Rapid non-genomic effects of corticosteroids and their role in the central stress response

Femke L Groeneweg, Henk Karst, E Ron de Kloet and Marian Joëls

Abstract

In response to a stressful encounter, the brain activates a comprehensive stress system that engages the organism in an adaptive response to the threatening situation. This stress system acts on multiple peripheral tissues and feeds back to the brain; one of its key players is the family of corticosteroid hormones. Corticosteroids affect brain functioning through both delayed, genomic and rapid, non-genomic mechanisms. The latter mode of action has long been known, but it is only in recent years that the physiological basis in the brain is beginning to be unravelled. We now know that corticosteroids exert rapid, non-genomic effects on the excitability and activation of neurons in (amongst others) the hypothalamus, hippocampus, amygdala and prefrontal cortex. In addition, corticosteroids affect cognition, adaptive behaviour and neuroendocrine output within minutes. Knowledge on the identity of the receptors and secondary pathways mediating the non-genomic effects of corticosteroids on a cellular level is accumulating. Interestingly, in many cases, an essential role for the ‘classical’ mineralocorticoid and glucocorticoid receptors in a novel membrane-associated mechanism is found. Here, we systematically review the recent literature on non-genomic actions of corticosteroids on neuronal activity and functioning in selected limbic brain targets. Further, we discuss the relevance of these permissive effects for cognition and neuroendocrine control, and the integration of this novel mode of action into the complex balanced pattern of stress effects in the brain.

Introduction

Stress has many faces. On the one hand, it is a highly adaptive response to disturbances in homeostasis. Decades of research have identified a complex, tightly balanced system in the brain and periphery that translates the effects of stress on specific tissues. On the other hand, stress is a potential risk factor for a large number of diseases, ranging from peripheral illnesses such as obesity and heart and vascular problems to many psychiatric disorders including major depression, schizophrenia, drug addiction and posttraumatic stress disorder (de Kloet et al. 2005, McEwen 2008, Yehuda 2009). Diseases can occur when the balance between the multiple players, phases and responsive tissues in the stress system is upset, so that the adaptive stress response converts into a maladapted, detrimental chain of events (de Kloet et al. 1999, McEwen 2001). Individual variations in this balance, due to genetic or environmental factors, determine whether an individual is resistant or sensitive to stress-related disorders (Kaffman & Meaney 2007, Oitzl et al. 2010). The key to understanding what causes the balance to shift away from adaptive towards detrimental effects of stress is a comprehensive understanding of the different players and phases involved in the stress response and their interactions with each other.

Corticosteroids play a major role in the response of the brain to stress. For many years, they were believed to be only responsible for the delayed and prolonged effects of stress, as opposed to monoamines and neuropeptides which were thought to establish rapid effects (de Kloet et al. 2005). While this is generally true, the picture is actually more complex. For instance, corticosteroids influence a wide range of behaviours and endocrine outputs within minutes, a time frame that is too rapid to be explained by genomic effects (de Kloet et al. 1999, Haller et al. 2008). In agreement, we and others recently established that corticosteroids rapidly alter neuronal activity and excitability in a number of brain areas, providing a physiological basis for the rapid effects on behaviour (Tasker et al. 2006, de Kloet et al. 2008). The existence of such a rapid mode of action raises many new questions. Where in the brain do these rapid effects take place? Which receptors and
pathways are involved in these effects? What are the functional consequences for cognition and neuroendocrine control? And, importantly, how are these rapid corticosteroid actions integrated with other components of the stress response? In this review, we discuss our current understanding of rapid actions of corticosterone in the brain, with emphasis on their functional significance for behaviour, cognition and neuroendocrine outputs.

**Setting the stage: the delicate balance of the stress response**

The release of corticosteroids is regulated by the hypothalamic–pituitary–adrenocortical (HPA) axis (see Fig. 1). Perception of a potentially threatening situation, be it physiological or psychological in nature, activates the paraventricular nucleus (PVN) of the hypothalamus. In the PVN, corticotrophin-releasing hormone (CRH) and vasopressin-containing neurons are activated and stimulate the pituitary to release ACTH, in turn this hormone induces release of corticosteroids from the adrenal glands. In humans, the main circulating corticosteroid is cortisol, while in rodents, only corticosterone prevails. Corticosteroids are released in hourly pulses that are highest in amplitude during the active period, thus causing an overall circadian release pattern (Young et al. 2004). This background of pulsatility is essential to keep the tissues responsive, e.g. to stress-induced peaks in corticosteroid release (Lightman & Conway-Campbell 2010).

Corticosteroids readily enter the brain and bind to two types of receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR; Reul & de Kloet 1985). These receptors are ligand–driven transcription factors. In unbound form, they reside in the cytoplasm; binding of corticosteroids triggers translocation of the receptor complex to the nucleus. Here, the activated receptor changes gene transcription directly by binding to recognition sites in the DNA or indirectly via interactions with other transcription factors (Beato & Sanchez-Pacheco 1996). Activation of the receptors results in waves of gene regulation, with both transactivation and transrepression of responsive genes (Datson et al. 2008).

Although the MR and GR share almost identical DNA-binding domains, they do not bind to the same sets of genes. For example, the set of genes that are over- or under-expressed after MR versus GR activation show only limited (<30%) overlap (Datson et al. 2001). Therefore, both receptors can exert distinct cellular actions. In addition, the two receptors show different localization patterns in the brain. The expression of the MR is mostly restricted to neurons in limbic areas, with moderate levels in the (prefrontal) cortex and the amygdala and high levels in the hippocampus (Reul & de Kloet 1985). The GR, on the other hand, is expressed ubiquitously throughout the brain, both in glia cells and neurons, with highest levels in the PVN and in the hippocampus (Reul & de Kloet 1985). Importantly, the MR has a sufficiently high affinity for corticosterone to remain activated even during the intervals between ultradian pulses and in the absence of stress (Reul & de Kloet 1985). This receptor has an established function in maintaining the integrity and stability of limbic circuits and plays a proactive role in maintaining homeostasis (Joëls et al. 2008). Conversely, the GR has a 10-fold lower affinity for corticosterone and becomes activated only after exposure to stress or during the circadian and ultradian peaks. The GR plays a reactive role in the stress response: it facilitates recovery of brain activity, distributes energy where it is needed and helps corticosterone levels to return to baseline through inhibition of the HPA-axis (de Kloet & Reul 1987). In cognition, the MR is involved in appraisal of novel situations, while activation of the GR facilitates the consolidation of stress-related information (de Kloet et al. 1999).

In addition to corticosteroids, many more hormones and transmitters are released after stress and affect brain function. For instance, stress leads to high levels of CRH, vasopressin and oxytocin in the hypothalamus. In the amygdala, CRH, adrenaline and noradrenaline are essential for translating stress-related signals into changes in amygdala-related functions. These systems do not act independently. For example, adrenergic activation in the amygdala is required for the enhancement of memory consolidation by GR activation (Roozendaal et al. 2006). In general, adrenergic actions seem to take place downstream from those of corticosteroids.
(Roozendaal et al. 2002). All of these hormones and transmitters together create the versatility of the stress response in the brain, a balance between activating and inhibiting systems in a multitude of brain areas that are themselves densely interconnected. It is in this fine-tuned balance that we must now also place the non-genomic actions of corticosterone.

Rapid effects of corticosterone in the brain

The rapid effects of corticosterone on brain and cognition have been the subject of several recent reviews (Dallman 2005, Tasker et al. 2006, Haller et al. 2008, de Kloet et al. 2008, Prager & Johnson 2009, Evanson et al. 2010a). However, over the last 2 years, a number of new studies have emerged that extend and challenge the existing views on the function and nature of these rapid effects. Here, we focus on the integration of these new findings with the existing theories on rapid corticosteroid signalling. The findings are discussed per brain area, i.e. the hypothalamus, pituitary, hippocampus, amygdala and frontal cortical areas. In the following sections, the major findings in these four different brain areas are summarized (see for overview Table 1).

Hypothalamus

The hypothalamic PVN is one of the core structures in the HPA-axis. PVN neurons express high levels of GR, but virtually no MR. Indeed, through GR activation in the PVN, corticosterone negatively feeds back on the HPA-axis in a delayed, genomic fashion (de Kloet et al. 1998). However, corticosterone also regulates HPA-axis activity in a more rapid time frame, through non-genomic actions (Jones et al. 1976, Dallman 2005). Importantly, a recent study showed that this rapid inhibition can be induced by local infusion of dexamethasone or a membrane-impermeable conjugate of dexamethasone with BSA (dex-BSA) into the PVN (Evanston et al. 2010b). This effect can be prevented by co-administration of an antagonist of the cannabinoid receptor type 1 (CB1; Evanson et al. 2010b). Thus, at the level of the PVN, corticosterone rapidly reduces HPA-axis activation in a non-genomic, membrane-associated manner, involving endocannabinoid signalling.

Insight in the neurobiological substrate of these fast effects was provided by Tasker et al. This group was the first to carry out detailed studies on the frequency of miniature excitatory postsynaptic currents (mEPSCs) in the PVN and the nearby supraoptic nucleus (SON; Di et al. 2003). An mEPSC reflects the postsynaptic current resulting from the spontaneous release of a single glutamatergic vesicle from a presynaptic terminal (Bekkers & Stevens 1989). Importantly, the frequency of these events (particularly in the absence of changes in mEPSC amplitude) is considered to be determined by presynaptic properties, reflecting changes in either release probability of the vesicles or changes in the number of synaptic contacts. Tasker et al. established that a high dose of corticosterone (between 100 nM and 1 μM) or its synthetic analogue dexamethasone reduces the frequency of mEPSCs in PVN neurons (Di et al. 2003, Mälcher-Lopes et al. 2006). This effect was detectable within 5 min and did not reverse when corticosterone was washed out. Effectively, the excitability of PVN neurons was reduced by application of corticosteroids in a rapid but prolonged manner. Rapid changes in mEPSC frequency induced by corticosterone could not be blocked by MR or GR antagonists (Di et al. 2003, 2009). Evidence was presented that these effects are non-genomic, membrane-initiated and involve G-protein-coupled signalling. Interestingly, rapid corticosteroid actions required retrograde endocannabinoid signalling and the CB1 receptor. The presumed cellular signalling pathway is visualized in Fig. 2A. Since the CB1 receptor is also required for rapid inhibition of the HPA-axis (Evanston et al. 2010b), the rapid inhibition of mEPSC frequency (and thus excitability) of PVN neurons could provide the cellular substrate for this phenomenon.

However, rapid inhibitory effects of corticosterone in the PVN are not restricted to vasopressin- and CRH-containing parvocellular neurons, but they are seen in all neuronal populations (parvocellular and magnocellular) in the PVN (Di et al. 2003, 2005, Tasker 2006). In the magnocellular neurons in the PVN and SON, a second effect was observed on the spontaneous release of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter. The frequency of mIPSCs (miniature inhibitory postsynaptic currents) was rapidly increased by dexamethasone, but this required even higher concentrations (1 μM or more; Di et al. 2005, 2009). Functionally, this suggests a more general role for the non-genomic effects of corticosterone in the hypothalamus, a coordinative role in rapidly reducing hypothalamic outputs that might otherwise interfere with the stress response (Tasker 2006).

Pituitary

Fast and delayed effects of corticosteroids have also been observed at the level of the anterior pituitary gland, where GR is abundantly expressed and MR levels are quite low (Reul et al. 1990). Already in the 1970’s and 1980’s, both rapid and delayed actions of corticosteroids on pituitary ACTH release were reported (Jones et al. 1972, Widmaier & Dallman 1984). Inhibition of ACTH release was seen as early as 1 min and as late as 2 h after corticosteroid administration. The latter is a genomic action mediated by GR-driven gene transcription, while the former action was insensitive to protein synthesis inhibitors and thus mediated by non-genomic pathways (Keller-Wood & Dallman 1984). Interestingly, the rapid inhibition of ACTH release was only seen when corticosterone levels were rapidly rising and not when they were already high, suggesting that this feedback is ratesensitive (Jones et al. 1972, Kaneko & Hiroshige 1978).

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Table 1 Rapid effects of corticosterone on neuronal functioning in the hypothalamus, hippocampus, amygdala and prefrontal cortex

<table>
<thead>
<tr>
<th>Effect</th>
<th>Receptor</th>
<th>Conc</th>
<th>Area</th>
<th>Delay in onset</th>
<th>Preparation</th>
<th>Signalling pathways</th>
<th>Remarks</th>
<th>References</th>
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<tbody>
<tr>
<td>Hypothalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mEPSC freq ↓</td>
<td>Other</td>
<td>100 nM</td>
<td>PVN and SON</td>
<td>5 min</td>
<td>Rat, ex vivo</td>
<td>Gzs, cAMP-PKA, ECB, CB1</td>
<td>(1), (3), NR</td>
<td>[1–4]</td>
</tr>
<tr>
<td>mIPSC freq ↑</td>
<td>Other</td>
<td>1 μM</td>
<td>PVN and SON</td>
<td>5 min</td>
<td>Rat, ex vivo</td>
<td>ECB, CB1</td>
<td>(1), (3)</td>
<td>[2,4]</td>
</tr>
<tr>
<td>eIPSC amplitude ↑</td>
<td>Unknown</td>
<td>1 μM</td>
<td>SON</td>
<td>7 min</td>
<td>Rat, ex vivo</td>
<td>Gβγ, NO release</td>
<td>(3)</td>
<td>[4]</td>
</tr>
<tr>
<td>2-AG and AEA levels ↑</td>
<td>Unknown</td>
<td>1 μM</td>
<td>Hypothalamus</td>
<td>10 min</td>
<td>Rat, ex vivo</td>
<td>PKA</td>
<td>(3, 4)</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mEPSC freq ↑</td>
<td>mMR</td>
<td>10 nM</td>
<td>CA1 &amp; DG</td>
<td>5 min</td>
<td>Mouse, ex vivo</td>
<td>ERK1/2</td>
<td>(1), (2), (3), (4), R</td>
<td>[5–8]</td>
</tr>
<tr>
<td>Ia current ↓</td>
<td>MR</td>
<td>100 nM</td>
<td>CA1</td>
<td>5 min</td>
<td>Mouse, ex vivo</td>
<td>G-proteins</td>
<td>(3), R</td>
<td>[6]</td>
</tr>
<tr>
<td>AMPAR mobility ↑</td>
<td>mMR</td>
<td>50 nM</td>
<td>CA1</td>
<td>2 min</td>
<td>Rat, embryonic culture</td>
<td>(1), (3)</td>
<td>[9]</td>
<td></td>
</tr>
<tr>
<td>mIPSC freq ↑</td>
<td>MR</td>
<td>30 nM</td>
<td>Ventral CA1</td>
<td>Not known</td>
<td>Rat, ex vivo</td>
<td>Mouse, embryonic culture</td>
<td>(3)</td>
<td>[10]</td>
</tr>
<tr>
<td>MR at membrane</td>
<td>mMR</td>
<td>–</td>
<td>Hippocampus</td>
<td>–</td>
<td>Mouse, embryonic culture</td>
<td>IFM, WB on synaptosomal fractions</td>
<td>[7]</td>
<td></td>
</tr>
<tr>
<td>Spine density ↑</td>
<td>mGR</td>
<td>100 nM</td>
<td>CA1</td>
<td>60 min</td>
<td>Rat, ex vivo</td>
<td>ERK1/2</td>
<td>(2), (3)</td>
<td>[11]</td>
</tr>
<tr>
<td>GR at membrane</td>
<td>mGR</td>
<td>–</td>
<td>Hippocampus</td>
<td>–</td>
<td>Mouse, ex vivo</td>
<td>WB on synaptosomal fractions</td>
<td>(2), (3)</td>
<td>[11]</td>
</tr>
<tr>
<td>Aspartate and glutamate levels</td>
<td>Other</td>
<td>600 ng/ml</td>
<td>local</td>
<td>20 min</td>
<td>Rat, in vivo</td>
<td>ERK1/2</td>
<td>(2), (3)</td>
<td>[12]</td>
</tr>
<tr>
<td>NMDA-dependent neurotoxicity ↑</td>
<td>Other</td>
<td>10 nM</td>
<td>Hippocampus</td>
<td>15 min</td>
<td>Rat, postnatal culture</td>
<td>ERK1/2, NR2A</td>
<td>(1), (3)</td>
<td>[13]</td>
</tr>
<tr>
<td>LTP induction ↑</td>
<td>Other</td>
<td>100 nM</td>
<td>CA1</td>
<td>10 min</td>
<td>Mouse, ex vivo</td>
<td>ERK1/2</td>
<td>(3)</td>
<td>[14]</td>
</tr>
<tr>
<td>sIPSC freq ↑</td>
<td>Other</td>
<td>25 nM</td>
<td>CA1</td>
<td>5 min</td>
<td>Mouse, ex vivo</td>
<td>G-proteins, NO</td>
<td>(1), (3)</td>
<td>[15]</td>
</tr>
<tr>
<td>NMDA-dependent current ↓</td>
<td>Not GR</td>
<td>100 nM</td>
<td>Hippocampus</td>
<td>Seconds</td>
<td>Rat, postnatal culture</td>
<td>cAMP-PKA</td>
<td>(1), (3), R</td>
<td>[16]</td>
</tr>
<tr>
<td>NMDA-dependent current prolonged</td>
<td>Not GR</td>
<td>1 μM</td>
<td>Hippocampus</td>
<td>Seconds</td>
<td>Rat, postnatal culture</td>
<td>(1), (3), R</td>
<td>[17]</td>
<td></td>
</tr>
<tr>
<td>AEA levels ↑</td>
<td>Unknown</td>
<td>3 mg/kg s.c.</td>
<td>Hippocampus</td>
<td>10 min</td>
<td>Rat, in vivo</td>
<td>ERK1/2</td>
<td>(1)</td>
<td>[18]</td>
</tr>
<tr>
<td>NMDA-dependent current ↓</td>
<td>Unknown</td>
<td>400 nM</td>
<td>CA1</td>
<td>Seconds</td>
<td>Mouse, ex vivo</td>
<td>G-proteins</td>
<td>(1)</td>
<td>[19]</td>
</tr>
<tr>
<td>Ca²⁺-currents ↓</td>
<td>Unknown</td>
<td>10 μM</td>
<td>CA1</td>
<td>4 min</td>
<td>Guinea pig, ex vivo</td>
<td>G-proteins, PKC</td>
<td>(20)</td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GR in membrane</td>
<td>mGR</td>
<td>–</td>
<td>BLA</td>
<td>–</td>
<td>Mouse</td>
<td>EM</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td>MR in membrane</td>
<td>mMR</td>
<td>–</td>
<td>BLA</td>
<td>–</td>
<td>Mouse</td>
<td>EM</td>
<td>(22)</td>
<td></td>
</tr>
<tr>
<td>mEPSC freq ↑</td>
<td>MmR</td>
<td>100 nM</td>
<td>BLA</td>
<td>15 min</td>
<td>Mouse, ex vivo</td>
<td>CB1</td>
<td>(1), (2), (3), (4), NR</td>
<td>[23]</td>
</tr>
<tr>
<td>mEPSC freq ↓</td>
<td>mGR</td>
<td>100 nM</td>
<td>BLA</td>
<td>15 min</td>
<td>Mouse, ex vivo</td>
<td>CB1</td>
<td>only after stress</td>
<td>[23]</td>
</tr>
<tr>
<td>AEA levels ↑</td>
<td>Unknown</td>
<td>3 mg/kg s.c.</td>
<td>Amygdala</td>
<td>10 min</td>
<td>Rat, in vivo</td>
<td></td>
<td></td>
<td>[18]</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate uptake ↑</td>
<td>Unknown</td>
<td>10 nM</td>
<td>Frontal cortex</td>
<td>5 min</td>
<td>Rat, cultured synaptosomes</td>
<td>G-proteins</td>
<td>No nuclei present</td>
<td>[24]</td>
</tr>
<tr>
<td>Calmodulin dynamics ↑</td>
<td>Unknown</td>
<td>30 nM</td>
<td>Cortex</td>
<td>15 min</td>
<td>Rat, cultured synaptosomes</td>
<td>G-proteins</td>
<td>No nuclei present</td>
<td>[25]</td>
</tr>
</tbody>
</table>

(1), Use of cort-BSA or dexam-BSA; (2), not prevented by protein inhibitor; (3), use of MR and GR antagonists; (4), use of MR- and GR-knockout mice; ‘unknown’, receptor was not examined; ‘other’, not the MR or GR; eIPSC/EPSC, evoked IPSC/EPSC; AEA, anandimide; 2-AG, 2-arachidonoylglycerol; sIPSC, spontaneous IPSC; mMR/mGR, membrane-associated MR/GR; ECB, endocannabinoids; CB1, cannabinoid receptor type 1; NO, nitric oxide; NR2A, NMDA receptor 2A subunit; PKC, protein kinase C; WB, western blot; IFM, immunofluorescent microscopy; EM, electron microscopy; NR, non-reversible; R, reversible. References: [1], Di et al. 2003; [2], Di et al. 2005; [3], Malcher-Lopes et al. 2006; [4], Di et al. 2009; [5], Karst et al. 2005; [6], Oljslagers et al. 2008; [7], Qiu et al. 2010; [8], Pasricha et al. 2011; [9], Goc et al. 2008; [10], Maggio & Segal 2009; [11], Komatsuzaki et al. 2005; [12], Venero & Borrell 1999; [13], Xiao et al. 2010; [14], Wiegert et al. 2006; [15], Hu et al. 2010; [16], Liu et al. 2007; [17], Takahashi et al. 2002; [18], Hill et al. 2010; [19], Sato et al. 2004; [20], French-Mullen 1995; [21], Johnson et al. 2005; [22], Prager et al. 2010; [23], Karst et al. 2010; [24], Zhu et al. 1998; [25], Sze & Iqbal 1994.
The cellular basis of the rapid effects is not well established and controversy remains about the receptor mediating the effects. On the one hand, pretreatment with a GR antagonist did not prevent the rapid effects of corticosterone on CRH-induced ACTH secretion in vivo (Hinz & Hirschelmann 2000). Also, in a pituitary-derived cell line, a membrane-binding place for dexamethasone and corticosterone was identified that did not have any affinity for the GR–antagonist RU486 (Maier et al. 2005). However, another line of evidence does suggest a role for the classical GR in mediating rapid feedback at the pituitary. Thus, a rapid and non-genomic translocation of annexin-I by dexamethasone was prevented by GR–antagonist treatment in a pituitary-derived cell line (Solito et al. 2003). This translocation of annexin-I was required for rapid inhibition of ACTH release (Buckingham et al. 2003, Tierney et al. 2003). Thus, corticosterone rapidly inhibits ACTH release from the pituitary, but whether this is due to a novel receptor or to the classical GR is still controversial. This rapid inhibition is also seen in control human subjects, while it is absent in depressed patients, suggesting that the rapid negative feedback is somehow associated with disease (Young et al. 1991).

**Hippocampus**

Adaptation to a stressful situation is a coordinated effort of the limbic system, consisting of the hippocampus, amygdala and prefrontal cortex (PFC; see Fig. 1). This, among other things, involves projections of these areas to and hence control over the PVN (Ulrich-Lai & Herman 2009). Collectively, these limbic areas also facilitate the formation of a memory trace of the event. Processing of contextual aspects depends predominantly on hippocampal function. The hippocampus expresses high levels of both MR and GR in all subfields (except its cornu ammonis-3 (CA3) region that mainly expresses MR; Reul & de Kloet 1985). Corticosterone exerts strong genomic effects on the activity and plasticity of all hippocampal subfields as well as on hippocampus-dependent memory (McEwen 2001, Kim & Diamond 2002, Mirescu & Gould 2006, Joels 2008). Low levels of corticosterone, through MR activation, facilitate plasticity and hippocampus-dependent memory (Diamond et al. 1992). By contrast, absence or very high levels of corticosterone inhibits plasticity; the latter is mediated through the GR (Alfarez et al. 2002, Kim et al. 2004).

Similar to neurons in the hypothalamus, hippocampal neurons spontaneously show mEPSCs. In a first study (Karst et al. 2005), the effect of corticosterone was examined on mEPSC frequency in the CA1 region of the hippocampus. It appeared that within 5 min of corticosterone administration, the frequency of mEPSCs is significantly enhanced, i.e. changed in a direction opposite to that observed in the PVN. The amplitude was unaffected (Karst et al. 2005, Olilsjagers et al. 2008; see Fig. 3B). This effect was recently reproduced by other investigators (Qiu et al. 2010) and granule neurons in the dentate gyrus respond similarly to corticosterone as CA1 neurons (Paricha et al. 2011). Similar to the corticosteroid effect in the hypothalamus, the rapid effect in the hippocampus does not depend on gene transcription and involves a membrane–located receptor (Karst et al. 2005).

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**Figure 2** Model of the present knowledge regarding the synaptic pathways of corticosterone-induced rapid effects on glutamatergic transmission. (A) Inhibition of glutamatergic transmission is initiated by postsynaptically located receptors; this can be either G-protein-coupled receptors (hypothalamus) or membrane-localized GRs (amygdala). Activation of these receptors by corticosterone induces activation of G-proteins and the cAMP-protein kinase A (PKA) pathway, which eventually induces synthesis of the retrograde messengers anandamide (AEA) and 2-arachidonoylglycerol (2-AG). In a retrograde mode of action 2-AG and AEA activate the cannabinoid receptor type 1 (CB1) at the presynaptic terminal, which in turn inhibits the release probability of glutamatergic vesicles. (B) Facilitation of glutamatergic transmission is initiated by both pre- and post-synaptically located membrane-MRs. Presynaptically, activation of the MR by corticosterone activates an extracellular signal-regulated kinase (ERK) pathway, resulting in stimulation of the release probability of glutamate vesicles. At the same time, postsynaptic activation of a membrane-associated MR inhibits potassium I_A-currents, and stimulates membrane diffusion of AMPA receptors. All three effects together result in a facilitation of glutamatergic transmission. __www.endocrinology-journals.org__
found to inhibit postsynaptic IA-currents, an effect that could involve MRs inserted into the presynaptic membrane. Importantly, the membrane-located MR appears to rapidly potentiate the excitability of hippocampal neurons via membrane-MRs located on both pre- and postsynaptic sites, thus priming the hippocampal circuit for subsequent stimulation by context-dependent factors.

However, not all rapid effects in the hippocampus involve the MR. First, a non-genomic increase in spine density of hippocampal neurons was found to depend on GRs rather than MRs (Komatsuzaki et al. 2005). Yet, other rapid corticosterone effects occur independent of MR or GR and therefore could be mediated by a novel (so far not identified) membrane-localized receptor. This applies to rapid stimulatory corticosterone effects on inhibitory transmission (Hu et al. 2010), on levels of extracellular excitatory amino acid (Venero & Borrell 1999), long-term potentiation (LTP) induction (Wiegert et al. 2006) and N-methyl-D-aspartic acid (NMDA)-dependent neurotoxicity (Xiao et al. 2010). Some studies also reported inhibitory actions of corticosterone on NMDA signalling (Sato et al. 2004, Liu et al. 2007). Apparently, corticosterone affects hippocampal signalling in multiple ways, involving membrane-located MRs, GRs and other, still unknown receptors. The existence of receptors with properties different from those of the MR and GR was also suggested by early biochemical studies (Orchini et al. 2000).

**Amygdala**

Stressful events invariably activate the amygdala, the brain’s principal emotional centre (Roozendaal et al. 2009). The amygdala expresses both MR and GR in its various subnuclei (Reul & de Kloet 1985) and amygdala-dependent memory, such as cued learning and emotional memory, is very sensitive to stress and corticosteroids (Roozendaal et al. 2009). Interestingly, genomic effects of corticosterone on the amygdala are generally opposite to those seen in the hippocampus, with enhanced activity in the former (Duvarc & Pare 2007, Mitra & Sapolsky 2008) and reduced activity and plasticity in the latter (Alfaz et al. 2002, 2009, Kim et al. 2004). In addition, the amygdala is one of the main targets of the adrenergic system. Many corticosteroid effects on amygdala functioning require adrenergic signalling (Roozendaal et al. 2009). This interaction might in part be mediated by non-genomic effects of corticosteroids. For instance, a systemic injection of corticosterone directly after a learning task rapidly (within 15 min) increased the levels of noradrenaline in the basolateral amygdala (BLA) and this was
correlated with the later facilitation of fear memory by corticosterone (McReynolds et al. 2010).

An important finding that raised interest in non-genomic actions of corticosterone in the amygdala was the demonstration of MR and GR at the plasma membrane in amygdalar neurons. Electron microscopic analyses were used for the detailed study of the subcellular distribution of the GR (Johnson et al. 2005) and MR (Prager et al. 2010) in the lateral amygdala. The GR was identified at the plasma membrane as well as in the nucleus and cytoplasm. GRs turned out to be present at both postsynaptic dendrites and presynaptic sites (Johnson et al. 2005). More recently, the same was shown for the MR (Prager et al. 2010).

The functional consequences of corticosterone on mEPSC frequency in the BLA and the central nucleus of the amygdala (CeA) were recently revealed (Karst et al. 2010). In the CeA, corticosterone had no effect on either frequency or amplitude of the mEPSCs. However, in the BLA, corticosterone induced a significant increase in mEPSC frequency, comparable to the effects found in hippocampus albeit slightly slower in onset (Karst et al. 2010; Fig. 3A and C). Comparable to the hippocampus, this enhanced mEPSC frequency after corticosterone treatment was MR-dependent and non-genomic in nature (Karst et al. 2010). However, in contrast to the hippocampus, the effect in the amygdala was not only slower in onset, but also persistent after washout of the hormone. One hour after a pulse of corticosterone, mEPSC frequency was still high (Karst et al. 2010). This lasting phase of the response was found to depend on protein synthesis and required the presence of both MR and GR (Karst et al. 2010).

The long-lasting effects of corticosterone were shown to also determine the responses of BLA neurons to subsequent pulses of the hormone. When BLA cells were exposed to a second pulse of corticosterone, mEPSC frequency was reduced (Karst et al. 2010; see Fig. 3C). Reduction in mEPSC frequency also occurred in tissue prepared from animals exposed to restraint stress prior to slice preparation. Interestingly, this rapid and non-genomic effect to renewed corticosteroid exposure depended on the GR rather than the MR (Karst et al. 2010). Similar to the hypothalamus (but in contrast to the hippocampus), it was shown to involve a postsynaptically localized GR and subsequent retrograde endocannabinoid signalling (Karst et al. 2010; see Fig. 2A). Thus, in a non-stressed animal, corticosterone seems to have a stimulatory effect in the (basolateral) amygdala. However, due to the long-lasting nature of these effects, a second exposure to corticosterone induces opposite effects, suggesting meta-plasticity of corticosteroid responses. These data suggest that the amygdala will respond differently to a stressor depending on the recent stress history of the organism.

Prefrontal cortex

The PFC is critically involved in complex behavioural control, such as behavioural inhibition, decision-making and working memory. It is extensively connected to the amygdala and receives afferents originating in the hippocampus (Arnsten 2009). Despite its important function, the PFC is underrepresented in studies on the effects of corticosteroids and stress. A number of studies have examined the effect of chronic stress or corticosterone exposure on the PFC. Under these conditions, LTP, dendritic complexity and PFC-dependent working memory were reduced in a genomic fashion (Arnsten 2009, Holmes & Wellman 2009). On the contrary, exposure to acute stress or corticosterone increased glutamatergic transmission and improved working memory performance (Yuen et al. 2009, 2010). These effects occurred with a delay of several hours and were shown to require gene transcription (Yuen et al. 2010). Thus, acute and chronic stress affect PFC plasticity and functionality in an opposite manner.

The only studies so far in the PFC that focused on rapid, non-genomic effects were performed in synaptosomes. In this preparation, corticosterone induced a rapid enhancement of glutamate uptake and of calcium-dependent calmodulin stabilization (Sze & Iqbal 1994, Zhu et al. 1998). Unfortunately, the receptors or pathways involved were not examined. In a recent study, Roozendaal and colleagues reported a putative membrane-GR-mediated effect of corticosterone in the insular cortex that is involved in memory acquisition. In this elegant study, administration of either corticosterone or cort-BSA directly into the insular cortex facilitated the acquisition of object recognition memory (Roozendaal et al. 2010). Although there are some concerns about the stability of cort-BSA in vivo, this is still indicative of a membrane-initiated effect. The effect was prevented by co-administration of a GR antagonist. The authors further proved that the facilitation of memory by membrane-GR activation was established through protein kinase A (PKA), cAMP response element-binding (CREB) and histone acetylation (Roozendaal et al. 2010). Taken together, rapid non-genomic actions of corticosterone are found in (some) prefrontal areas; so far, they seem to be mostly excitatory (as are the sub-acute genomic effects) and could have implications for higher-order learning in complex tasks. However, the data are still very sparse.

Functional implications of rapid corticosteroid effects in the brain

Taking all results into account, we can distinguish some interesting general features of the rapid effects of corticosterone in the brain (see also Fig. 2 and Table 1). i) It is important to notice that all non-genomic effects are permissive or conditional effects. In none of the studies, corticosterone induced any activity on its own, instead it facilitates or inhibits signalling of ion channels, receptors and neurotransmitters. Thus, it increases or decreases the threshold for activation of these neurons by context-dependent factors. Therefore, which effects (in which brain areas) will be most pronounced during a stressful encounter depends on the context. ii) We
see a distinctive pattern with a general increase in excitability for some areas (hippocampus, amygdala and potentially the PFC) and a decrease in others (the hypothalamus). iii) While some responses are transient (mostly in the hippocampus), other effects are prolonged (hypothalamus, pituitary and amygdala). The brain circuitry activated by stress will thus be different depending on the delay after the stressor. iv) In general, the inhibitory effects on hypothalamic functioning seem to require a higher dose of corticosterone than most effects in other brain areas. If so, the set of responses seen after a mild stressor may be different from that of a more severe stressor, the latter having an additional negative effect on PVN-related responses (Prager & Johnson 2009). v) Finally, a number of rapid corticosterone effects require the presence of classical MR and GR inserted in or attached to the plasma membrane, while other effects are mediated through as yet unknown (G-protein coupled) receptors. In general, MR-mediated effects tend to stimulate excitation, while GR-mediated effects can also be inhibitory (see Fig. 2).

We refer to these five general points when we consider the potential functional consequences of rapid corticosteroid actions in the brain for HPA-axis regulation and cognition, also taking the ultradian release pattern into consideration. Finally, we address the integration of these rapid effects with the rest of the brain’s response to stress.

**Regulation of the HPA-axis**

Corticosteroids exert rapid, as well as delayed, inhibitory feedback at the core structures of the HPA-axis: the PVN of the hypothalamus (Evanson et al. 2010b) and the pituitary gland (Jones et al. 1972, Hinz & Hirschelmann 2000). In the pituitary, this seems to be caused by both GR-dependent (Buckingham et al. 2003) and GR-independent (Hinz & Hirschelmann 2000) rapid signalling pathways. In the PVN, the rapid suppression of glutamatergic transmission by corticosterone could well underlie (amongst others) fast suppression of the HPA-axis in a GR-independent manner (Tasker 2006). As mentioned earlier, this hypothesis is backed up by the effectiveness of intra-PVN infusions of dexamethasone or dex-BSA on HPA-axis activity in a rapid time frame (Evanson et al. 2010b).

In addition, extra-hypothalamic structures also control the activity of the HPA-axis. For instance, the hippocampus and PFC exert negative feedback on the HPA-axis through (indirect) projections to the PVN, while the amygdala has a stimulatory influence on the PVN and thus HPA-axis (Ulrich-Lai & Herman 2009). Rapid non-genomic corticosterone actions in these areas may affect this limbic control over the HPA-axis. This also enables a role for the MR, absent from the hypothalamus, in the regulation of HPA-axis activation. Indeed, MRs in the hippocampus are important to determine the threshold of the stress response (Reul et al. 2000, Joëls et al. 2008). In agreement, treatment of rats with MR agonists induced a rapid suppression of both ACTH and corticosterone release (Atkinson et al. 2008). Thus, not only can corticosterone inhibit HPA-axis activation directly through its genomic and non-genomic effects at core structures of the axis, but it can also provide a second layer of control at limbic areas that enables a subtler and context-dependent rapid trans-synaptic regulation of the HPA-axis.

**Adaptation of behaviour and cognition**

In addition to regulation of the HPA-axis through (trans-synaptic) connections to the PVN, the limbic circuitry is vital for adaptation to stressful events and the formation of memory of these events (Fig. 1). Many actions of corticosterone, for example facilitation of memory consolidation, are dependent on gene transcription, through activation of the genomic GR (and MR; Oitzl et al. 2001). However, corticosterone also affects behaviour and memory in a rapid and presumably non-genomic manner. Thus, rapid effects of corticosterone have been described for a number of adaptive behaviours, including rapid facilitation of novelty-induced locomotion (Sandi et al. 1996a,b), context-dependent aggression (Mikics et al. 2004) and risk assessment behaviour (Mikics et al. 2005). These effects were all observed within 7 min and the latter two were proven to be independent of gene transcription, see also Table 2. In all cases, an injection with corticosterone rapidly increased a specific type of behaviour that is seen as adaptive in that context (i.e. aggression towards an intruder, or locomotion and risk assessment in a novel environment). Interestingly, the MR has been repeatedly reported to be involved in these types of behaviour, involving novelty reactivity and coping strategies (Oitzl & de Kloet 1992, Sandi & Rose 1994, Berger et al. 2006, Joëls et al. 2008, Brinks et al. 2009). As these behavioural effects are induced rapidly and only with stress doses of corticosterone, they always seemed incompatible with the constitutively active genomic MR. The lower affinity membrane-MR could prove to be the logical substrate for these effects. Unfortunately, this role of the membrane-MR has not been studied directly yet. There is circumstantial evidence for involvement of MRs in novelty behaviour. This comes from a study using knockout mice for the limbic system-associated membrane protein (LSAMP). These mice showed increased novelty reactivity and impaired learning (Catania et al. 2008, Qiu et al. 2010), and associated with this, a reduction in non-genomic MR function in the hippocampus (Qiu et al. 2010).

In behavioural studies on the regulation of memory, the GR is reported to have a predominant function in memory consolidation, while the MR is mostly involved in memory retrieval and learning strategies (Oitzl & de Kloet 1992, de Kloet et al. 1999). A similar convergence of functions is seen in the rapid domain. First, a rapid facilitation of memory consolidation by corticosterone was shown to depend on the (presumably membrane localized) GR in the cortex (Roozendaal et al. 2010). Second, application of antagonists for endocannabinoid signalling in the amygdala was reported to block corticosterone-induced effects on memory consolidation (Campolongo et al. 2009). Together, this suggests
that the membrane-GR–mediated and endocannabinoid-dependent inhibition of neuronal excitability (see Fig. 2A and Karst et al. (2010)) might be implicated in memory consolidation. In contrast, corticosterone effects on memory retrieval seem to be MR mediated. Administration of corticosterone 30 min before a memory retrieval task impaired retrieval of information in a non-genomic, hippocampal-dependent and MR–mediated manner (Khaksari et al. 2007, Sajadi et al. 2007). Finally, acute stress or cort-BSA infusion into the hippocampus induced a shift in memory retrieval tested 5 or 15 min later; however, this study did not investigate the receptor involved (Chauveau et al. 2010). Rapid, in addition to delayed, corticosteroid effects thus seem to be involved in all phases of the memory process, i.e. acquisition, consolidation and retrieval. In general, the GR seems to potentiate consolidation via both rapid and delayed (genomic) pathways. Conversely, the MR seems to have a specific (non-genomic) role during memory retrieval, possibly as a mechanism to focus attention to a new stressor. Taken together, in its role as rapid corticosteroid sensor, the MR facilitates adaptive behaviour in the context of the stressor while inhibiting behaviours that are no longer relevant.

### Implication of ultradian pulses

Corticosterone does not reach the brain in high amounts during a stressful situation only, but also during ultradian peaks (Droste et al. 2008). Rapid non-genomic corticosterone actions might have an additional function in translating these pulses into ultradian alterations in brain function. Indeed, rapid feedback on the HPA-axis (Windle et al. 1998), aggressive behaviour (Haller et al. 2000) and novelty reactivity, all depend on the phase of an ultradian pulse the animal is in. In a recent study by Sarabdjitsingh et al. (2010) ultradian pulses were manipulated experimentally. Exposure to noise stress induced a stronger ACTH release and higher behavioural reactivity when animals were stressed during the rising phase of an ultradian corticosterone pulse compared with animals exposed to the same stressor during the falling phase (Sarabdjitsingh et al. 2010). These responses were seen within minutes, indicating that non-genomic mechanisms must have been involved. In the brain, these effects were associated with increased activity of the amygdala and decreased activity of the PVN (recorded by c-fos expression) during the rising phase, compared with the falling phase (Sarabdjitsingh et al. 2010), reminiscent of the corticosteroid effects seen for mEPSC frequency in PVN and amygdala. Hypothetically, during the rising phase of an ultradian pulse, non-genomic pathways are activated in limbic areas, which in turn could affect stress-related behaviour.

### Integration of non-genomic and genomic effects

In several cases, rapid non-genomic corticosteroid actions were shown to transgress into more lasting effects, integrating two temporal domains (rapid and delayed) which, until recently, were each linked to different classes of stress hormones, i.e. monoamines (and to some extent neuro-peptides) on the one hand and corticosteroids on the other hand. For example, rapid effects in the hypothalamus are long lasting (Di et al. 2003) and thus HPA-axis feedback will be inhibited over a long period of time. Indeed, dexamethasone infusions in the PVN exert both rapid and delayed negative feedback actions on the HPA-axis activity (Dallman et al. 1994, Dallman 2005). Similarly, the increased excitability in the BLA starts as a non-genomic MR–dependent phenomenon and eventually evolves into a genomic phenomenon that also requires the GR (Karst et al. 2010). At a cognitive level, the facilitation of memory consolidation by cort-BSA injections in the insular cortex (Roozendaal et al. 2010) is evoked by a membrane-associated effect that evolves into a genomic effect through activation of the transcription factor CREB (Roozendaal et al. 2010). Finally, rapid corticosterone effects on aggressive and risk assessment behaviour are independent of gene transcription immediately after

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**Table 2** Rapid effects of corticosteroids on behaviour and cognitive performance

<table>
<thead>
<tr>
<th>Effect</th>
<th>Receptor</th>
<th>Conc</th>
<th>Area</th>
<th>Delay in onset</th>
<th>Pathways</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered memory retrieval by cort-BSA or acute stress</td>
<td>Unknown</td>
<td>0–3 nmol local</td>
<td>Dorsal hippocampus</td>
<td>10 min</td>
<td>PKA, CREB histone acetylation</td>
<td>(1)</td>
<td>Chauveau et al. (2010)</td>
</tr>
<tr>
<td>Memory consolidation †</td>
<td>mGR</td>
<td>3 ng local</td>
<td>Insular cortex</td>
<td>24 h</td>
<td>PKA, CREB histone acetylation</td>
<td>(1), (3)</td>
<td>Roozendaal et al. (2010)</td>
</tr>
<tr>
<td>Memory retrieval ‡</td>
<td>MR</td>
<td>1 mg/kg i.p.</td>
<td>–</td>
<td>30 min</td>
<td>Opioids</td>
<td>(2), (3)</td>
<td>Khaksari et al. (2007) and Sajadi et al. (2007)</td>
</tr>
<tr>
<td>Risk assessment †</td>
<td>Unknown</td>
<td>0–5 mg/kg i.p.</td>
<td>–</td>
<td>7 min</td>
<td>MR</td>
<td>(2)</td>
<td>Mikics et al. (2005)</td>
</tr>
<tr>
<td>Aggressive behaviour †</td>
<td>Unknown</td>
<td>0–5 mg/kg i.p.</td>
<td>–</td>
<td>7 min</td>
<td>MR</td>
<td>(2)</td>
<td>Mikics et al. (2004)</td>
</tr>
<tr>
<td>Locomotion †</td>
<td>Other</td>
<td>2–5 mg/kg i.p.</td>
<td>–</td>
<td>7 min</td>
<td>NO</td>
<td>(2), (3)</td>
<td>Sandi et al. (1996a,b)</td>
</tr>
</tbody>
</table>

(1), use of cort-BSA or dex-BSA; (2), not prevented by protein inhibitor; (3), use of MR and GR antagonists; ‘unknown’, receptor was not examined; ‘other’, not the MR or GR; mGR, membrane-associated GR; NO, nitric oxide.
corticosterone injection but develop into transcription-dependent effects later on (Mikics et al. 2004, 2005). Thus, many non-genomic effects of corticosterone are tightly linked to later genomic actions. At least in one case (Karst et al. 2010), the initial non-genomic action is required for the subsequent genomic phase, suggesting that both phases work in coordination.

However, non-genomic and genomic actions can also be integrated if they occur independent from one another. In the hippocampus, the initial enhanced mEPSC frequency is quickly reversed: when corticosteroid levels drop, the effects are immediately lost (Karst et al. 2005). Supposedly, a brief period of enhanced excitability is followed by a refractory period with an increased threshold for the induction of new signals; the latter depends on genomic GR signalling (Alfarez et al. 2002, 2009, de Kloet et al. 2008, Krugers et al. 2010).

A similar dichotomy was seen with respect to LTP induction in the hippocampus. Corticosterone given immediately before LTP induction stimulated LTP induction (Wiegert et al. 2006), while corticosterone applied hours earlier inhibited the induction of the same type of LTP (Diamond et al. 1992, Pavlides et al. 1993). The initial rapid facilitation of signalling might help the organism to appraise the novel situation, subsequently the genomic phase will take over and restore the activity of the circuits to regain homeostasis (Joëls et al. 2006).

Overall, this implies that the temporal pattern of activation by corticosterone is different for the various areas. As summarized in Fig. 4, both the hippocampus and amygdala are more sensitive for incoming signals during stress or corticosterone exposure, while activity in the PVN is rapidly inhibited. In a delayed fashion, the hippocampus will switch to a state where the threshold for activation is elevated, while activation thresholds in the amygdala and hypothalamus do not differ between the two time-domains. Hypothetically, this can have consequences for the cognitive functions associated with these brain areas. For example, as the amygdala is involved in emotional memory formation, the prolonged activation in this area might support efficient encoding of emotional aspects of a stressful event, which could explain the preferential memory of emotional over neutral, hippocampal-dependent information (Buchanan & Lovallo 2001, Karst et al. 2010). Finally, it seems that a second exposure of corticosterone switches amygdalar excitability back to its pre-stress state (Karst et al. 2010). This mechanism could protect the amygdala from inappropriately prolonged activation (McEwen 2001, Karst et al. 2010). For the PFC, the limited data so far suggest that its sensitivity to activation is elevated by corticosterone in both an acute and more prolonged manner. However, as the data for the PFC is still sparse, we have not included it in Fig. 4.

Critical evaluation of approaches

Reviewing the available literature also raises some mechanistical points of interest. First of all, the proof of membrane localization of the investigated receptors is based, for a large part, on the effectiveness of the membrane-impermeable cort-BSA conjugate (see also Tables 1 and 2). The use of this conjugate is debated as a proportion of the steroid could dissociate from BSA and thus pass the plasma membrane (Stevis et al. 1999, Harrington et al. 2006), although the amount of steroid released does seem to be very low (Yang et al. 2010). For oestradiol, a new, stronger conjugate has been constructed that shows improved stability (Harrington et al. 2006, Yang et al. 2010). A similar improved conjugate is not yet available for corticosterone, but would be of great significance for the field. In addition, it is of importance to perform dose–response curves for corticosterone and cort-BSA: if both hormone substrates are equally effective, it is unlikely that the small percentage of free steroid from the BSA-conjugate induces the effect. So far, this seems to be the case (Karst et al. 2005, Xiao et al. 2010), although one study did report a 10-fold lower effectiveness of cort-BSA compared with free corticosterone (Qi et al. 2005). Furthermore, for single-cell patch clamp studies, corticosterone can be applied intracellularly (Liu et al. 2007, Oljajgers et al. 2008). As thus applied corticosterone is ineffective, this is a further indication of true membrane localization of the receptor. Finally, the identification of the MR and the GR at the membrane with electron microscopy and cell fractionation studies further strengthens the concept of membrane localization of the MR and GR (see Table 1).

A further point of interest is the use of selective antagonists to deduce if the GR and MR are involved in rapid
corticosteroid effects. The consequence of the membrane-association of steroid receptors for ligand binding is not well understood. There is evidence that membrane association can affect the ligand-binding domain of steroid receptors and thus change ligand specificity especially for synthetic agonists and antagonists (Norman et al. 2004). So far, the classical antagonists for the MR (spironolactone) and GR (RU486) have successfully antagonized some rapid corticosterone actions in the brain, and ligand specificity thus seems unaltered (Karst et al. 2005, 2010). However, in the periphery, there are indications of an altered selectivity for antagonists for the membrane-associated MR. Here, classical MR antagonists (spironolactone and canrenone) did not block non-genomic actions of aldosterone, while two types of open E-ring MR antagonists (RU28318 and potassium canrenoate) did prevent these effects (Alzamora et al. 2000, Mihailidou & Funder 2005). Thus, ligand selectivity of the membrane-MR might differ between tissues. Owing to the uncertainty on the effectiveness of antagonists on membrane-associated corticosteroid receptors, we recommend the use of MR- and GR-knockout mice and tissue thereof as a definite test for MR or GR involvement.

Finally, a number of rapid corticosterone effects could not be blocked by classical MR and GR antagonists (see Tables 1 and 2) and have therefore been postulated to be mediated by a different class of membrane-bound receptors, probably G-protein-coupled receptors (GPCR). Indeed, a number of non-MR or -GR membrane binding sites were found in the brain of multiple species (Guo et al. 1995, Orchilnik et al. 2000, Maier et al. 2005). However, the identity of these receptors has proven hard to resolve and, as of yet, no novel corticosteroid receptors have been cloned. In addition, as mentioned, it would be valuable to check rapid effects of corticosteroids in MR- and GR-knockout mice to determine if all effects now appointed to GPCRs are truly MR and GR independent.

Concluding remarks

The existence of rapid effects of corticosterone has been known for over 50 years; however, it is only in the last 10 years that these effects have been studied in more detail. Yet, there are still many unanswered questions.

First, we cannot appreciate the consequences of non-genomic effects of corticosteroids when they are studied in isolation, instead we must view these effects in the context of the complete stress response. Exactly how rapid non-genomic and genomic actions are integrated to collectively accomplish the behavioural response to stress awaits further investigation, as discussed in the previous section.

Second, through its non-genomic effects, corticosterone acts in the same time-domain as other transmitters and hormones released after stress, e.g. catecholamines or CRH. This gives ample opportunities for crosstalk between the various stress hormones (Joëls & Baram 2009). For example, activation of the noradrenergic system in the amygdala is required for effects of corticosterone to take place (Roozendaal et al. 2002, 2006). However, at this time, relatively little is known about the mechanism by which corticosteroids alter responsiveness to other stress factors and if non-genomic corticosterone signalling is involved.

Equally important is the molecular basis of rapid corticosteroid effects. A comparison of the available data (see Tables 1 and 2) suggests that many pathways are shared across brain areas. For example, multiple studies have proven involvement of G-proteins and the signal-regulated kinase–CREB pathway. Importantly, these same pathways are also activated by rapid signalling of other steroid receptors, such as the oestrogen receptor (ER), androgen receptor (AR), progesterone receptor (PR; reviewed in (Hammes & Levin 2007, Vasudevan & Pfaff 2007, Levin 2008)) and by rapid aldosterone signalling through the MR in peripheral tissues (Grossmann & Gekle 2009). Information gathered in these related fields could serve as an important guideline for investigation of the signalling partners of corticosteroids in the brain. For instance, rapid signalling of both corticosterone (Di et al. 2009) and oestradiol (Boulware et al. 2007) in neurons suggest that the specific type of G-protein that is engaged in the hormonal actions is an important determinant of the subsequent signalling cascade and the physiological outcome.

Finally, evidence for membrane localization of the MR and GR is quite convincing. However, as steroid receptors are not transmembrane proteins, they will require assistance for their translocation to the membrane. Again regulation of this phenomenon seems to be shared by most, if not all, steroid receptors and could serve as a guideline for the stress receptors. The regulation of membrane localization of the ERα is especially well understood. Specific point mutations in the ERα sequence that either disrupt binding to the scaffolding protein caveolin-1 (Razandi et al. 2003) or that prevent palmitoylation of the receptor (Acconcia et al. 2005) disrupt the membrane localization and rapid signalling of the ERα. Importantly, the domain required for palmitoylation was found in many steroid receptors and mutation of key amino acids in this sequence disrupts the membrane localization of the PR, AR and ERβ as well (Pedram et al. 2007). Thus, in most steroid receptors, membrane translocation regulation seems comparable. Intriguingly, the GR shares the preserved sequence for palmitoylation and would be postulated to be palmitoylated and transported to the membrane, but the MR lacks an essential amino acid and cannot be palmitoylated at this sequence. Whether this means that the MR is palmitoylated at another sequence or whether this receptor translocates to the membrane through a different pathway is still unknown. Preliminary evidence from the periphery does suggest that both GR and MR membrane translocation involves caveolin-1: the GR directly binds caveolin-1 (Matthews et al. 2008) and the MR is localized in lipid rafts (where caveolin-1 is also present; Grossmann et al. 2010). In the brain, the regulation of membrane translocation of the MR and GR is still undiscovered territory and more studies on this subject are clearly needed.
Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Non-genomic effects of corticosteroids

F L GROENEWEG and others


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