# Intracellular non-genomic effects of glucocorticoids in the brain and other tissues

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A.M. Smink[#], R. Fornari[^] and B. Roozendaal[^]
[#] University of Groningen, student bachelor Biomedical sciences
[^] Department of Neuroscience, Section Anatomy, University Medical Center Groningen, University of Groningen

## Abstract

Evidence obtained from many different types of experiments indicates that during emotionally arousing experiences the adrenal stress hormone corticosterone play a critical role in memory consolidation. Classically, glucocorticoids (GCs), as corticosterone, act through genomic receptors. Nevertheless, there is increasing evidence that GCs also use several other, non-genomic pathways to induce an effect. Several effects of GCs cannot be regulated by a genomic pathway, for example some physiological and behavioral effects that occur as a result of GCs release appear too fast to be mediated through a genomic action. The non-genomic mechanisms of GCs during memory consolidation are poorly understood. This review is about non-genomic mechanisms of GCs in the brain and other tissues, in order to understand the glucocorticoid mechanisms on cellular level during emotional memory consolidation. In order to obtain insight in possible non-genomic mechanisms of GCs in the brain, non-genomic effects of glucocorticoids on the calcium content in different peripheral cell types will be reviewed. Calcium transport is of vital important for almost all the cells in an organism and in a large number of cells GCs influence this transport mechanism non-genomically. These non-genomic mechanisms observed in peripheral cell types are similar to some mechanisms that are observed in the brain during memory consolidation. These studies indicate that GCs may act also through non-genomic mechanisms in the brain, like in the periphery, to interfere with synaptic transmission and therefore, with memory consolidation.

**Keywords:** glucocorticoids, glucocorticoids receptor, mineralocorticoids, mineralocorticoid receptor, non-genomic mechanism, calcium content, protein kinases, epithelium.

## Introduction

Emotionally arousing experiences tend to leave significant impressions. From our own experiences and from research data it is known that either positive or negative emotional experiences, like car accidents, graduation, weddings or a first boyfriend, may produce long lasting memories. Evidence obtained from many different types of experiments indicates that adrenal stress hormones, released during these emotionally arousing experiences, play a critical role in memory consolidation [24].

Stress responses provoke an activated state of our body in order to restore homeostasis and to facilitate the adaption [1,2]. In this process the body is being prepared for a classic 'fight or flight' response [2,3]. This response is based on the activation of the sympatho-adrenomedullary and the hypothalamic-pituitary-adrenal (HPA) systems. Essential to the stress response are neurons in the paraventricular nucleus (PVN) of the hypothalamus, which contains neuroendocrine neurons for synthesis and secretion of different types of hormones, including corticotropin-releasing-hormone (CRH) [25]. CRH influence the HPA axis by stimulating the adrenocorticotropic hormone (ACTH) secretion by the anterior lobe of the pituitary gland in the general circulation. ACTH persuade the adrenal cortex to produce glucocorticoids (GC; corticosterone in de rat; cortisol in humans). These glucocorticoids are produced in a circadian rhythm and are known to influence different tissues in the periphery during stress to prepare the body for the 'fight or flight' reaction and maintain homeostasis [4]. Corticosteroids are lipophilic, so dissolve easily in lipid membranes and readily cross the blood-brain barrier. Thus, corticosteroids not only affect the periphery but

also induce a number of effects in the brain through different specific mechanisms and pathways. Corticosteroids are also part of the negative feedback loop of the HPA axis, they have an inhibitory effect on CRH release when binding to specific receptors in the hypothalamus. However, in other brain areas like the basolateral amygdala (BLA) corticosteroids do not have this inhibitory effect, but stimulate neuronal activity [25]. The activated hypothalamus also induces the enhanced activity of the sympathic nervous system during stress. The sympathic nervous system stimulates the adrenal medulla to release epinephrine, which also influences different tissues [25].

Although epinephrine and corticosterone activate other specific mechanisms and pathways in the brain, they converge in regulating memory consolidation by influencing central noradrenergic mechanisms [24]. McGaugh and coworkers have shown that norepinephrine (NE) regulates memory consolidation during stressful and emotional situations [5, 27]. Extensive evidence indicates that corticosterone also modulates synaptic response during memory consolidation, so glucocorticoids and NE could act synergistically in regulating memory formation [2]. Several animal and human studies obtained evidence for a role of glucocorticoids in the formation of long lasting memories of emotional events. These studies showed that glucocorticoids strengthen the consolidation of memories of emotionally arousing experiences. In contrast, no strengthen consolidation appears after less arousing or neutral experiences [6]. These data indicate that activation of the noradrenergic system during arousing experiences is essential in enabling glucocorticoid modulation of memory consolidation. Additional data indicates that the amygdala, specifically the BLA, is a critical site of the interaction between norepinephrine and glucocorticoids for this effect.

Classically, glucocorticoids act through intracellular receptors. The classic genomic model for steroids (including glucocorticoids) implies that steroids regulate biological functions by mechanisms that involve gene transcription, mRNA and protein synthesis [14]. According to this model, glucocorticoids diffuses through the cell membrane, enter the cytoplasm, and bind to the intracellular steroid receptor. This activated receptor translocate into the nucleus, where it bind to DNA and regulate gene transcription and protein synthesis [1,2]. There are two intracellular receptors for glucocorticoids: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). These receptors are colocalised in the cytoplasm, were they play a critical role in behavioral adaptation, learning and memory processes [1]. Corticosterone binds to cytosolic GRs or MRs, forming a complex that bind heat shock proteins, and the activated GRs or MRs homodimerize. The heat shock proteins dissociate and the homodimer of GRs or MRs translocate to the nucleus, where it binds to glucocorticoid response elements which are part of the DNA. The glucocorticoid response element recruit cofactors and histone-modifying elements in order to start transcription and protein synthesis [2].
MR binds corticosterone with high affinity and therefore the receptors are saturated at basal corticosterone levels [11]. This receptor appears to be involved in the behavior towards novel objects and in the reactivity to this information [12]. MRs are secreted to maintain hydromineral homeostasis by acting on the nervous system and on renal and vascular tissues [1,16]. The affinity of GR towards corticosterone is ten times less than the affinity of MRs [10, 13]. Therefore, this receptor is only activated after stressful situations and at the circadian peak when the glucocorticoid concentration is high [11]. This means that in order to activate GRs a higher concentration glucocorticoids have to be induced by stress [2]. GR activation in the hippocampus promotes behavioral adaptation [7]. Although activation of MR provides maintenance of hydromineral homeostasis, GR activation provides rehabilitation of disturbed homeostasis after stress.

To examine whether the strengthen of memory consolidation induced by glucocorticoids depend on the activation of GRs or MRs, several experiments with mice, rats and chicks were done. A GR-knockout mice showed an impaired memory function [8]. The same impairment was observed in rats and chicks treated with an antagonist for the GR. The MR antagonist did not produced any impairment, so that suggest that MR is not involved in regulation of the glucocorticoid effects on memory consolidation. The glucocorticoid receptors can influence gene transcription both trough

DNA binding-dependent and DNA binding-independent mechanisms. Experiments with mice which have a mutation in the GR that impair DNA binding showed that the enhanced effect of glucocorticoids on memory is depended on the DNA binding capacity of the GR [9].

Nevertheless, there is increasing evidence that glucocorticoids also use several other, non-genomic pathways to induce an effect. Several effects cannot be explained by the genomic pathway, for example some physiological and behavioral effects that occur as a result of glucocorticoids release appear too fast to be mediated through a genomic action. This review is about the non-genomic pathway of glucocorticoids. These non-genomic, rapid effects of glucocorticoids are caused by activation of several downstream signaling cascades and several neurotransmitters (e.g. norepinephrine and endocannabinoids) which eventual enhance memory consolidation and reduce memory retrieval and working memory. Nevertheless, the non-genomic mechanisms of glucocorticoids during memory consolidation are poorly understood. This review is about non-genomic mechanisms of glucocorticoids in the brain and other tissues, in order to understand the glucocorticoid mechanisms on cellular level during emotional memory consolidation.

**Non-genomic effects of glucocorticoids**

Glucocorticoids can initiate responses at or near the cell surface, they can change signaling pathways used by membrane receptors and there have been found membrane receptors for these steroids. Roozendaal and coworkers did some tests with corticosterone and BSA (Bovine Serum Albumin), a membrane-impermeable protein, which prevent corticosterone diffusion through the membrane into the cytoplasm. They showed that even when corticosterone can not enter the cell it has an influence on memory consolidation, so it must have membrane bound receptors which can induce the memory enhancing effects. Moreover, when gene transcription and protein synthesis are blocked or not present, glucocorticoids can cause responses independently of the genomic pathway [14]. Aldosterone has in vitro effect on the sodium exchange in dog erythrocytes, these cells lack a nucleus and thus a genomic response cannot appear. The effects of aldosterone in these cells must depend on non-genomic pathways [15].

The non-genomic effects of glucocorticoids can be classified in three groups, based on the variety of the concerned receptors: effects not mediated by a receptor, effects mediated by cytosolic receptors, and effects mediated by membrane-bound receptors [16]. Glucocorticoids signaling not mediated by a receptor frequently have direct effects on cell membranes. Physicochemical properties of the plasma membrane could be modified by glucocorticoids in order to alter the membrane permeability to ions [16]. Non-genomic actions of glucocorticoids mediated through the classical, cytosolic GR are suggested by the association of kinases with the GR complex in the cytoplasm of the cell. Binding of GCs to the cytosolic GR cause dissociation of the kinase from the GR complex to phosphorylate its targets and induce a signaling cascade. Most of the time non-genomic effects are caused by GC binding to a membrane-bound receptor. The class of membrane-bound receptor effects could be subdivide in three categories: effects mediated by membrane-bound GR, effects mediated by a membrane-bound non-GR glucocorticoid receptor, and effects mediated by proteins that are not primarily receptors [16]. A great amount of evidence supporting a modified form of the classical GR as a membrane-bound receptor which mediates some of the rapid effect of GCs, for instance, in immune cells. However, current evidence of non-genomic effects of GCs in the central nervous system suggests that receptors other than GR may also be involve, because some of the non-genomic effects could not be eliminated by a specific GR antagonist. The effects mediated by proteins that are not primarily receptors include, for example, the effects of GCs directly on proteins in the cell membrane like ion channels [16].

Most organs and physiological systems are sensitive to glucocorticoids. This review will point out the non-genomic effects of GCs on the calcium content in different cell types in order to obtain insight in possible non-genomic mechanisms of glucocorticoids in the brain.

**Non-genomic effects of glucocorticoids on intracellular calcium content**

Calcium transport is of vital importance for almost all the cells in an organism and in a large number of cells GCs influence this transport mechanism non-genomically [26]. Glucocorticoids induce opposite effects on the intracellular calcium concentration ($[Ca^{2+}]i$). In cortical collecting ducts [19] and vascular smooth muscle cells [20] the $[Ca^{2+}]i$ increase through GC treatment, whereas the $[Ca^{2+}]i$ in rat thymocytes [21], human leukocytes, airway smooth muscle cells [22], human lymphoblast's [23] and bronchial epithelial cells [18] decreases after GC treatment.

*Glucocorticoid effect on intracellular calcium in bronchial epithelium*
Urbach and coworkers [18] showed that the synthetic glucocorticoid dexamethasone decrease the $[Ca^{2+}]i$ in bronchial epithelial cells. Their data suggest that the $[Ca^{2+}]i$ is modulated via non-genomic signaling since blocking the classical GR or MR and the transcription pathway do not affect the effect of dexamethasone. Treatment with the cyclic- AMP (cAMP) or the cAMP-dependent protein kinase (PKA) inhibitors block the effect of dexamethasone, and therefore, it is assumed that cAMP and PKA play an important role in the GC pathway in bronchial epithelium. Inhibition of the dexamethasone effect by $Ca^{2+}$ ATPase pump inhibitor, suggest that dexamethasone stimulates $Ca^{2+}$ ATPases of intracellular stores in order to release calcium. Dexamethasone also reduced the calcium dependent $Cl^-$ secretion, induced by apical exposure to ATP, by 30%. Urbach and coworkers where the first who approved that dexamethasone impair the effect of apical ATP, leading to inhibition of the $Cl^-$ secretion in human bronchial epithelial cells [18]. The rapid inhibition of $Cl^-$ secretion induced by dexamethasone is associated with a rapid decrease in the $[Ca^{2+}]i$ via a non-genomic pathway which not include the classical glucocorticoid receptor.

Dexamethasone has a rapid non-genomic inhibitory effect on the intracellular $Ca^{2+}$ signaling in human airway epithelium. Aldosterone, known as a mineralocorticoid [19], induced a similar effect on airway epithelial cells [52], and therefore, it may involve the same intracellular signaling pathway. Aldosterone can not stimulate a further response, when dexamethasone have been added first and had induced a rapid response. This indicates that aldosterone and dexamethasone interact through the same receptor in order to induce a rapid response on the intracellular calcium concentration [18]. As aldosterone is a MR agonist [19], both, aldosterone and dexamethasone probably interfere with the MR. However, the effect of aldosterone was also not blocked by the MR antagonist, which suggest a non-genomic mechanism for the intracellular calcium content in airway epithelium, that is not mediated by the classical mineralocorticoid receptor.

Other glucocorticoids like triamcylone acetonide and budesonide do not produce a decrease in the calcium concentration in airway epithelium. The difference in producing the non-genomic effect between these glucocorticoids could be explained by the relative lipophilicity. Dexamethasone has a lower relative lipophilicity compared with triamcylone acetonide and budesonide. This suggest that because of the lower lipophilicity, dexamethasone can interact directly with the membrane in order to induce the non-genomic effect [18].
Other steroids, like cortisol, progesterone, estradiol and estrogen, also decrease $[Ca^{2+}]i$ through activation of $Ca^{2+}$ ATPases which affect the membrane fluidity [28, 29].

Verrière and coworkers [34] indicate that dexamethasone also stimulate rapidly the $Na^+/H^+$ exchange in bronchial epithelial cells via a non-genomic mechanism. This response is not mediated through the glucocorticoid or mineralocorticoid nuclear receptor (showed with receptor antagonists), but acts via PKA and the mitogenactivated protein kinase (ERK 1/2 MAP kinase) signaling pathway. Regulation of the intracellular pH in airway epithelium is dependent on several acid extruders such as the $Na^+/H^+$ exchanger and $H^+$/ATPase pump. The $Na^+/H^+$ exchanger inhibitor EIPA showed that the effect of dexamethasone is dependent on the $Na^+/H^+$ exchange and when this exchange is blocked the basal pHi rapidly decreases. This indicate that the regulation of the intracellular pH of bronchial epithelial cells is dependent of the $Na^+/H^+$ exchange. The activation of $Na^+/H^+$ exchange occur through protein phosphorylation. Experiments with PKA and ERK 1/2 MAP kinase inhibitors showed that the effect of dexamethasone on the pHi is

dependent on these kinases. Dexamethasone activates the $Na^+/H^+$ exchange through up-regulation of PKA and ERK 1/2 MAP kinase [34].

In brief, in the bronchial epithelium, GCs with a low lipophilicity directly interact with the cell membrane in order to interfere with the $Ca^{2+}$ ATPase pump. Dexamethasone seems to block the effect of ATP, and decrease the intracellular calcium concentration which rapidly inhibits the $Cl^-$ secretion. It is assumed that cAMP and PKA play an important role in this pathway. Dexamethasone also influence the $Na^+/H^+$ exchange in bronchial epithelial cells via up-regulation of PKA and ERK 1/2 MAP kinase in order to regulate the pHi. In this way, GCs can recover the intracellular pH after an acid load.

*Glucocorticoid effect on intracellular calcium in cortical collecting duct epithelium*
In the distal nephron, particularly the cortical collecting duct (CCD), the glucocorticoid aldosterone promotes electrogenic salt reabsorption by several mechanisms in the epithelium [19]. These mechanisms influence eventually apical $Na^+$ channels, $K^+$ channels and the basolateral $Na^+/K^+$ pump and are activated by aldosterone binding to the mineralocorticoid receptor. The activated receptor complex diffuse into the nucleus, where it activates transcription and translation of new proteins which can alter the membrane permeability. The effect onset latency is one till two hours, so these effects are classified as the genomic response of aldosterone in CCD cells. The onset latency of the non-genomic response is much faster, namely seconds till minutes. The non-genomic response has been found in epithelia of the distal colon [30] and sweat gland [31]. However, the aldosterone signal transduction pathway is not yet fully understood, multiple intracellular signaling pathways are found in smooth muscle cells [32], endothelial cells and leukocytes [33]. Also the receptors and transport targets are multiple. These pathways involve $Ca^{2+}$ exchange, inositol triphosphate ($IP_3$), $Na^+/H^+$ exchange and protein kinase C (PKC).

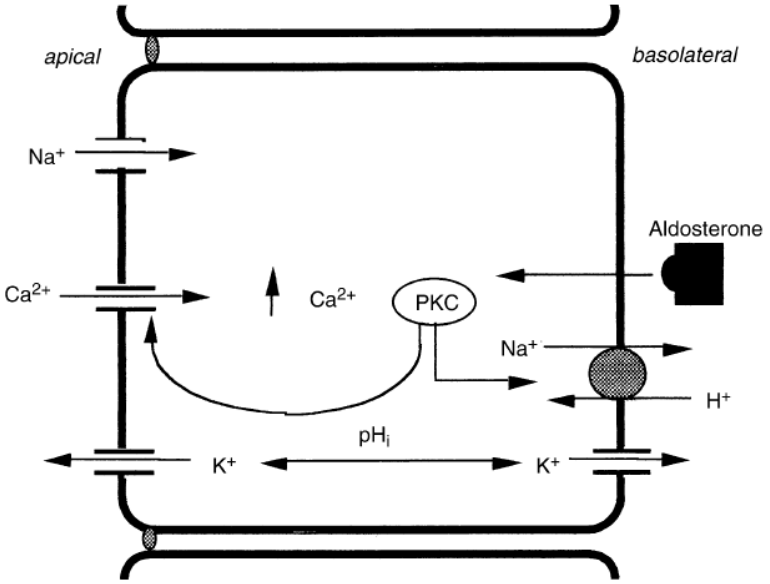


**Figure 1: Cortical Collecting Duct (CCD) cell model for the non-genomic pathway of corticosteroids [19].** The corticosteroid Aldosterone binds to cytosolic or membrane-bound unidentified 'receptor', which activates protein kinase C (PKC) in order to increase the intracellular calcium concentration. The $Ca^{2+}$/PKC complex regulate the basolateral $Na^+/H^+$ exchange, and the resultant intracellular alkalinization activates basolateral $K^+$ channels. The decrease of $K^+$ prepares the cell for the later genomic up-regulation of sodium absorption.

Harvey and Higgins described a non-classical pathway for aldosterone in mouse CCD cells, these cells do not express the classic mineralocorticoid receptor [19]. In contrast to the results in airway epithelium, aldosterone increases the intracellular calcium levels in these CCD cells. This effect is independent of protein synthesis and thus non-genomic, because addition of the transcription inhibitor actinomycin D do not affect the aldosterone effect. This also applies for addition of the MR antagonist spironolactone, which is logical because the CCD cells do not express the classical mineralocorticoid receptor. This suggest a role for an unidentified receptor molecule. The increase in calcium through aldosterone is blocked by a protein kinase C inhibitor

chelerythrine chloride. So PKC plays a central role in calcium modulation, in turn affecting basolateral $Na^+/H^+$ exchange. These data are processed in a non-genomic model for CCD cells (Fig. 1). The decrease of $K^+$ prepares the cell for the later genomic up-regulation of sodium absorption [19]. The calcium increase is possible because these cells have the capacity to mobilize calcium from $IP_3$-sensitive intracellular pools. However, the study of Harvey and Higgins also show that basolateral sodium is needed to induce the $[Ca^{2+}]i$ increase. Addition of the sodium transport inhibitor amiloride had opposite effects: addition to the basolateral side of the cell caused an increased $[Ca^{2+}]i$ ; in contrast, addition to the apical side of the cell caused a decreased $[Ca^{2+}]i$.

There is also seen an increase in $[Ca^{2+}]i$ by the steroids progesterone and estradiol in mouse CCD cells, whereas the glucocorticoid hydrocortisone had no effect [19]. In the renal epithelium, the glucocorticoid dexamethasone also has an enhancing effect on the intracellular calcium concentration, in contrast to the effect of dexamethasone on airway epithelium as seen above.

In summary, aldosterone activates epithelium absorptive transport via a non-genomic response, which includes PKC activation, basolateral $Na^+/H^+$ exchange and an increase in intracellular calcium. This response is not mediated via activation of the classical MR, but aldosterone binds to cytosolic or membrane-bound unidentified receptor in order to induce the effect.

*Glucocorticoid effect on intracellular calcium in colon epithelial cells*
Studies with human distal colon cells showed, just like in the cortical collecting duct, that the intracellular calcium concentration rapidly increases after stimulation with aldosterone [30, 47]. The calcium influx is regulated in some way by PKC, because inhibition of PKC activity by chelerythrine chloride abolished the effect of aldosterone on the calcium influx [47]. However, glucocorticoids like hydrocortisone and dexamethasone do not show any effect on $[Ca^{2+}]i$. These results are also found in isolated rat distal colonic crypts [48], and in a PKC dependent verapamil-sensitive $Ca^{2+}$ channel in the human colonic epithelial cell line T84 [49].

Researchers examined the role of aldosterone on ion transport control in the distal colon, interested in the influence of aldosterone in the absorption and secretion mechanisms. Their results suggest that these mechanisms are regulated by $K^+$ recycling [30]. Basolateral $K^+$ recycling via $K_{ATP}$ channels seems important for maintenance of cellular electrochemical homeostasis during $Na^+$ absorption, meanwhile $K_{Ca}$ channels are necessary for $Cl^-$ secretion. Changes in the intracellular calcium concentration by aldosterone have opposite effects on the $K_{ATP}$ and $K_{Ca}$ channels. Increasing the $[Ca^{2+}]i$ activates the $K_{Ca}$ channels and histamine release for $Cl^-$ secretion. Inhibition of this channel blocked the $Cl^-$ secretion, showing that the $K_{Ca}$ channels are needed for this effect. Inhibition of the $K_{ATP}$ channel makes no difference in $Cl^-$ secretion after aldosterone stimulation. On the other hand, inhibition of the $K_{ATP}$ channel block $Na^+$ absorption and inhibition of the $K_{Ca}$ channel have no effect on $Na^+$ absorption. So the $K_{ATP}$ channel is needed for regulation of $Na^+$ absorption.

Moreover, it was shown that the rapid effect of aldosterone on $K^+$ channels depends on PKC activation and arachidonic acid metabolism [30]. Aldosterone can bind directly to PKC for activation of the non-genomic cascade, but the involvement of arachidonic acid would imply that there is unidentified membrane-associated receptor. Also an important role in this cascade is for $Na^+/H^+$ exchange, because inhibition of this exchange abolish the aldosterone effect.

Briefly, not all the glucocorticoids have an effect on the $[Ca^{2+}]i$ in colon epithelial cells. However, aldosterone induce a rapid increase in the $[Ca^{2+}]i$ which involve PKC signaling. Ion transport in the distal colon, including secretion and absorption mechanisms, is regulated by the $K^+$ channels. It has been shown that the rapid effect of aldosterone on $K^+$ channels depends on PKC activity and arachidonic acid metabolism.

*Glucocorticoid effect on intracellular calcium on adrenocortical cells*
Corticosterone enhances the calcium signaling and pregnenolone synthesis in adrenocortical cells after stimulation with ACTH [35]. This effect on calcium appears within seconds after stimulation and blockade of the protein synthesis do not affect this effect, thus this suggest a non-genomic pathway. ACTH-induced steroidogenesis was suppressed by inhibition of $Ca^{2+}$ channels, this indicate that calcium is very important for the steroidogenesis by ACTH in adrenocortical cells. These data suggest that calcium may be a second messenger for ACTH. Other evidences showed that also ATP [36] and Angiotensin II [37, 38, 39] require an increased calcium level to induce steroidogenenis. It is thought that calcium is important for these processes because it increases the availability of cholesterol to cytochrome P450scc, which is the rate-limiting step in genesis [35]. Calcium elevation also can stimulate enzymes involved in the synthesis of NADPH, which also improve steroidogenesis. Additionally, corticosterone can stimulate cAMP and arachidonic acid metabolites which might also contribute to the enhancing effect on steroidogenesis just like calcium. Experiments with classic glucocorticoid receptor antagonists revealed that the effect of corticosterone on adrenocortical cells is not mediated via the classical receptors, because the effect was not affected by the antagonists. However, corticosterone-BSA also increase calcium levels in adrenocortical cells, which suggest that glucocorticoids probably bind to an unidentified receptor on the cell membrane. In fact, it was demonstrated that corticosterone binds to a specific class of proteins with a binding constant of 77 nm [35].

The main conclusion of the research after the corticosterone effect on adrenocortical cells is that corticosterone almost certainly activates ACTH-induced calcium signalling and steroidogenesis [35]. The effect is achieved by corticosterone binding to an unidentified receptor on the cell membrane of the adrenocortical cells.

**Glucocorticoids effects in the brain**

Just like in the peripheral tissues as described above, at neuronal membranes glucocorticoids may act through membrane receptors, and signal transduction through these receptors may allow for rapid modulation of synaptic transmission as well as modulation of membrane ion currents [2].

Regarding glucocorticoids effects on memory, it has been demonstrated that in the BLA, glucocorticoids facilitate a downstream cascade, including cAMP and protein kinases to eventually enhance memory consolidation. As mention in the introduction, the enhancing effects of glucocorticoids are dependent of the noradrenergic system. NE stimulates the β-adrenergic receptor, which activates the cAMP/PKA pathway in order to increase the levels of phosphorylated c-AMP response-element binding protein (pCREB) [40, 41]. There is thought that glucocorticoids affect the norepinephrine and pCREB signaling in order to enhance memory consolidation via chromatin modification [6]. Nevertheless, blocking GR activity or PKA do not result in memory consolidation. This indicates that the activation of GR and PKA is necessary for long-term memory formation.

Glucocorticoids also interact with another additional downstream mechanism which includes cAMP and PKA, namely the endocannabinoid system. Cannabinoid (CB) receptors are highly expressed in the BLA, Campolongo and coworkers suggest that these receptors are also involved in the regulation of memory consolidation [42]. Their findings showed an enhanced or impaired emotional memory consolidation after intra-BLA infusion of a CB1 receptor agonist or antagonist, respectively. The CB1 receptor antagonist also blocked the memory enhancing effect of corticosterone. Administration of corticosterone increases the endocannabinoids levels in the amygdala [43]. These data have led to a endocannabinoids model named the Tasker model (Fig. 2) [44]. Postsynaptic activation of membrane-bound GRs activates the cAMP/PKA cascade which eventually induces the synthesis of endocannabinoids. Endocannabinoids bind to presynaptic CB1 receptors on GABAergic neurons, where they inhibit the inhibition of NE neurons [44, 45, 46]. The NE neuron is no longer suppressed, and therefore NE release is enhanced. As previously said, NE can increase memory consolidation.

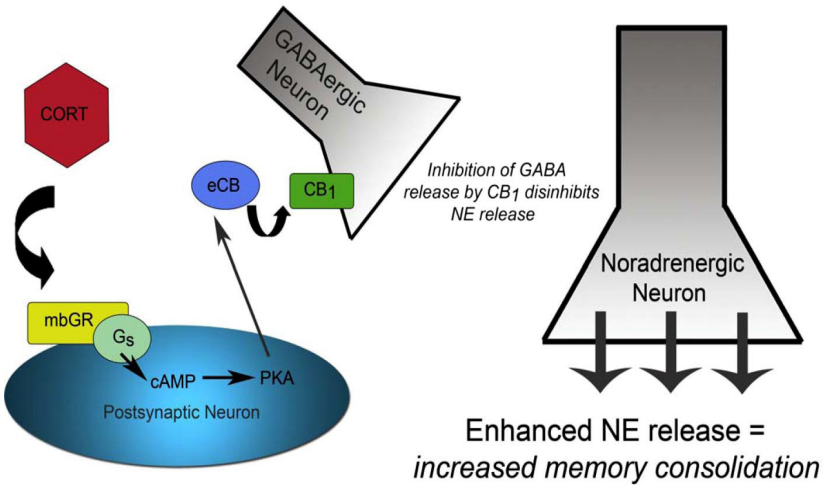


**Figure 2: the Tasker model [44].** Corticosterone (CORT) binds to membrane bound glucocorticoid receptors (mbGR) which activate the cAMP/PKA pathway to induce the synthesis of endocannabinoids (eCB). The endocannabinoids bind to the presynaptic cannabinoid receptor one (CB1) of GABAergic neurons to inhibit the GABA release. Noradrenergic neurons are no longer suppressed by GABA so there would be more NE release, representing an increased memory consolidation.

The BLA interacts with the medial prefrontal cortex (mPFC) during memory consolidation. The mPFC is a brain region which is involved in higher-order cognitive and affective processing as well as executive function [50, 51]. Recently is found that memory enhancement by a GR agonist requires a rapid increase in the phosphorylation of extracellular signal-regulated kinase 1/2 (pERK 1/2) in the BLA and the mPFC [52]. These intracelullar mechanism of glucocorticoids is similar to the one observed in bronchial epithelial cells, where dexamethasone cause an upregulation of PKA and ERK 1/2 MAP kinase to influence the $Na^+/H^+$ exchange in order to regulate the pHi.

**Discussion**

The non-genomic mechanisms of glucocorticoids affect the function of several cell types in diverse tissues and systems. Glucocorticoids activate intracellular signaling pathways in epithelium such as those involving calcium and protein kinases to modulate at the end the basolateral membrane ion transporters, particularly $Na^+/H^+$ exchange and $K^+$ channels. In this way, the epithelial cells shift to an absorptive transport mode.
Glucocorticoids furthermore affect the membrane transport of other ions, like $K^+$, $Na^+$, $H^+$ and $Cl^-$. Discrepancies between the different tissues can be caused by different interactions between $Ca^{2+}$ and other ion transport. Also the varying protein kinase responses observed in the different cell types may be explained by tissue and transport pathway specificity. Diverse effects are induced by the different glucocorticoids, so the mechanism not only depends on the target but also on the structure and the lipophilicity of the glucocorticoid.

It is important to look very critically to the classification of a non-genomic effect, because it could differ between articles. Makara and Haller developed a set of three criteria to separate genomic and non-genomic effects [17,16]. These criteria include rapidity of onset, independence of the genome and independence from classical nuclear steroid receptors. These criteria are developed on the basis of the following arguments. A certain time is needed for the effects of the genomic pathway to occur, if the rapidity of onset is elevated there must be concluded that the effect is caused independent of the genomic pathway. If the effects persist either in the presence of inhibitors of translation or transcription, or occur in cells where the genome is lacking, it may be conclude that the effect is independent of the genomic pathway. According to these authors, MR and GR are nuclear receptors, who in general act via genomic signaling like gene translation or transcription. Effects which occur without activating these receptors are most likely independent of the genomic pathway [17,16].
However, these criteria are not always faultless in making a distinction between genomic en non-genomic effects. The genomic effects are characterized by a latency of onset lasting several hours, but some genomic gene transcription could occur very rapid in a few minutes, so there is

an overlap between genomic and non-genomic onset rapidity. There are examples of systems that appear to be free of genome, but still have some form of translation and effects mediated by mitochondrial genes may be even faster than those mediated by the nuclear DNA. The third criteria is very debatable, nowadays there is growing amount of evidence which indicates that the classical GR also play a role in non-genomic signaling, and is expressed on the cell membrane [17]. In general, effects which are not affected by inhibition of both receptors suggest a non-genomic mechanism. However, some studies only block one of the classical receptors and then conclude if the effect is genomic or not. This is not sufficient, because the effect can also appear via the other classical receptor. The effects produced by glucocorticoids conjugated with BSA are also considered to be non-genomic. But this may provide false positive evidence, because this BSA conjugated GCs can activate the receptors on the cell membrane. Taken together, the systems are actually too complicated to make such a simple distinction between genomic and non-genomic.

Another discussion point is the dose of glucocorticoids in the different studies. If the doses of glucocorticoids are within physiological range, the effects are comparable with the normal situations in an organism. However, some studies have used much higher doses, which is only interesting for the pharmacology. Most of the researchers did not measured the glucocorticoid levels after administration. So in the majority of the studies it can not be determined if the effects are physiological or pharmacological, and unfortunately, no comparison can be made.

As seen above there are multiple interactions between glucocorticoid-sensitive proteins, so it is difficult to determine the primary site of action. The binding of GCs to a membrane protein appears to be non-genomic and there seems to be a relation between the non-genomic and genomic mechanisms. There is a hypothesis which says that the non-genomic effects are induced in order to prepare the cell to the forthcoming genomic effects, and another hypothesis also argue that the non-genomic mechanisms fill the temporal gab between the immediate need for change and the slow expression of genomic effects [26].

This review obtained some insight into the non-genomic mechanisms of glucocorticoids. It seems that GCs have a broad spectrum of effects on cellular level. In peripheral tissues changes in the intracellular calcium content activate different mechanisms, protein kinases such as PKA, PKC and ERK 1/2 MAP play an important role in these mechanisms. These pathways are also rapidly activated by GCs when the classic receptors GR or MR are blocked, which strongly suggest an unidentified receptor which is involved in the non-genomic effect. In neuronal membranes and synapses GCs also act through membrane receptors in order to activate a signal transduction cascade which includes the same protein kinases. This transduction cascade allows rapid modulation of synaptic transmission and membrane ion transport. During memory consolidation the protein kinases, which are mentioned above, are also activated by GCs in some parts of the brain. Concluding, the non-genomic mechanisms of GCs observed in peripheral cell types are similar to some mechanisms that are observed in the brain during memory consolidation. Taken together, all these studies indicate that GCs may act also through non-genomic mechanisms in the brain, like in the periphery, to interfere with synaptic transmission and therefore, with memory consolidation. Much more work has to be done to explore all the details of the non-genomic pathway.

## References


1. de Kloet, E.R. (2000) Stress in the brain. *Eur J Pharmacol* 405: 187-198
2. Parger, E.M., Johnson, L.R. (2009) Stress at the synapse: signal transduction mechanisms of adrenal steroids at neuronal membranes. *Neuroscience* 2
3. Cannon, W.B. (1929) Bodily changes in pain, hunger, fear, and rage: an account of recent researches into the function of emotional excitement.
4. Chrousos, G.P., Kino, T. (2009) Glucocorticoid signalling in the cell: expanding clinical implications to complex human behavioral and somatic disorders. *Ann N Y Acad Sci.* 1179: 153-166
5. Tully, K., Li, Y., Tsvetkov, E. Bolshakov, V.Y. (2007) Norephinephrine enables the induction of associative long-term potentiation at thalamo-amygdala synapses. *Proc Natl Acad Sci USA* 104: 14146-14150
6. Roozendaal, B., Hernandez, A., Cabrera, S.M., Hagewoud, R., Malvaez, M., Stefanko, D.P., Haettig, J., Wood, M.A. (2010) Membrane-associated glucocorticoid activity is necessary for modulation of long-term memory via chromatin modification. *The journal of Neuroscience* 30(14): 5037-5046
7. Oitzl, M.S., de Kloet, E.R. (1992) Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. *Behav Neurosci* 106: 62-71
8. Oitzl, M.S., de Kloet, E.R., Joels, M., Schmid, W., Cole, T.J. (1997) Spatial learning deficits in mice with a targeted glucocorticoid receptor gene disruption. *Eur J Neurosci* 9: 2284-2296
9. Oitzl, M.S., Reichardt, H.M., Joels, M., de Kloet, E.R. (2001) Point mutation in the mouse glucocorticoid receptor preventing DNA binding imparis spatial memory. *Proc Natl Acad Sci USA* 98: 12790-12795
10. Reul, J.M., de Kloet, E.R. (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology* 117: 2505-2511
11. Roozendaal, B. (2000) Glucocorticoids and the regulation of memory consolidation. *Psychopharmacology (Berl)* 198: 551-563
12. Oitzl, M.S., Fluttert, M., de Kloet, E.R. (1994) The effect of corticosterone on reactivity to spatial novelty is mediated by central mineralocorticosteroid receptors. *Eur. J. Neurosci* 6: 1072-1079
13. de Kloet, E.R. (1991) Brain corticosterone receptor balance and homeostatic control. *Front Neuroendocrinol* 12: 95-164
14. Moore, F.L., Evans, S.J. (1999) Steroid hormones use non-genomic mechanisms to control brain functions and behaviors: A review of evidence. *Brain Behav Evol* 54: 41-50
15. Wehling, M. (1997) Specific, nongenomic actions of steroid hormones. *Annu Rev Physiol* 59: 365-393
16. Evanson, N.K., Herman, J.P., Sakai, R.R., Krause, E.G. (2010) Nongenomic actions of adrenal steroids in the central nervous system. *Journal of Neuroendocrinology*
17. Makara, G.B., Haller, J. (2001) Non-genomic effects of glucocorticoids in the neural system. Evidence, mechanisms and implications. *Progress in Neurobiology* 65: 367-390
18. Urbach, V., Walsh, D.E., Mainprice, B., Bousquet, J., Harvey, B.J. (2002) Rapid non-genomic inhibition of ATP-induced $Cl^-$ secretion by dexamethasone in human bronchial epithelium. *J Physiol* 545: 869-878
19. Harvey, B.J., Higgins, M. (2000) Nongenomic effect of aldosterone on $Ca^{2+}$ in M-1 cortical collecting duct cells. *Kidney International* 57: 1395-1403
20. Steiner, A., Vogt, E., Locher, R., Vetter, W. (1988) Stimulation of the phosphoinositide signalling system as a possible mechanism for glucocorticoid action in blood pressure control. *Journal of Hypertension* 6: 366-368
21. Buttgereit, F., Krauss, S., Brand, M.D. (1997) Methylprednisolone inhibits uptake of $Ca_{2+}$ and $Na_+$ ions into concanavalin A-stimulated thymocytes. *Biochemical* Journal 1326: 329-332
22. Chhabra, S.K., Khanduja, A., Jain, D. (1999) Decreased sodiumpotassium and calcium adenosine triphosphatase activity in asthma: modulation by inhaled and oral corticosteroids. *Indian Journal of Chest Disease and Allied Sciences 41*: 15-26
23. Gardner, J.P., Zhang, L. (1999) Glucocorticoid modulation of $Ca_{2+}$ homeostasis in human B lymphoblasts. *J Physiol* 514: 385-396
24. McGaugh, J.L., Roozendaal, B. (2002) Role of adrenal stress hormones in forming lasting memories in the brain. *Current opinion in Neurobiology* 12: 205-210
25. Bear, M.F., Connors, B.W., Paradiso, M.A. (2007) Neuroscience, exploring the brain. (Third edition) USA: Lippincott Williams & Wilkins.

26. Haller, J., Mikics, E., Makara, G.B. (2008) The effect of non-genomic glucocorticoid mechanisms on bodily functions and the central neural system. A critical evaluation of findings. *Frontiers in Neuroendocrinology* 29: 273-291
27. McGaugh, J.L. (2000) Memory: a century of consolidation. *Science* 287: 248-251
28. Massa, E.M., Morero, R.D., Bloj, B., Farias, R.N. (1975) Hormone action and membrane fluidity: effect of insulin and cortisol on the Hill coefficients of rat erythrocyte membranebound acetylcholinesterase and ($Na^+ + K^+$) ATPase. *Biochemical and Biophysical Research Communications* 66: 115-122
29. Whiting, K.P., Restall, C.J., Brain, P.F. (2000) Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechaisms. *Life Sciences* 67-743-757
30. Maguire, D., MacNamara, B., Cuffe, J.E., Winter, D., Doolan, C.M., Urbach, V., O'Sullivan, G.C., Harvey, B.J. (1999) Rapid responses to aldosterone in human distal colon. *Steroids* 64: 51-63
31. Hegarty, J.M., Harvey, B.J. (1999) Aldosterone accelerates a $Na^+/H^+$ exchange dependent pH recorvery after acid loading in cultured human eccrine sweat gland epithelial cells. *J Physiol* 517P: 20P
32. Christ, M., Meyer, C., Sippel, K., Wehling, M. (1995) Rapid aldosterone signaling in vascular smooth muscle cells: Involvement of phosholipase C, diacylglycerol and protein kinase C. *Biochem Biophys Res Commun* 213: 123-129
33. Christ, M., Eisen, C., Aktas, J., Theisen, K., Wehling, M. (1993) The inositol-1,4,5-triphosphate system is involved in rapid effects of aldosterone in human mononuclear leukocytes. J Clin Endocrinol 77: 1452-1457
34. Verrière, V.A., Hynes, D., Faherty, S., Devaney, J., Bousquet, J., Harvey, B.J., Urbach, V. (2005) Rapid effects of dexamethasone on intracellular pH and $Na^+/H^+$ exchanger activity in human bronchial epithelial cells. *The Journal of Biological Chemistry* 43: 35807-35814
35. Chiyo, T., Yamazaki, T., Aoshika, K., Kominami, S., Ohta, Y. (2007) Corticosterone enhances adrenocorticotropin-induced calcium signals in bovine adrenocortical cells. Endocrinology 144: 3376-3381
36. Niitsu, A. (1992) Calcium is essential for ATP-induced steroidogenesis in bovine adrenocortical fasciculate cells. *Jpn J Pharmacol* 60: 269-274
37. Foster, R., Lobo, M.V., Rasmussen, H., Marusic, E.T. (1981) Calcium: its role in the mechanism of action of angiotensin II and potassium in aldosterone production. *Endocrinology* 109: 2196-2201
38. Bird, I.M., Meikle, I., Williams, B.C., Walker, S.W. (1989) Angiotensin II-stimulated cortisol secretion is mediated by a hormone-sensitive phospholipase C in bovine adrenal fasciculate/reticularis cells. *Mol Cell Endocrinol* 64: 45-53
39. Guillon, G., Trueba, M., Joubert, D., Grazzini, E., Chouinard, L., Côté, M., Payet, M.D., Manzoni, O., Barberis, c., Robert, M., Gallo-Payet, N. (1995) Vasopressin stimulates steroid secretion in human adrenal glands : comparison with angiotensin-II effect. *Endocrinology* 136: 1285-1295
40. Roozendaal, B., Quirarte, G.L., McGaugh, J.L. (2002) Glucocorticoids interact with the basolateral amygdala beta-adrenoceptor—cAMP/PKA system in influencing memory consolidation. *Eur J Neurosci* 15: 553-560
41. Roozendaal B, Okuda S, Van der Zee EA, McGaugh JL (2006) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proc Natl Acad Sci USA* 103: 6741-6746
42. Campolongo, P., Roozendaal, B., Trezza, V., Hauer, D., Schelling, G., McGaugh, J.L., Cuomo, V. (2009) Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. *Proc Natl Acad Sci USA* 106: 4888-4893
43. Hill, M.N., Karatsoreos, I.N., Hillard, C., McEwen, B.S. (2010) Rapid induction of limbic endocannabinoid signalling by glucocorticoid hormones in vivo. *Psychoneuroendocrinol* in press
44. Hill, M.N., McEwen, B.S. (2009) Endocannabinoids: the silent partner of glucocorticoids in the synapse. *Proc Natl Acad Sci USA* 106: 4579-4580
45. Di, S., Malcher-Lopes, R., Halmos, K.C., Tasker, J.G. (2003) Nongenomic glucocortiod inhibition via endocannabinoids release in the hypothalamus: a fast feedback mechanism. *J Neurosci* 23: 4850-4857
46. Coddington, E., Lewis, C., Rose, J.D., Moore, F.L. (2007) Endocannabinoids mediate the effects of acute stress and corticosterone on sex behaviour. *Endocrinology* 148: 493-500

47. Doolan, C.M., O'Sullivan, G.C., Harvey, B.J. (1998) Rapid effects of corticosteroids on cytosolic protein kinase C and intracellular calcium concentration in human distal colon. *Mol Cell Endocrinology* 138: 71-79
48. Doolan, C.M., Harvey, B.J. (1996) Modulation of cytosolic protein kinase C and calcium ion activity by steroid hormones in rat distal colon. *J Biol Chem* 271: 8763-8767
49. Doolan, C.M., Harvey, B.J. (1997) Rapid effects of steroid hormones on free intracellular calcium in the colonic epithelial cell line T84. *J Physiol Proc* 499: 5
50. Davidson, R.J. (2002) Anxiety and affective style: role of prefrontal cortex and amygdala. *Biol Psychiatry* 51: 68-80
51. Quirk, G.J., Gehlert, D.R. (2003) Inhibition of the amygdala: key to pathological states? *Ann NY Acad* Sci 985: 263-272
52. Urbach, V., Harvey, B.J. (2001) Rapid and non-genomic reduction of intracellular [$Ca^{2+}$] induced by aldosterone in human bronchial epithelium. *Journal of Physiology* 537: 267-275