins include other transcription factors that may either tether the receptor to the DNA, or that bind in the vicinity of GR and MR to tune the transcriptional response. In addition, transcriptional coregulator proteins constitute the actual signal transduction pathway to the transcription machinery. We review the current evidence for involvement of individual coregulators in GR-dependent effects on stress responses, and learning and memory. We discuss the use of *in vitro* and in silico tools to predict those coregulators that are of importance for particular brain processes. Finally, we discuss the potential of selective receptor modulators that may only allow a subset of all interactions, thus allowing more selective targeting of glucocorticoid-dependent processes in the brain.

Key words: brain, stress, coregulators, steroid hormone receptors, p160, CBP/p-300.

Contents

INTRODUCTION

Adrenal glucocorticoid hormones produce profound and diverse effects in the brain in relation to cognition and behavior, as well as in the periphery in a plethora of processes such as immune responses, metabolism and development (Sapolsky et al., 2000; de Kloet et al., 2005). Their effects are mediated by two different receptor types: the very high-affinity mineralocorticoid receptor (MR), and the somewhat lower affinity glucocorticoid receptor (GR). Together these receptors coordinate normal circadian activity and – by virtue of the reactivity of the hypothalamus pituitary adrenal (HPA) axis that drives secretion of the endogenous glucocorticoids – the response and adaptation to stress (Conway-Campbell et al., 2012).

Glucocorticoid hormones modulate the initial response to stressors *a.o.* by affecting appraisal of stimuli and biasing response strategies. Because of its high affinity for cortisol and corticosterone MR is occupied to a substantial degree even under basal conditions, and it plays an important role in initial behavioral responsiveness (Oitzl and de Kloet, 1992). Relevant brain regions in rodents include the hippocampus, prefrontal cortex and certain nuclei of the amygdala (de Kloet et al., 2005).

*Corresponding author. Address: Leiden University Medical Centre, Department of Endocrinology, Albinusdreef 2, Building 1, C4-82, 2333 Leiden, The Netherlands. Tel: +31-0-71-5261833l; fax: +31-0-71-5248136.

E-mail address: I.Zalachoras@lumc.nl (I. Zalachoras).

Abbreviations: AF-1, activation function-1; AF-2, activation function 2; cKO, conditional knockout; CRTCs, CREB-regulated transcriptional coregulators; DBD, DNA binding domain; ER, estrogen receptor; GR, glucocorticoid receptor; GREs, glucocorticoid response elements; HATs, histone acetyltransferases; HPA, hypothalamus pituitary adrenal; ILPFC, infralimbic prefrontal cortex; LBD, ligand binding domain; MR, mineralocorticoid receptor; PCAF, p300/CBP-associated factor; PR, progesterone receptor; RIP 140, receptor interacting protein 140; SGRMs, selective glucocorticoid receptor modulators; SRC, steroid receptor coactivator.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.03.038

Recovery from and adaptation to stressors takes place *a.o.* via glucocorticoid-dependent plasticity in circuits involved in endocrine regulation as well as learning and memory (de Kloet et al., 2005) and response selection. Because of its lower affinity, the GR gets substantially occupied at hormone levels reached at the circadian peak (Reddy et al., 2012) and after stress. Accordingly, it is the receptor most prominently involved in the transcriptional consequences of stress-induced elevation of glucocorticoid levels. GR shows a widespread distribution in the brain and affects, for example, limbic and hypothalamic structures, as well as the classical diffuse modulatory (e.g. serotonergic and dopaminergic) neurotransmitter systems (Roozendaal et al., 2008; Rodrigues et al., 2009). Importantly, in order to affect neuronal plasticity, GR has to modulate the activity of other signaling pathways, and this likely occurs through interaction with other transcription factors such as CREB, which are activated via neuronal signaling pathways (Roozendaal et al., 2010). GR and MR expression in the brain may be sex and age dependent, while disturbances in GR and MR levels have been correlated with psychiatric disorders (Qi et al., 2012; Wang et al., 2013).

In this review we will focus on the genomic effects mediated by MR and GR, and link these to other proteins that are directly involved in transcriptional changes associated with neuronal plasticity. Of note, glucocorticoids can also have fast non-genomic effects through several downstream pathways (Karst et al., 2005; Gutièrrez-Mecinas et al., 2011) that seem to be mediated by MR and GR. These have been reviewed elsewhere (Groeneweg et al., 2011, 2012). These fast effects can interact with classical slow-onset genomic effects (Karst et al., 2010), and it will be of great interest to see how many of the well-known effects of glucocorticoids on the brain and behavior depend (in part) on non-genomic mechanisms.

MOLECULAR STRUCTURE AND FUNCTION OF MR AND GR

Nuclear receptors consist of functional domains that can be directly coupled to their function as transcription factors. The central DNA binding domain (DBD) is necessary for the specific binding associated with particular target genes. The DBD of both MR and GR recognizes 15 nucleotide sequences known as glucocorticoid response elements (GREs). The DBD is flanked by the N-terminus that contains the transcriptional output 'activation function-1' (AF-1), and a carboxy-terminal ligand binding domain (LBD), which also contains 'activation function 2' (AF-2) (Nicolaides et al., 2010; Veleiro et al., 2010).

In the absence of ligand, MR and GR are bound to chaperone protein complexes in the cytoplasm. Upon ligand binding, a conformational change takes place that leads to the dimerization of the nuclear receptor and its translocation to the nucleus. There, with the assistance of coregulators the nuclear receptor can bind to GREs on the DNA and activate or repress the expression of specific genes. The receptors are thought to mainly form homodimers, act as monomers in conjunction with other, non-receptor, transcription factors, or heterodimerize with other steroid receptors (Pearce, 1994; Chen et al., 1997). Activity of receptors depends of course on the type and local concentration of the ligand (Awasthi and Simons, 2012; Yang and Fuller, 2012) and on the pattern of ligand exposure in time (Walker et al., 2012). However, additional regulation can take place at multiple levels. These may include the expression levels of the receptor (Noguchi et al., 2010), its posttranslational modifications (Nicolaides et al., 2010), its interactions with molecular chaperones in the cytoplasm (Touma et al., 2011), dimerization and translocation to the nucleus (Fitzsimons et al., 2008), DNA binding and its interactions with proteins involved in transcription, either transcription factors or coregulator proteins (de Kloet et al., 2009).

Transcription factors that bind to regulatory DNA in conjunction with GR (and to a much lesser extent MR) are being discovered at a substantial rate by genome-wide localization of receptor binding using ChIP-sequencing, and subsequent statistical analysis of DNA motifs that overlap with or surround the receptor binding sites. Some of the identified transcription factors will bring the receptors to the DNA by way of 'tethering' mechanisms, like those involved in classic transrepression in the immune system (De Bosscher et al., 2008). There are also those transcription factors that bind in the vicinity (within hundreds of base pairs) of the steroid receptors, and are in some way involved in modulating their function. In generic cell lines, AP-1 has been shown to act as a 'pioneer' and make the DNA accessible for GR binding through chromatin modification (Biddie Simon et al., 2011). The exact nucleotide content of the GRE is associated with GR's dependence on such priming mechanisms. It is also conceivable, or even likely, that factors that bind in the vicinity of MR and GR interact functionally in larger complexes on the DNA, analogous to what happens at composite GREs where GR binds directly adjacent to other transcription factors (Webster and Cidlowski, 1999). In the rat hippocampus, it has been shown that GC-rich motifs for transcription factors MAZ1 and SP1 occur in conjunction with GR binding to the DNA, suggesting either a pioneering function, or a functional interaction with these factors (Datson et al., 2011). Recently, the first ChIP sequencing data for GR were published for neuronally differentiated PC12 cells. Interestingly, GR binding occurred in the vicinity of AP-1 sites, as expected, but the authors also described recognition sites for a number of completely new transcription factors in the vicinity of GR binding. These data suggest that the effects of GR (and MR) are modified by other signaling pathways that we are just beginning to discover (Polman et al., 2012).

The other class of nuclear proteins that interact with MR and GR are the nuclear receptor coregulators on which we will focus in the rest of this review.