

Review

Non-genomic Effects of Glucocorticoids: An Updated View

Reynold A. Panettieri,¹ Dedmer Schaafsma,² Yassine Amrani,³ Cynthia Koziol-White,¹ Rennolds Ostrom,⁴ and Omar Tliba^{5,*}

Glucocorticoid (GC) anti-inflammatory effects generally require a prolonged onset of action and involve genomic processes. Because of the rapidity of some of the GC effects, however, the concept that non-genomic actions may contribute to GC mechanisms of action has arisen. While the mechanisms have not been completely elucidated, the non-genomic effects may play a role in the management of inflammatory diseases. For instance, we recently reported that GCs ‘rapidly’ enhanced the effects of bronchodilators, agents used in the treatment of allergic asthma. In this review article, we discuss (i) the non-genomic effects of GCs on pathways relevant to the pathogenesis of inflammatory diseases and (ii) the putative role of the membrane GC receptor. Since GC side effects are often considered to be generated through its genomic actions, understanding GC non-genomic effects will help design GCs with a better therapeutic index.

Mechanism of Action of GCs

GCs primarily mediate their effects by activating the ubiquitously expressed intracellular **GC receptor** (GR; see [Glossary](#)) [1]. In its inactive state, the GR resides in the cytoplasm and, upon ligand activation, it translocates to the cell nucleus to interact with GC response elements, thereby producing **genomic actions** that alter protein expression. Interestingly, evidence suggests that GCs also manifest almost immediate **non-genomic actions** on several signaling processes [2]. GC non-genomic effects involve nonspecific interactions with the cell membrane, or specific interactions with cytosolic GRs (cGRs) or membrane-bound GRs (mGRs) (Table 1). This review article summarizes the current knowledge on non-genomic effects of GCs, with a focus on GR-mediated events and GR-associated signaling pathways. Where appropriate, potential links to inflammatory diseases are highlighted in the main text and their potential impact is discussed in Boxes 1 and 2.

GCs Exert Rapid Effects on Levels of Intracellular Calcium

Studies suggest that GC rapidly (within seconds) modulates basal intracellular calcium ($[Ca^{2+}]_i$) levels and agonist-induced **calcium mobilization** (Tables 2 and 3).

Effects of GCs on Intracellular Calcium Homeostasis

GCs can increase or decrease cytosolic calcium depending on the cell type. Evidence from non-immune cells, such as primary or immortalized human bronchial epithelial cells, consistently demonstrate that acute exposure to GCs, and to a lesser extent to the mineralocorticoid aldosterone, reduces basal $[Ca^{2+}]_i$ [3,4]. Similarly, in rat thymocytes [5] and mouse neuroblastoma cells [6] $[Ca^{2+}]_i$ decreased after acute exposure to GCs, and in cichlid fish pituitary cells cortisol inhibited $[Ca^{2+}]_i$ and reduced prolactin secretion [7]. However, in immune cells, it is

Highlights

GC genomic and non-genomic effects involve distinct mechanisms of action but play complementary roles in mediating the anti-inflammatory effects of GCs.

GCs are mostly used in asthma as a ‘controller’ therapy because of their delayed effects, but since GCs recently have been shown to ‘rapidly’ enhance the effects of bronchodilators, they could be used also as a ‘rescue’ therapy, especially in combination with β_2 agonists.

Compelling evidence proposed the emerging role of (airway) structural cells as a major target for GC non-genomic effects that act through poorly understood, cell-specific mechanisms.

Both inflammatory pathways and non-inflammatory pathways such as calcium mobilization, muscle tone, and reactive oxygen species are targets for the GC non-genomic effects.

Designing a GC able to solely act through non-genomic pathways may prevent some of the GC side effects often engendered by GC genomic effects.

¹Department of Medicine, Rutgers Institute for Translational Medicine and Science, Robert Wood Johnson School of Medicine, New Brunswick, NJ, USA

²Science Impact, Winnipeg, Manitoba R3L 2S9, Canada

³Department of Infection, Immunity and Inflammation, Institute for Lung Health, Leicester Biomedical Research Center Respiratory, Leicester, UK

⁴Department of Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Irvine, CA, USA

⁵Department of Biomedical Sciences, College of Veterinary Medicine, Long Island University, Brookville, NY, USA

*Correspondence: omar.tliba@liu.edu (O. Tliba).

Table 1. Various Criteria (Either Alone or in Combination) Used to Distinguish Genomic Effects from Non-genomic Effects of GCs

GC effect	Acute (simultaneous or within 30 min)	Chronic (delayed)
Genomic effects	–	+
Inhibitory effects of cycloheximide or actinomycin D	–	+
GR involvement	– or +	+
Inhibitory effects of RU486	– or +	+
Type of GR involved	None, membrane GR or cytosolic GR	Cytosolic GR
GR-independent mechanisms	GC interaction with membrane	None

Box 1. Calcium Regulation, ASM Tone, and Asthma Pathogenesis

Because ASM serves as the pivotal tissue regulating bronchomotor tone, changes in the pathways regulating ASM contractile properties may play an important role in the development of abnormal lung function in asthma. Abnormal GPCR-associated calcium homeostasis and ASM shortening may represent one such mechanism. Changes could occur at different levels of the contraction cascade including (i) $[Ca^{2+}]_i$ release from internal stores, (ii) myosin light chain kinase activity, (iii) myosin light chain phosphorylation (pMLC), and (iv) actin–myosin crossbridge cycling leading to cell shortening. Changes in ASM shortening could also be due to changes in sensitivity of the contractile apparatus to $[Ca^{2+}]_i$ initiated by the small GTPase RhoA, which activates ROCK to inactivate myosin light chain phosphatase (MLCP). Decreased MLCP activity results in an increase in pMLC levels for a given level of $[Ca^{2+}]_i$ and thus enhances ASM contractility. It is important to note that there are other parallel pathways where actin polymerization also mediates agonist-induced ASM shortening independently from Ca^{2+} and pMLC but potentially through the phosphorylation of other proteins such as vinculin. Collectively, this evidence suggests that ASM contractile function can mediate airway hyperresponsiveness in chronic airway inflammatory diseases by involving, at least in part, changes in Ca^{2+} regulatory pathways.

Box 2. Role of NOS/NO Signaling in Asthma Pathogenesis

Altered NO production has been implicated in the development of both acute and chronic allergen-induced airways hyperresponsiveness. Production of NO occurs through the action of nitric oxide synthase (NOS), of which three isoforms have been identified thus far: two constitutive (c)NOS isoforms referred as neuronal (n), endothelial (e) NOS, and one inducible isoform called (i) NOS. Upon activation, cNOS isoforms produce relatively low amounts of NO, whereas iNOS can produce high and potentially damaging levels of NO. NO generated by eNOS is associated with beneficial bronchodilatory effects in allergic asthma, but iNOS-derived NO is generally considered detrimental, as it has been linked to, for instance, epithelial damage, inflammatory cell infiltration, and mucus hypersecretion. These detrimental effects are largely due to the accumulation of RNS, including peroxynitrite, which are reaction products of NO and superoxide anions. Since NOS/NO signaling and RNS play key roles in chronic airway inflammatory diseases, including asthma and COPD, an acute role for GC/GR signaling and (inducible and/or endothelial) NOS activity can be envisioned. In-depth studies are warranted to determine whether such functional interaction exists and whether targeting it would provide any therapeutic benefit for asthma.

unclear whether GCs genuinely exert non-genomic effects on basal $[Ca^{2+}]_i$. For example, while acute exposure to GCs was reported to decrease $[Ca^{2+}]_i$ in leukocytes, these leukocytes were obtained from donors who were treated with oral prednisolone for 7 days [8], potentially confounding the results of the study. Similarly, studies in human lymphoblasts show that cortisol markedly reduced basal $[Ca^{2+}]_i$ only after 48 h of treatment [9]. These data argue against a role for non-genomic effects of GCs in altering basal $[Ca^{2+}]_i$ in immune cells.

With regard to the lungs, evidence supports that a variety of GCs differentially modulate basal $[Ca^{2+}]_i$ upon immediate exposure. For instance, the acute inhibitory effects of dexamethasone (within 30 s) on basal $[Ca^{2+}]_i$ in bronchial epithelial cells were comparable to those of triamcinolone acetonide and hydrocortisone, but not budesonide [10]. Interestingly, the GR antagonist RU486 and the protein synthesis inhibitor cycloheximide failed to prevent these acute GC effects, suggesting the involvement of GR-independent and non-genomic pathways. These

Glossary

Calcium mobilization: intracellular process triggered by external stimuli (e.g., contractile agonists) where calcium is released to be engaged in different cellular functions such as increased muscle reactivity and contraction. Calcium is usually acquired from extracellular sources (calcium influx) or intracellular stores (e.g., endoplasmic reticulum).

Genomic action: action that modulates the expression of genes. It involves transcriptional processes where an activated transcriptional factor translocates to the nucleus and binds gene promoters to modulate their expression. Such processes require certain time (i.e., they require some time as opposed to immediate or quick or acute effects) and are delayed.

Glucocorticoid receptor (GR): a nuclear receptor that acts as a receptor and a transcriptional factor. It is primarily located in the cytosol. Glucocorticoids, through their lipophilicity, diffuse across the cell membrane to bind to GRs in the cytosol. Such binding promotes the translocation of GR to the nucleus where it binds gene promoters to modulate their expression. As described in this review article, evidence demonstrates a membrane version of GR, not acting as a transcriptional factor, but rather as a membrane receptor modulating the acute non-genomic effects of GC.

Muscle reactivity: the ability of the muscle to respond to contractile agonists. It is impaired during pathophysiological conditions such as asthma.

Non-genomic action: action that does not modulate the expression of genes. It does not involve transcriptional processes or protein synthesis. Such action promotes rapid effects on events proximal to the cell membrane to activate certain signal transduction pathways.

Side effects of GC: because of their wide range of actions (e.g., effects on the immune system, metabolism, skeletal muscle, bone and eyes, to name a few), GC exerts, in addition to its intended effect, some harmful effects, especially when used at a high dose and over the long term as in asthma patients. Such effects usually require the genomic actions of GR.

Table 2. Examples of Various Cell Types Where GCs Were Reported to Have Non-genomic Effects Due to Their Rapid Onset, Insensitivity to GR Blockade (RU486), and Protein Synthesis Inhibition (Cycloheximide)

Cell type	GC(s)	Refs
Human bronchial epithelial cells	Dexamethasone Triamcinolone Hydrocortisone	[3,4,10]
Rat thymocytes	Methylprednisolone	[5]
Mouse neuroblastoma cells	Corticosterone	[6,14]
Cichlid fish pituitary cells	Cortisol	[7]
Mouse cortical collecting duct cells	Dexamethasone Aldosterone	[11]
Rat vascular smooth muscle cells	Aldosterone Cortisol Dexamethasone	[12,13,25]
Rat B103 neuroblastoma cells	Hydrocortisone	[15]
Guinea-pig cochlear spiral ganglion neurons	Dexamethasone	[16]
Rat hippocampal neurons	Corticosterone Dexamethasone BSA-conjugated cortisol	[19]
Mouse skeletal C2C12 cells	Dexamethasone	[20]
Guinea pig tracheal tissues	Budesonide	[22]
Murine airway smooth muscle cells	Dexamethasone	[23]
Guinea pig mouse model of allergic asthma	Budesonide	[24]
Human vascular endothelial cells	Dexamethasone	[30]

observed effects could be due to the various degrees of lipophilicity among GCs as well as to direct interactions of GCs with the cell membrane [10]. Non-genomic mechanisms have been proposed mostly based on the use of pharmacological inhibitors. Urbach and colleagues found that the rapid GC effects involved pathways regulated by the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) type Ca^{2+} -ATPase pump, adenylyl cyclase, and protein kinase A (PKA), but not protein kinase C (PKC) [10]. Collectively, these studies show the complexity of mechanisms involved in the rapid, GR-independent effects of GCs on $[\text{Ca}^{2+}]_i$, which likely occurs through an adenylyl cyclase/PKA-mediated stimulation of a thapsigargin-sensitive Ca^{2+} -ATPase [10].

Conversely, acute stimulatory effects of GC on basal calcium levels have been documented. A brief exposure to GC can increase $[\text{Ca}^{2+}]_i$ in several cell types. For example, in mouse cortical collecting duct cells, dexamethasone and aldosterone increased $[\text{Ca}^{2+}]_i$. Interestingly, the effect of aldosterone was mediated by a non-genomic activation of the PKC pathway as evidenced by the abolishment of its effect on basal $[\text{Ca}^{2+}]_i$ in the presence of the PKC inhibitor chelerythrine chloride, but not the mRNA synthesis inhibitor actinomycin D [11]. Similarly, in rat vascular smooth muscle cells, GCs rapidly increased $[\text{Ca}^{2+}]_i$ [12] potentially through GC-mediated increases in inositol (1,4,5)-triphosphate (IP3) levels associated with the translocation of the calcium- and lipid-dependent PKC from the cytosolic to the membranous compartment [13]. In these cells, while the administration of epinephrine by itself had little effect on IP3 levels, epinephrine potentiated the rapid response induced by cortisol [13]. Collectively, these findings highlight a role of PKC in the rapid increase of basal $[\text{Ca}^{2+}]_i$ by GCs.

Table 3. Examples of Signaling Pathways Activated by GCs via Nongenomic Mechanisms That Were Acute and Sensitive (or Insensitive) to GR Blockade (RU486), and Insensitive to Protein Synthesis Inhibition (Cycloheximide)

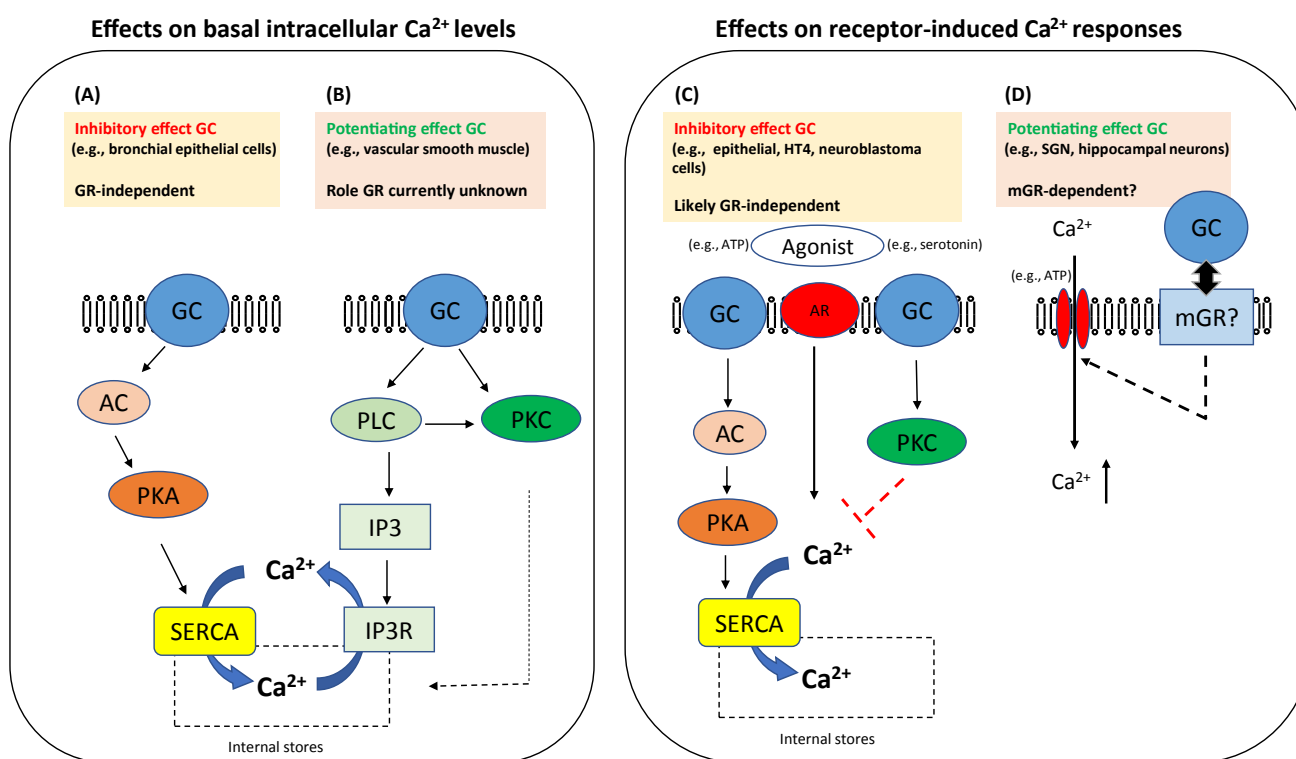
Signaling pathway(s)	Cell type	GC(s)	Refs
PKA SERCA Ca ²⁺ -ATPases Adenylyl cyclase	Human bronchial epithelial cells	Dexamethasone	[10]
PKC	Mouse cortical collecting duct cells	Dexamethasone Aldosterone	[11]
IP3 accumulation PKC	Rat vascular smooth muscle cells	Dexamethasone Aldosterone	[12]
PKA	HT4 neuroblastoma cells	Corticosterone	[6]
PKC	Rat B103 neuroblastoma cells	Corticosterone	[15]
CaMKII AMPK	Mouse skeletal myotubes	Dexamethasone	[20]
PKC	Tracheal smooth muscle tissues	Cortisol	[21]
Rho kinase	Rat vascular smooth muscle cells	Dexamethasone	[25]
ROS/RNS (NO synthase)	Human breast cancer cells	Cortisol	[26]
NO pathways	Guinea pig cochlear spiral ganglion neurons Human vascular endothelial cells Human umbilical endothelial cells	Dexamethasone	[16,30,33]
ERK1/2, p38 MAPK, JNK	PC12 cells Rat vascular smooth muscle cells	Dexamethasone	[25,37]
Src tyrosine kinase	Human breast cancer cells A549 cells	Cortisol Dexamethasone	[26,40]
PI3K/Akt	Human vascular endothelial cells	Dexamethasone	[30,33]

Effects of GCs on Agonist-Induced Calcium Mobilization

The effects of GCs on agonist-induced calcium mobilization are variable depending on the agonist, the extracellular stimuli, and the cell type. Evidence suggests that GCs rapidly inhibit, at least partially, the ability of ATP to increase $[Ca^{2+}]_i$ in some cell types. In human bronchial epithelial cells, for example, 15-min exposure to 1 nM dexamethasone markedly reduced ATP-induced increases in $[Ca^{2+}]_i$. The ATP-induced Ca^{2+} response was independent of extracellular calcium but did involve a Ca^{2+} mobilization from thapsigargin-sensitive intracellular stores [10]. Similarly, in murine HT4 neuroblastoma cells, acute (5-min) pre-incubation with corticosterone dose-dependently inhibited $[Ca^{2+}]_i$ signals induced by ATP [6]. Unlike in human bronchial epithelial cells, the Ca^{2+} response induced by ATP in these cells relies on Ca^{2+} influx across the plasma membrane and Ca^{2+} release from intracellular stores [6]. Inhibition of PKA abrogated the inhibitory action of corticosterone on ATP-induced Ca^{2+} elevation, whereas little influence was observed with respect to PKC inhibition. Additional studies demonstrated that these GC inhibitory effects were unaffected by GR blockade. These key findings obtained from studies in HT4 cells suggest that GC activates membrane-initiated, non-genomic, PKA-dependent, PKC-independent pathways [6, 14]. In contrast, in rat B103 neuroblastoma cells, the inhibitory effects of corticosterone on serotonin-induced peak $[Ca^{2+}]_i$ were found to be PKC dependent [15]. Together, these studies suggest that the mechanisms mediating the acute non-genomic effects of GC on agonist-evoked calcium mobilization are stimuli and cell type dependent.

In contrast to human bronchial epithelial and murine HT4 neuroblastoma cells, pretreatment of guinea pig cochlear spiral ganglion neurons (SGNs) with dexamethasone for 10 min enhanced ATP-induced Ca^{2+} mobilization [16]. This effect was prevented in the presence of a GR antagonist and mediated by rapid Ca^{2+} influx through activation of ionotropic purinergic

P2X receptors [16]. Of note, all P2X subtypes are expressed in SGN, albeit to different extents [17,18]. Similarly, in rat hippocampal neurons, pretreatment with corticosterone or dexamethasone for 10–20 min prolonged *N*-methyl-D-aspartate (NMDA)-induced transient elevation in $[Ca^{2+}]_i$ [19]. Importantly, the steroid effect was reversed by the removal of corticosterone, indicating that the steroid effect was not due to irreversible impairment of Ca^{2+} extrusion from the neurons. Thapsigargin and cycloheximide had little effect on the potentiating effect of corticosterone, excluding the involvement of a thapsigargin-sensitive Ca^{2+} -ATPase or *de novo* protein synthesis, respectively. Interestingly, the GC effect was reproduced by the use of a membrane-impermeable bovine serum albumin (BSA)-conjugated cortisol, suggesting that mGR likely underlies the rapid non-genomic effects of GCs [19]. However, canonical genomic actions of GCs can also alter Ca^{2+} mobilization. In human lymphoblasts, while cortisol reduced basal $[Ca^{2+}]_i$ (as indicated above), Ca^{2+} mobilization induced by platelet-activating factor is enhanced only by chronic treatment (48 h) with cortisol [9] (Figure 1).



Trends in Pharmacological Sciences

Figure 1. Acute Non-genomic Effects of Glucocorticoids on Basal and Agonist-Induced Ca^{2+} Responses. Glucocorticoids (GCs) have been described to differentially affect basal intracellular Ca^{2+} ($[Ca^{2+}]_i$) homeostasis. Depending on the cell type studied and the GC applied, GCs can either reduce or augment basal $[Ca^{2+}]_i$. (A) GCs may decrease $[Ca^{2+}]_i$ by activating AC/PKA-mediated mechanisms, likely through events taking place at the cell membrane level and independent of GR stimulation, ultimately leading to SERCA activation (thapsigargin-sensitive Ca^{2+} -ATPase). (B) Conversely, GCs can activate PLC/IP3- and PKC-dependent signaling cascades, resulting in enhanced basal $[Ca^{2+}]_i$; the involvement of GR in this process is currently unknown. (C) Agonist-induced increases in $[Ca^{2+}]_i$ can be counteracted by GC-mediated activation of AC/PKA-induced stimulation of SERCA pumps as described in ATP-stimulated cells. In contrast, a functional role for PKC was determined in the effects of GC on serotonin-induced Ca^{2+} responses, suggesting that the acute inhibitory mechanisms of GCs are highly agonist specific. (D) Limited studies are available on acute potentiating effects by GCs on agonist-induced Ca^{2+} responses; in neuronal cells, it was suggested that these effects are mediated via the rapid activation of Ca^{2+} influx through ionotropic ATP-gated purinergic 2X receptors. Whether GC-mediated membrane receptors are involved in this pathway remains to be further investigated (mGR?). These responses rely on the presence of external Ca^{2+} . Abbreviations: AC, adenylyl cyclase; AR, agonist receptor; GR, glucocorticoid receptor; IP3, inositol (1,4,5)-triphosphate; IP3R, IP3, inositol (1,4,5)-triphosphate receptor; mGR, membrane glucocorticoid receptor; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; SERCA, sarco/endoplasmic reticulum Ca^{2+} -ATPase; SGN, spiral ganglion neuron.

GCs Rapidly Modulate Skeletal and Smooth Muscle Function

Several studies have reported variable acute effects of GCs on **muscle reactivity** and tone. The specific example of airway smooth muscle (ASM) cells in the pathogenesis of inflammatory diseases is highlighted in [Box 1](#). In mouse skeletal myotubes (C2C12 immortalized myoblasts), treatment with dexamethasone (for less than 20 min) reduced glucose uptake induced by electrical pulse stimulation-mediated contraction, in a Ca^{2+} /calmodulin protein kinase II (CaMKII)- and AMP-activated protein kinase (AMPK)-dependent manner [20]. The effects were unaffected by blockade of GR (RU486) or inhibition of protein synthesis (cycloheximide), indicating a rapid non-genomic and GR-independent effect. In another study, cortisol synergized with isoprenaline in reducing tracheal spasms in response to histamine [21]. The spasmolytic effect was fully prevented in the presence of RU486 (implicating a GR-dependent pathway) and partially reduced by PKC inhibition, but it was unaffected by actinomycin D (excluding *de novo* RNA synthesis), again suggesting a non-genomic, GR-mediated signaling pathway involving PKC [21].

Other studies support a role for GCs in rapidly reducing ASM tone. Pretreatment with budesonide (within 15 min) suppressed histamine-induced isometric tension in guinea pig tracheal rings and shrinkage in individual tracheal ASM cells, effects that were unaffected by cycloheximide (suggesting non-genomic actions by budesonide) [22]. Unlike the findings by Wang and colleagues [21], these budesonide effects were insensitive to RU486, excluding classic GR involvement [22]. Similarly, in murine ASM cells, exposure to dexamethasone for 10 min decreased basal $[\text{Ca}^{2+}]_i$ and reduced peak elevations in $[\text{Ca}^{2+}]_i$ induced by acetylcholine, effects that were insensitive to GR blockade and cycloheximide [23]. Consistently, studies using an *in vivo* guinea pig model of asthma, an established model to study allergen-induced asthmatic reactions and airway hyperresponsiveness [24], revealed a beneficial effect on ovalbumin-induced changes in lung resistance and compliance by acutely inhaled budesonide. The protective effects of budesonide were evident within 10 min, suggesting a non-genomic course of action [25]. In summary, GCs have acute spasmolytic actions in ASM that can require both GR-dependent and -independent pathways and potentially PKC-mediated signaling.

A recent study in rat vascular smooth muscle cells under conditions of lipopolysaccharide-induced septic shock showed that dexamethasone treatment for 10 min promotes norepinephrine (NE)-induced phosphorylation of key proteins associated with contraction [26]. While no significant effect on myosin light chain 20 (MLC20) phosphorylation was observed after exposure to either dexamethasone or NE alone, the combined treatment markedly enhanced phospho-MLC20, an effect that was unaltered by GR blockade with RU486. Interestingly, inhibition of Rho kinase (ROCK) with Y27632 completely reversed the potentiating effects of dexamethasone on NE-induced phospho-MLC20. Together, these findings could be of clinical significance and indicate that the impaired vascular response to NE observed in septic shock may be restored by short-term exposure to dexamethasone through non-genomic activation of ROCK activity [26].

GCs Exert Rapid Effects on Reactive Oxygen Species/Reactive Nitrogen Species

Studies demonstrated a rapid effect of GCs on reactive oxygen species (ROS) generation and the involvement of nitric oxide (NO) in mediating some GC effects. An example of the role NO/ROS in the pathogenesis of inflammatory disease is highlighted in [Box 2](#). In breast cancer cells, cortisol rapidly increased levels of ROS and reactive nitrogen species (RNS) (as early as 15 min) and induced DNA damage. The GR antagonist RU486 blocked the cortisol effect, while N^{ω} -nitro-L-arginine methyl ester (L-NAME) and 1400W dihydrochloride demonstrated the

involvement of nitric oxide synthase (NOS) and inducible NOS (iNOS), respectively. The pharmacological inhibition of Src by PP2 prevented GC-induced RNS elevation, suggesting the ability of GC to rapidly stimulate Src- and iNOS-dependent release of damaging RNS levels [27].

Rapid effects of GCs on endothelial NOS (eNOS), an important mediator of vascular integrity with anti-inflammatory, -ischemic, and -atherogenic properties, have been described as well [28–30]. Indeed, the treatment of human vascular endothelial cells with dexamethasone rapidly enhanced (as early as 10 min), in a concentration-dependent manner, eNOS activity, NO production, and NO-dependent vasorelaxation [31]. These GC effects were abrogated by RU486, phosphoinositide 3-kinase (PI3K) inhibitors wortmannin and LY292002, or L-NAME, but not by the transcriptional inhibitor actinomycin D.

Additional evidence supporting rapid effects of GCs on NOS/NO showed an augmented ATP-induced, NOS-dependent NO release in guinea pig type I spiral ganglion neurons by dexamethasone that was thought to be a consequence of ATP-induced $[Ca^{2+}]_i$ [16]. Similarly, GR-mediated increases in $[Ca^{2+}]_i$, eNOS phosphorylation, and NO production were observed in human umbilical vein endothelial cells [32]. Interestingly, NO production increased $[Ca^{2+}]_i$ originating from intracellular and extracellular Ca^{2+} sources [32].

The PI3K/Akt pathway is critical in the activation of NO signaling, e.g., phosphorylation of eNOS [33], and the involvement of this pathway in the rapid effects of GCs has been documented [33]. For example, dexamethasone rapidly increased (within 20 min), in a dose-dependent manner, GR-dependent phosphorylation and activation of PI3K as demonstrated by phosphorylation of Akt and glycogen synthase kinase-3, indicating that GCs can functionally activate PI3K and downstream targets in human endothelial cells [31]. The potential clinical relevance of these observations was confirmed in two different mouse models of ischemic injury (i.e., transient myocardial ischemia and transient focal cerebral ischemia), wherein GC exerted rapid protective effects (within 30 min) via GR-dependent activation of PI3K and eNOS pathways as evidenced by the administration of RU486, wortmannin and L-NAME, respectively [31,32]. Additional studies in COS-7 cells demonstrated a key role for GR in GC-induced activation of the PI3K/Akt pathway. When cells were transfected with a dimerization-defective GR mutant (A458T, a construct that is unable to bind DNA and transactivate GC target genes), acute dexamethasone stimulation still activated the PI3K/Akt pathway [34]. Together, these findings suggest the involvement of a non-transcriptional/non-genomic mechanism in the GR-dependent activation of PI3K/Akt by GCs.

Since NO signaling plays a key role in chronic airway inflammatory diseases, such as asthma and chronic obstructive pulmonary disease (COPD) [35], we believe that the crosstalk between GC and NO signaling warrants further investigation to determine whether the rapid effects of GC on NO signaling would be beneficial or detrimental in disease pathogenesis.

GCs Exert Acute Effects on Inflammatory and Apoptotic Pathways

Evidence shows rapid non-transcriptional actions of GCs on inflammation both in transformed cells and immune cells. In transformed cells, such as A549 adenocarcinoma cells, acute exposure (as early as 1 min) to dexamethasone rapidly inhibited epidermal growth factor (EGF)-induced arachidonic acid release, an important mediator of inflammation [36]. This inhibitory effect was due to hindering the recruitment of Grb2, p21ras, and Raf to the EGF receptor (EGFR) through a GR-dependent (RU486-sensitive) and transcription-independent (actinomycin D-insensitive) mechanism. The inhibition of Grb2 recruitment was accompanied by

lipocortin-1 recruitment to EGFR in the cell membrane. Subsequently, lipocortin-1 competitively inhibited Grb2 binding to EGFR, thereby blocking the recruitment of critical signaling molecules necessary for EGF actions [36].

The acute effects of GCs on inflammatory pathways were also observed in immune cells, such as human neutrophils, where acute exposure (5 min) to methylprednisolone or hydrocortisone significantly inhibited *N*-formyl-methionyl-leucyl-phenylalanine-induced neutrophil degranulation, effects that were not prevented by RU486 or cycloheximide treatments, suggesting the involvement of GR-independent and non-genomic pathways [37]. Also, in murine macrophages acutely treated with dexamethasone (30 min), toll-like receptor (TLR)9-induced activation of different inflammatory signaling pathways, such as those involving nuclear factor (NF)- κ B and mitogen-activated protein kinases (MAPKs), was dramatically suppressed [38]. After TLR9 engagement, IL-1R-associated kinase 1 (IRAK1) is recruited to the cell membrane. A critical step in activating the TLR signaling cascade is the ubiquitination of IRAK1 through its physical interaction with the E3 ligase β -transducin repeat-containing protein (β -TrCP). Such ubiquitination and degradation of IRAK1 promotes the trafficking of the 'TNFR-associated factor 6-TAK1 adaptor proteins-transforming growth factor β -activated kinase 1' complex to the cytosol to subsequently induce MAPK and NF- κ B activation. Dexamethasone inhibition of IRAK1 ubiquitination did not occur in the presence of RU486, suggesting the involvement of GR-dependent mechanisms [38]. Further investigation of the molecular mechanisms revealed that by physically interacting with IRAK1 GR interferes with the interaction between β -TrCP and IRAK1, thereby impeding its ubiquitination, a critical step in the activation of the TLR9-dependent inflammatory cascade [38].

Rapid GC treatment can also exert proinflammatory action in other cell types. For example, in PC12 cells (cell line derived from rat adrenal gland), corticosterone induced rapid activation (within 15 min) of extracellular signal-regulated kinase (ERK)1/2, p38, and c-Jun NH₂-terminal kinase (JNK) in a PKC-dependent manner [39,40]. The activation of MAPK pathways after GC treatment appears to be mediated by the putative mGR, since corticosterone-BSA can rapidly (with 15 min) activate all MAPKs [39,40]. Similarly, in rat vascular smooth muscle cells, dexamethasone either alone or in combination with NE rapidly (within 10 min) induces ERK1/2 and p38 MAPK activities [26]. Thus, in certain cells, GCs can activate MAPK in a non-genomic manner.

In CCRF-CEM cells, a cell line derived from human T cells (from pediatric acute lymphoblastic leukemia patients), sensitivity to acute dexamethasone-induced cell death was determined in the presence and absence of phosphodiesterase (PDE) inhibitors [41]. Nonspecific PDE and specific PDE4 inhibition reversed steroid resistance and markedly increased sensitivity to dexamethasone. This effect is likely due to increased cAMP levels, consistent with abundant documentation on interactions between GR and cAMP pathways in the induction of apoptosis in lymphoid cells by both [42,43]. To date, the mechanisms of cAMP-induced apoptosis are unclear, but the presence of GR appears to be required, even in the absence of GCs. For instance, in parental T cells, elevation of cAMP, with either forskolin or dibutyryl-cAMP, induced apoptotic cells death, whereas GR-deficient cells were insensitive to the apoptotic effects of cAMP elevation. When GR expression was reconstituted by transfection, not only was GC sensitivity restored but also the sensitivity to cytolytic effects induced by cAMP was promoted [42].

The effects of GCs on the mitochondrial control of cell metabolism and apoptosis have been extensively reviewed elsewhere [44–47]. For instance, Sekeris and colleagues were the first to discover the presence of GR in mitochondria [48]. Through its acute non-genomic effects, GCs

promote mitochondrial apoptotic pathways, resulting in the disruption of the mitochondrial membrane potential and the release of proapoptotic factors such as cytochrome C [49]. Importantly, the translocation of GR from the cytoplasm to the mitochondria correlates with the sensitivity of a given cell type to GC-induced apoptosis [50,51]. In line with this, recent studies in mouse thymocytes showed that short-term treatment with GC induces a direct interaction of GR with the proapoptotic Bcl2 family member-associated proteins such as Bim [52]. Such interaction subsequently activates Bax, thereby decreasing the mitochondrial membrane potential, cytochrome C release, and caspase-9 activation. However, it is important to note that the effects of GC on the mitochondria control of apoptosis also involve genomic pathways. For example, in murine neuronal stem cells, dexamethasone was able to augment 2,3-methoxy-1,4-naphthoquinone-induced apoptosis, wherein a large percentage of studied genes involved in the mitochondrial respiratory chain and some encoding for antioxidant enzymes were downregulated by long-term treatment with GC [53]. These events allowed GCs to increase cellular sensitivity to oxidative stress, thereby promoting neurotoxicity. This is clinically relevant as it can occur during prenatal exposure of the fetal brain to excess GCs [53].

Potential Role of a Putative mGR in Mediating the Rapid Effects of GCs

As described previously, the rapid non-genomic effects can, at least in part, be mediated through a putative mGR. Over the years, caveolin-1 (Cav-1), the major protein component of caveolae, has been implicated as a scaffold for the organization of several cytoplasmic signal complexes at the plasma membrane [54,55]. In lung epithelial cells (A549), dexamethasone treatment leads to a rapid (within 2 min) phosphorylation of Cav-1 and protein kinase B (PKB)/Akt in a Src-dependent manner [56]. Subcellular fractionation revealed colocalization of GR and Src to caveolin-containing membrane fractions [56]. Interfering with caveolae/caveolin (by disruption of lipid raft formation, impairment of function using dominant-negative caveolin, downregulation of Cav-1 using short hairpin RNA, or genetic ablation of Cav-1) prevented acute (within 2 min) GC-induced PKB phosphorylation. Of note, caveolin downregulation had little effect on GC-mediated transactivation, supporting the existence of a putative mGR. Further functional studies in caveolin knockout cells revealed considerable inhibition of GC-mediated cell growth arrest, suggesting that membrane-proximal signals acutely initiated by GC are required to mediate delayed effects (anti-proliferative effects) previously ascribed exclusively to the nuclear actions of GR [56]. Further evidence supporting a role for caveolae in mGR function stems from studies of membrane nuclear receptors such as the estrogen receptor (ER) [57], showing requirement of Cav-1 in mediating acute cellular actions. Indeed, using epitope proximity ligation assays, Watson and colleagues demonstrated interactions of ER α with Cav-1. Interestingly, the use of nystatin, which binds to cholesterol and disrupts caveolar structures, blocked estrogen-induced rapid (5-min) ERK activation in pituitary tumor cells [57]. Together, these findings indicate a critical role of Cav-1 in acute nuclear receptor/steroid signaling.

While the expression of mGR has been demonstrated in a myriad of cell types [58], the colocalization and crosstalk between mGR and Cav-1 are variable and highly cell specific. Indeed, in U2-OS and MCF-7 cells, double recognition proximity ligation assays demonstrated the physical association of Cav-1 with the mGR [58]. However, studies in human CD14⁺ monocytes showed that mGR and Cav-1 are not colocalized and that overexpression of the recombinant Cav-1 transcript in human K562 chronic myelogenous leukemia cells did not affect mGR expression/appearance, suggesting that in these specific cell lines Cav-1 is not the limiting factor for mGR expression/appearance, without ruling out the possibility that it is a component of the transport machinery of GR from the cytosol to the membrane [59]. Palmitoylation, a critical post-translational modification occurring through the addition of fatty acid

(e.g., palmitic acid) on amino acid residues of membrane proteins, plays a major role in the subcellular trafficking of proteins between membrane compartments [60]. Interestingly, the involvement of palmitoylation in the recruitment of other nuclear receptors, such as ER, to the plasma membrane has been reported [61]. Recent studies investigated whether this process is necessary for the recruitment of GR to the membrane and its colocalization with Cav-1 in COS-7 cells. Treatment of cells with the palmitoylation inhibitor 2-bromopalmitate had little effect on membrane localization of GR and its colocalization with Cav-1, and little influence on the acute effects of GC on MAPK signaling pathways. In addition, human GR α did not undergo S-palmitoylation, rendering this process unlikely to modulate membrane recruitment of GR [62]. Future studies on the mechanisms underlying GR recruitment to caveolae-rich parts and its potential association with Cav-1 are warranted, specifically in airway cells.

Several studies have reported an interaction of mGR with other membrane receptors, particularly G protein-coupled receptors (GPCRs) [63]. Zhang and colleagues demonstrated the involvement of mGR- and GPCR-dependent mechanisms in the rapid effect (as early as 1 min) of corticosterone on NMDA-evoked currents in hippocampal neurons [63] and further suggested that mGR may couple to multiple G proteins, including G_s and G_{q/11}. Other studies indicate that mGR directly elicits the activation of downstream intracellular signaling pathways. For instance, corticosterone might act via mGR to rapidly elicit PKC-dependent activation of ERK1/2 MAPK pathway (with 15 min) in PC12 cells [39]. Interestingly, proteomic analysis of the lymphoma cell line CCRF-CEM identified 128 proteins that were differentially regulated by the specific activation of mGR using BSA-conjugated cortisol for a short-term period (5 and 15 min) [58]. These actions were unique to mGR, as no activation of cGR target genes, such as GILZ, were observed. The majority of networks rapidly activated by mGR were mainly involved in cellular growth and cancer (after 5-min treatment with BSA-conjugated cortisol), cellular development, or hematological system development and function (after 15-min treatment with BSA-conjugated cortisol). Ingenuity pathway analysis provided strong evidence that mGR is involved in proapoptotic, immune-modulatory, and metabolic pathways that are also regulated by GCs through cGR, suggesting that acute mGR stimulation can trigger rapid early priming events, ultimately paving the way for the slower genomic activities by GCs [58].

Concluding Remarks and Future Perspectives

Although we have some insight into how GCs regulate different signaling pathways in a non-genomic manner, future in-depth investigations are warranted to further unravel details of these complex interactions. Indeed, key questions (see Outstanding Questions) still need careful consideration, and additional research must address several important issues: (i) the differential nature of non-genomic effects of GC in immune cells versus non-immune/structural cells; (ii) differences between non-genomic effects of various steroids based on their lipophilicity [10]; (iii) the fact that not all non-genomic effects are GR mediated (RU486 insensitive) and may be due to nonspecific interactions of GC with the cell membrane [2]; (iv) the possibility that non-genomic and genomic effects are interconnected, where the acute non-genomic effects pave the way for the slower genomic activities of GCs [58]; and (v) the significant role of Cav-1, and possibly other scaffolding/anchoring proteins, as a modulator of mGR activation, wherein the relative numbers of mGRs associated with Cav-1 are critical in mediating non-genomic effects of GCs [58,64,65]. Since **side effects** associated with GC therapy are often generated through its genomic actions [66], uncovering the non-genomic actions of GC with beneficial effects will likely lead to the development of compounds that selectively activate non-genomic signaling and thus have improved therapeutic profiles.

Outstanding Questions

What is the functional role of non-genomic effects of GC in chronic airway inflammatory disease, such as asthma and COPD, both in *in vitro* and in *in vivo* models of asthma?

Are non-genomic immediate effects necessary and/or sufficient to promote the delayed and lasting genomic effects of GC?

What are the roles of mGR, nonspecific interactions of GC with cell membranes, and cGR in mediating the non-genomic effects of GC in airway cells?

What is the origin of mGR in airway cells? Does mGR translocate from the cytosol to the cell membrane in a caveolin-dependent manner or interact with other membrane receptors or ion channels to mutually modulate their respective functions?

Does the expression of mGR change in disease or after exposure to proinflammatory cytokines and how do these changes in mGR expression, if any, affect GC non-genomic effects?

Which strategy is optimal to benefit clinically from the non-genomic effects of GC?

Does the fact that side effects are often associated with the genomic effects of GC mean that a steroid solely producing non-genomic effects will have a better therapeutic index?

Acknowledgments

This work was funded by National Institutes of Health grants 7R01HL111541-07 (O.T.), HL 2P01HL114471-06 (R.A.P.), and GM107094 (R.O.).

References

- Lemanske, R.F., Jr and Busse, W.W. (2003) 6. Asthma. *J. Allergy Clin. Immunol.* 111, S502–S519
- Song, I.H. and Buttgereit, F. (2006) Non-genomic glucocorticoid effects to provide the basis for new drug developments. *Mol. Cell. Endocrinol.* 246, 142–146
- Urbach, V. and Harvey, B.J. (2001) Rapid and non-genomic reduction of intracellular $[Ca^{2+}]_i$ induced by aldosterone in human bronchial epithelium. *J. Physiol.* 537, 267–275
- Urbach, V. *et al.* (2006) Rapid anti-secretory effects of glucocorticoids in human airway epithelium. *Steroids* 71, 323–328
- Buttgereit, F. *et al.* (1997) Methylprednisolone inhibits uptake of Ca^{2+} and Na^+ ions into concanavalin A-stimulated thymocytes. *Biochem. J.* 326 (Pt 2), 329–332
- Han, J.Z. *et al.* (2005) Inhibition of ATP-induced calcium influx in HT4 cells by glucocorticoids: involvement of protein kinase A. *Acta Pharmacol. Sin.* 26, 199–204
- Borski, R.J. *et al.* (1991) Cortisol rapidly reduces prolactin release and cAMP and $45Ca^{2+}$ accumulation in the cichlid fish pituitary *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 88, 2758–2762
- Chhabra, S.K. *et al.* (1999) Decreased sodium-potassium and calcium adenosine triphosphatase activity in asthma: modulation by inhaled and oral corticosteroids. *Indian J. Chest Dis. Allied Sci.* 41, 15–26
- Gardner, J.P. and Zhang, L. (1999) Glucocorticoid modulation of Ca^{2+} homeostasis in human B lymphoblasts. *J. Physiol.* 514 (Pt 2), 385–396
- Urbach, V. *et al.* (2002) Rapid non-genomic inhibition of ATP-induced Cl^- secretion by dexamethasone in human bronchial epithelium. *J. Physiol.* 545, 869–878
- Harvey, B.J. and Higgins, M. (2000) Nongenomic effects of aldosterone on Ca^{2+} in M-1 cortical collecting duct cells. *Kidney Int.* 57, 1395–1403
- Wehling, M. *et al.* (1995) Nongenomic effects of aldosterone on intracellular Ca^{2+} in vascular smooth muscle cells. *Circ. Res.* 76, 973–979
- Steiner, A. *et al.* (1988) Stimulation of the phosphoinositide signalling system as a possible mechanism for glucocorticoid action in blood pressure control. *J. Hypertens. Suppl.* 6, S366–S368
- Han, J.Z. *et al.* (2002) Evoked intracellular Ca^{2+} elevations in HT4 neuroblastoma cells. *Neuroreport* 13, 1089–1094
- Han, J.Z. *et al.* (2002) A rapid, nongenomic action of glucocorticoids in rat B103 neuroblastoma cells. *Biochim. Biophys. Acta* 1591, 21–27
- Yukawa, H. *et al.* (2005) Acute effects of glucocorticoids on ATP-induced Ca^{2+} mobilization and nitric oxide production in cochlear spiral ganglion neurons. *Neuroscience* 130, 485–496
- Brandle, U. *et al.* (1999) Gene expression of P2X-receptors in the developing inner ear of the rat. *Neurosci. Lett.* 273, 105–108
- Xiang, Z. *et al.* (1999) P2X receptor immunoreactivity in the rat cochlea, vestibular ganglion and cochlear nucleus. *Hear. Res.* 128, 190–196
- Takahashi, T. *et al.* (2002) Corticosterone acutely prolonged N-methyl-D-aspartate receptor-mediated Ca^{2+} elevation in cultured rat hippocampal neurons. *J. Neurochem.* 83, 1441–1451
- Gong, H. *et al.* (2016) Dexamethasone rapidly inhibits glucose uptake via non-genomic mechanisms in contracting myotubes. *Arch. Biochem. Biophys.* 603, 102–109
- Wang, C. *et al.* (2012) Glucocorticoid decreases airway tone via a nongenomic pathway. *Respir. Physiol. Neurobiol.* 183, 10–14
- Sun, H.W. *et al.* (2006) Rapid inhibitory effect of glucocorticoids on airway smooth muscle contractions in guinea pigs. *Steroids* 71, 154–159
- Sun, H.W. *et al.* (2010) Rapid inhibitory effect of glucocorticoids on peak of $[Ca^{2+}]_i$ and PLC in airway smooth muscle. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 26, 440–443
- Meurs, H. *et al.* (2006) A guinea pig model of acute and chronic asthma using permanently instrumented and unrestrained animals. *Nat. Protoc.* 1, 840–847
- Zhou, J. *et al.* (2003) Rapid nongenomic effects of glucocorticoids on allergic asthma reaction in the guinea pig. *J. Endocrinol.* 177, R1–R4
- Zhang, T. *et al.* (2013) Dexamethasone induces rapid promotion of norepinephrinemediated vascular smooth muscle cell contraction. *Mol. Med. Rep.* 7, 549–554
- Flaherty, R.L. *et al.* (2017) Glucocorticoids induce production of reactive oxygen species/reactive nitrogen species and DNA damage through an iNOS mediated pathway in breast cancer. *Breast Cancer Res.* 19, 35
- Ishida, A. *et al.* (1997) Induction of the cyclin-dependent kinase inhibitor p21(Sdi1/Cip1/Waf1) by nitric oxide-generating vasodilator in vascular smooth muscle cells. *J. Biol. Chem.* 272, 10050–10057
- Limbourg, F.P. and Liao, J.K. (2003) Nontranscriptional actions of the glucocorticoid receptor. *J. Mol. Med. (Berl.)* 81, 168–174
- Loscalzo, J. (1995) Nitric oxide and vascular disease. *N. Engl. J. Med.* 333, 251–253
- Hafezi-Moghadam, A. *et al.* (2002) Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase. *Nat. Med.* 8, 473–479
- Leung, K.W. *et al.* (2009) Protopanaxadiol and protopanaxatriol bind to glucocorticoid and oestrogen receptors in endothelial cells. *Br. J. Pharmacol.* 156, 626–637
- Dimmeler, S. *et al.* (1999) Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399, 601–605
- Limbourg, F.P. *et al.* (2002) Rapid nontranscriptional activation of endothelial nitric oxide synthase mediates increased cerebral blood flow and stroke protection by corticosteroids. *J. Clin. Invest.* 110, 1729–1738
- Meurs, H. *et al.* (2003) Arginase and asthma: novel insights into nitric oxide homeostasis and airway hyperresponsiveness. *Trends Pharmacol. Sci.* 24, 450–455
- Croxtall, J.D. *et al.* (2000) Glucocorticoids act within minutes to inhibit recruitment of signalling factors to activated EGF receptors through a receptor-dependent, transcription-independent mechanism. *Br. J. Pharmacol.* 130, 289–298
- Liu, L. *et al.* (2005) Rapid non-genomic inhibitory effects of glucocorticoids on human neutrophil degranulation. *Inflamm. Res.* 54, 37–41
- Kong, F. *et al.* (2017) Inhibition of IRAK1 ubiquitination determines glucocorticoid sensitivity for TLR9-induced inflammation in macrophages. *J. Immunol.* 199, 3654–3667
- Qiu, J. *et al.* (2001) Rapid activation of ERK1/2 mitogen-activated protein kinase by corticosterone in PC12 cells. *Biochem. Biophys. Res. Commun.* 287, 1017–1024
- Li, X. *et al.* (2001) Corticosterone-induced rapid phosphorylation of p38 and JNK mitogen-activated protein kinases in PC12 cells. *FEBS Lett.* 492, 210–214
- Ogawa, R. *et al.* (2002) Inhibition of PDE4 phosphodiesterase activity induces growth suppression, apoptosis, glucocorticoid sensitivity, p53, and p21(WAF1/CIP1) proteins in human acute lymphoblastic leukemia cells. *Blood* 99, 3390–3397
- Kiefer, J. *et al.* (1995) Functional glucocorticoid receptor expression is required for cAMP-mediated apoptosis in a human leukemic T cell line. *J. Immunol.* 155, 4525–4528

43. McConkey, D.J. *et al.* (1993) Cyclic AMP potentiates glucocorticoid-induced endogenous endonuclease activation in thymocytes. *FASEB J.* 7, 580–585
44. Gavrilova-Jordan, L.P. and Price, T.M. (2007) Actions of steroids in mitochondria. *Semin. Reprod. Med.* 25, 154–164
45. Psarra, A.M. *et al.* (2009) Interaction of mitochondrial thioredoxin with glucocorticoid receptor and NF-kappaB modulates glucocorticoid receptor and NF-kappaB signalling in HEK-293 cells. *Biochem. J.* 422, 521–531
46. Psarra, A.M. and Sekeris, C.E. (2009) Glucocorticoid receptors and other nuclear transcription factors in mitochondria and possible functions. *Biochim. Biophys. Acta* 1787, 431–436
47. Psarra, A.M. *et al.* (2006) The mitochondrion as a primary site of action of steroid and thyroid hormones: presence and action of steroid and thyroid hormone receptors in mitochondria of animal cells. *Mol. Cell. Endocrinol.* 246, 21–33
48. Demonacos, C. *et al.* (1993) Import of the glucocorticoid receptor into rat liver mitochondria *in vivo* and *in vitro*. *J. Steroid Biochem. Mol. Biol.* 46, 401–413
49. Palinkas, L. *et al.* (2008) Developmental shift in TcR-mediated rescue of thymocytes from glucocorticoid-induced apoptosis. *Immunobiology* 213, 39–50
50. Boldizar, F. *et al.* (2010) Emerging pathways of non-genomic glucocorticoid (GC) signalling in T cells. *Immunobiology* 215, 521–526
51. Sionov, R.V. *et al.* (2006) Glucocorticoid-induced apoptosis revisited: a novel role for glucocorticoid receptor translocation to the mitochondria. *Cell Cycle* 5, 1017–1026
52. Prenek, L. *et al.* (2017) The regulation of the mitochondrial apoptotic pathway by glucocorticoid receptor in collaboration with Bcl-2 family proteins in developing T cells. *Apoptosis* 22, 239–253
53. Mutsaers, H.A. and Tofighi, R. (2012) Dexamethasone enhances oxidative stress-induced cell death in murine neural stem cells. *Neurotox. Res.* 22, 127–137
54. Gosens, R. *et al.* (2008) Caveolae and caveolins in the respiratory system. *Curr. Mol. Med.* 8, 741–753
55. Williams, T.M. and Lisanti, M.P. (2004) The caveolin proteins. *Genome Biol.* 5, 214
56. Matthews, L. *et al.* (2008) Caveolin mediates rapid glucocorticoid effects and couples glucocorticoid action to the antiproliferative program. *Mol. Endocrinol.* 22, 1320–1330
57. Watson, C.S. *et al.* (2012) Estrogen- and xenoestrogen-induced ERK signaling in pituitary tumor cells involves estrogen receptor-alpha interactions with G protein-alpha and caveolin I. *Steroids* 77, 424–432
58. Vernocchi, S. *et al.* (2013) Membrane glucocorticoid receptor activation induces proteomic changes aligning with classical glucocorticoid effects. *Mol. Cell. Proteomics* 12, 1764–1779
59. Spies, C.M. *et al.* (2006) Membrane glucocorticoid receptors are down regulated by glucocorticoids in patients with systemic lupus erythematosus and use a caveolin-1-independent expression pathway. *Ann. Rheum. Dis.* 65, 1139–1146
60. Czuba, L.C. *et al.* (2018) Post-translational modifications of transporters. *Pharmacol. Ther.* Published online June 30, 2018. <http://dx.doi.org/10.1016/j.pharmthera.2018.06.013>
61. Li, L. *et al.* (2003) Plasma membrane localization and function of the estrogen receptor alpha variant (ER46) in human endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4807–4812
62. Nicolaidis, N.C. *et al.* (2017) The role of S-palmitoylation of the human glucocorticoid receptor (hGR) in mediating the nongenomic glucocorticoid actions. *J. Mol. Biochem.* 6, 3–12
63. Zhang, Y. *et al.* (2012) Glucocorticoid acts on a putative G protein-coupled receptor to rapidly regulate the activity of NMDA receptors in hippocampal neurons. *Am. J. Physiol. Endocrinol. Metab.* 302, E747–E758
64. Tsuji, Y. *et al.* (2005) Differential-expression and tyrosine-phosphorylation profiles of caveolin isoforms in human T cell leukemia cell lines. *Int. J. Mol. Med.* 16, 889–893
65. Tsuji, Y. *et al.* (2006) Quantification of caveolin isoforms using quantitative real-time RT-PCR, and analysis of promoter CpG methylation of caveolin-1alpha in human T cell leukemia cell lines. *Int. J. Mol. Med.* 18, 489–495
66. De Bosscher, K. *et al.* (2010) Classic glucocorticoids versus non-steroidal glucocorticoid receptor modulators: survival of the fittest regulator of the immune system? *Brain Behav. Immun.* 24, 1035–1042