Review

Science review: Mechanisms of impaired adrenal function in sepsis and molecular actions of glucocorticoids

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Abstract

This review describes current knowledge on the mechanisms that underlie glucocorticoid insufficiency in sepsis and the molecular action of glucocorticoids. In patients with severe sepsis, numerous factors predispose to glucocorticoid insufficiency, including drugs, coagulation disorders and inflammatory mediators. These factors may compromise the hypothalamic–pituitary axis (i.e. secondary adrenal insufficiency) or the adrenal glands (i.e. primary adrenal failure), or may impair glucocorticoid access to target cells (i.e. peripheral tissue resistance). Irreversible anatomical damages to the hypothalamus, pituitary, or adrenal glands rarely occur. Conversely, transient functional impairment in hormone synthesis may be a common complication of severe sepsis. Glucocorticoids interact with a specific cytosolic glucocorticoid receptor, which undergoes conformational changes, sheds heat shock proteins and translocates to the nucleus. Glucocorticoids may also interact with membrane binding sites at the surface of the cells. The molecular action of glucocorticoids results in genomic and nongenomic effects. Direct and indirect transcriptional and post-transcriptional effects related to the cytosolic glucocorticoid receptor account for the genomic effects. Nongenomic effects are probably subsequent to cytosolic interaction between the glucocorticoid receptor and proteins, or to interaction between glucocorticoids and specific membrane binding sites.

Keywords adrenal cortex hormones, glucocorticoid receptor, sepsis

Introduction

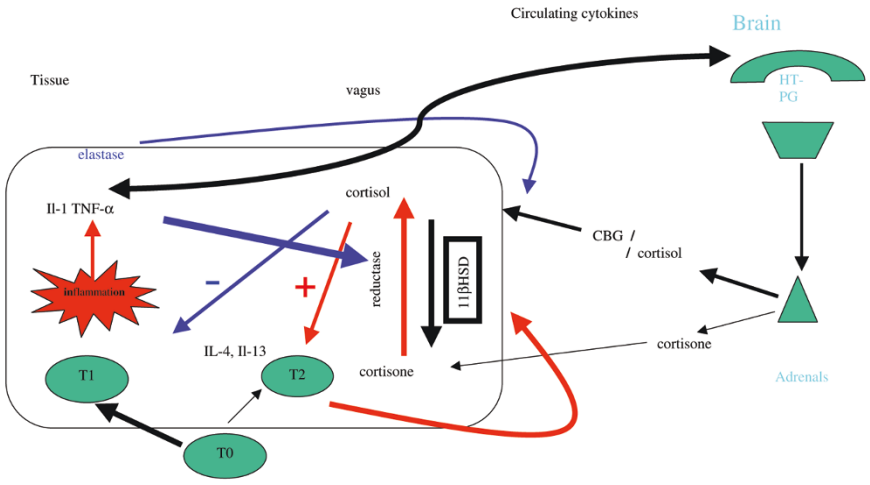
The hypothalamic–pituitary adrenal axis is a key component of the host response to sepsis, as was suggested almost a century ago following observations of apoplectic adrenal glands in fatal meningococcaemia [1,2]. In animals, removal of the adrenal cortex but sparing the medulla results in less resistance to challenge with endotoxin [3]. In recent years, advances in our understanding of the role played by glucocorticoid insufficiency in the pathogenesis of septic shock resulted in increased use of glucocorticoid replacement therapy. In a previous review article [4] we described the clinical aspects of adrenal dysfunction in sepsis, as well as the role of cortisol replacement in the management of septic shock. In the present review we detail the mechanisms of glucocorticoid insufficiency that are active during sepsis and the molecular actions of glucocorticoids.

Methods

We attempted to identify all relevant studies, regardless of language or publication status (published, unpublished, in press and in progress). We searched the following electronic databases: Medline (1966 to December 2003), Embase (1974 to December 2003) and Lilacs (www.bireme.br; accessed December 2003). Search terms used were as follows: 'septic

ACTH = adrenocorticotrophic hormone; CBG = cortisol-binding globulin; CRH = corticotropin-releasing hormone; GR = glucocorticoid receptor; 11β-HSD = 11β-hydroxysteroid dehydrogenase; hsp = heat shock protein; IL = interleukin; LPS = lipopolysaccharide; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor-κB; NOS = nitric oxide synthase; SRC = steroid receptor coactivator; TNF = tumour necrosis factor.

Figure 1



Crosstalk between the immune system and the neuroendocrine axis. 11β-HSD, 11β-hydroxysteroid dehydrogenase; CBG, cortisol-binding globulin; HT, hypothalamus; IL, interleukin; PG, pituitary gland; TNF, tumour necrosis factor.

shock', 'sepsis', 'adrenal insufficiency', 'steroids', 'corticosteroids', 'adrenal cortex hormones', 'hydrocortisone' and 'glucocorticoids'. We also checked the reference lists of all trials identified using these methods. Reports were selected on the basis of relevance to the specific topics covered.

Mechanisms of glucocorticoid insufficiency

During an acute illness such as sepsis, circulating pro-inflammatory cytokines, including IL-6, tumour necrosis factor (TNF)-α and IL-1β, stimulate the production of corticotropin-releasing hormone (CRH) and of adrenocotricotrophic hormone (ACTH; corticotropin; Fig. 1). Simultaneously, vagal afferent fibres detect the presence of cytokines such as IL-1β and TNF-α, as well as other factors that are as yet unknown, at the site of inflammation and activate the hypothalamic–pituitary axis. Numerous other factors also contribute toward upregulating ACTH synthesis, such as the noradrenergic system, vasopressin, serotonin, angiotensin and vasoactive intestinal peptide [5]. Subsequently, ACTH increases cortisol release from the adrenal glands, which then binds to a specific carrier – cortisol-binding globulin (CBG) – that is synthetized by the liver and to albumin in order to reach the target tissues. Under normal conditions, 90–95% of plasma cortisol in humans is bound to CBG, and it is generally accepted that the CBG-bound cortisol has restricted access to target cells [6,7]. At inflammatory sites, elastase produced by neutrophils liberates cortisol from CBG, allowing localized delivery of cortisol [7]. Then, cortisol can freely cross the cell's membrane, or it may interact with specific membrane binding sites. Alternatively, cortisol is inactivated by conversion to cortisone by the 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2.

Dysfunction at any of these steps eventually results in diminished cortisol action. Thus, it can be anticipated that glucocorticoid insufficiency may be related to a decrease in glucocorticoid synthesis (i.e. adrenal insufficiency) or to reduced access of glucocorticoid to target tissues and cells.

Decreased glucocorticoid synthesis

Upon ACTH stimulation, glucocorticoids are synthesized by the adrenal cortex from cholesterol. The cholesterol required for steroidogenesis is derived from local cholesterol synthesis from acetate (about 20%) and from exogenous sources (the remaining 80%) [8]. Cholesterol is converted to 21-carbon glucocorticoids and 19-carbon weak androgens in serial enzymatic steps. A small amount of corticosterone is stored as a sulphate conjugate in the adrenal cortex [9]. However, the amount of glucocorticoid found in adrenal tissue is not sufficient to account for the initial rise in cortisol that occurs following stress, and it is not sufficient to maintain normal rates of secretion for more than a few minutes in the absence of continuing biosynthesis. Thus, the rate of secretion is directly proportional to the rate of biosynthesis. In other words, any disruption in glucocorticoid synthesis will immediately result in glucocorticoid insufficiency. Adrenal insufficiency can be considered primary or secondary, although this categorization is often artificial within the context of critical illness.

Secondary adrenal failure

Sepsis may result in decreased CRH or ACTH synthesis by inducing irreversible anatomical damage to the hypothalamus or the pituitary gland. The anterior and posterior hypophysial arteries are derived from the internal carotid arteries. The

Table 1

Drug related glucocorticoid insufficiency

Mechanisms	Drugs
Primary adrenal insufficiency	
Haemorrhage	Anticoagulant therapy (heparin, warfarin)
Cortisol synthesis enzyme inhibition	Aminogluthethimide
	Ketoconazole
	Fluconazole
	Etomidate
	Dexmedetomidine
Cortisol metabolism activation	Phenobarbital
	Phenytoin
	Rifampin
Secondary adrenal insufficiency	
Suppression of CRH and ACTH synthesis	Glucocorticoid therapy (systemic or topical)
	Megestrol acetate
	Medroxyprogesterone
	Ketorolac tromethamine
	Antidepressant drugs (e.g. imipramine)
	Opiate drugs
Peripheral resistance to glucocorticoids	
Interaction with glucocorticoids receptor	Mifepristone
Inhibition of the glucocorticosteroid-induced gene transcription	Antipsychotic drugs (e.g. chlorpromazine)
	Antidepressant drugs (e.g. imipramine)

ACTH, adrenocorticotrophic hormone; CRH, corticotropin-releasing hormone.

arterial branches to the pars tuberalis and the primary plexus of the portal vessels in the median eminence are derived from the internal carotid and posterior communicating arteries. The venous blood passes to surrounding venous sinuses in the dura mater or in the basisphenoid bone. In many cases the arterial supply to the pars distalis is reduced or even absent, and the portal vessels may be only routes by which blood can be supplied to the anterior pituitary gland. Consequently, pituitary necrosis is a well known complication of dramatic cardiovascular collapse, as occurs in Sheehan's syndrome during the postpartum period. Within this context, glucocorticoid insufficiency is usually associated with deficiency in thyroid and growth hormones and in vasopressin. Necrosis or haemorrhage of the hypothalamus or of the pituitary gland have been reported in sepsis as a result of prolonged hypotension or severe coagulation disorders [10].

Sometimes, sepsis may exacerbate chronic known or latent secondary adrenal insufficiency, which may be due to hypothalamic or pituitary tumours, chronic inflammation, or congenital ACTH deficiency. Secondary adrenal insufficiency may also follow drug therapy (Table 1) [11]. Previous treatments with glucocorticoids induce prolonged suppression of CRH and ACTH synthesis, and result in slow onset secondary adrenal insufficiency that may outlast exposure to this treatment [12]. The duration of suppression of the hypothalamic–pituitary axis after a single dose of a glucocorticoid depends on the anti-inflammatory potency and duration of the glucocorticoid preparation, hydrocortisone being the least suppressive agent and dexamethasone the most [13]. Although systemic glucocorticoid administration is more likely to suppress the hypothalamic–pituitary axis than local treatments, adrenal insufficiency has been observed even after topical administration of glucocorticoids [14]. It is thought that after 20–30 mg/day prednisone (or equivalent) for 5 days, the hypothalamic–pituitary axis is highly likely to be suppressed [15]. Thus, patients with sepsis who have previously been treated with glucocorticoids should be considered adrenal insufficient. It may be more cost-effective to treat all such patients with systematic replacement therapy than to target treatment at those patients who are identified by endocrine tests.

Opiate receptors are known to modulate ACTH/cortisol synthesis. In normal individuals administration of an opiate agonist results in a fall in plasma cortisol levels, although it induces hypotension. In contrast, administration of naloxone, an opiate antagonist, increases plasma ACTH and cortisol to levels similar to those that occur in insulin-induced hypoglycaemia [16]. Anaesthesia with high-dose diazepam and fentanyl inhibits the early increase in ACTH and cortisol

that occurs in response to surgery, suggesting that these drugs act at the level of the hypothalamus [17,18]. Given that these drugs are commonly used for sedation in critically ill patients, one may expect that these drugs contribute, at least partly, to adrenal insufficiency in patients with sepsis.

During sepsis, suppression of CRH synthesis may also result from neuronal apoptosis, which may be triggered by elevation in substance P [19] or inducible nitric oxide synthase (NOS) in the hypothalamus [20]. Circulating proinflammatory mediators such as TNF-α may block CRH-induced ACTH release [21]. Likewise, local expression of TNF-α and IL-1β may interfere with CRH and ACTH synthesis [20].

Primary adrenal failure

In sepsis, primary adrenal failure may result from bilateral necrosis and haemorrhage of the adrenals, as reported by Waterhouse [1] and Friderichsen [2]. Adrenal blood flow is about 6–7 ml/min per gram of tissue. Three small arteries derived from the inferior phrenic artery, the renal artery and the aorta form rich plexuses in the cortex and supply the gland. The plexuses are continuous with the sinuses of the medulla, which drain into the central vein of the medulla. The right adrenal vein drains into the inferior vena cava and the left into the renal vein. Hence, the rich blood supply required by the organ and the limited venous drainage (a single vein) predispose to extensive haemorrhage [22]. Experiments in animals has shown that the ACTH-stimulated (stressed) adrenal gland is more susceptible to haemorrhage [23]. Bilateral adrenal haemorrhage may be found in about 1–1.8% of autopsied patients [24] and in up to 30% of nonsurvivors from septic shock [25]. The main risk factors for hemorrhagic primary adrenal failure are increase in serum urea nitrogen of 25 mg/dl or more, positive blood cultures, shock, coagulation disorders, and anticoagulant therapy.

Sepsis may exacerbate chronic known or latent primary adrenal insufficiency, which is usually caused by autoimmune adrenalitis in developed countries and tuberculous adrenalitis in developing countries [26]. Other infectious diseases, including viral and fungal infections, may also cause chronic primary adrenal insufficiency, particularly in immunosuppressed patients. For example, morphological evaluation of adrenal glands from 128 autopsied patients with the AIDS identified compromised adrenals in 99.2% of cases, with distinct pathological features and infectious agents [27]. Cytomegalovirus is by far the commonest pathogen involved in adrenal dysfunction in AIDS patients [27,28]. Finally, genetic disorders, tumoural and nontumoural adrenal infiltration, and bilateral adrenalectomy are less common causes.

Numerous drugs that are commonly used in acutely ill patients are known to decrease cortisol synthesis (Table 1). These drugs may block enzymatic steps such as inhibition of the adrenal P450 cholesterol side-chain cleavage enzyme by aminogluthethimide [29], or partial or full inhibition of the adrenal 11β-hydroxylase by etomidate [30], ketoconazole [31] or high-dose fluconazole [32]. Etomidate inhibits steroidogenesis by blocking mitochondrial cytochrome P450 enzymes, and this effect may persist as long as 24 hours after a single dose of etomidate in critically ill patients [17]. Dexmedetomidine, a highly selective and potent α_2 agonist, is increasingly used for postoperative sedation and analgesia [33]. It is an imidazole compound and *in vitro* and *in vivo* animal studies have shown that dexmedetomidine inhibits cortisol synthesis at a concentration that is higher than those obtained during anaesthesia in humans [34]. In addition, it has recently been shown that dexmedetomidine may be used for short-term (i.e. 24 hours) postoperative sedation in the intensive care unit without altering adrenal function [35].

During severe sepsis, circulating proinflammatory cytokines such as TNF-α may inhibit ACTH-induced cortisol release [36]. Neutrophil-derived corticostatins such as α-defensins compete with ACTH on their binding sites and exert an inhibitory effect on the adrenal cells [37]. This phenomenon may explain the blunted response to exogenous ACTH that is observed in about 50% of patients with severe sepsis [38]. In less sick patients, ACTH resistance may be better unmasked by the low dose (1 μg) than by the traditional 250 μg ACTH test [39].

Finally, cortisol metabolism may be accelerated by drug competition. Indeed, the main enzymes involved in cortisol metabolism – the microsomal 6β-hydroxylase and the cytosolic 4-ene-reductase, members of the cytochrome 3A subfamily – may be inhibited by a number of drugs (Table 1), including ketoconazole and cyclosporine [40], clarithromycin [41] and antiepileptic drugs such as phenytoin [42] and phenobarbital [43].

Decreased glucocorticoid delivery and action

Decreased glucocorticoid access to tissues

CBG is a member of the serine protease inhibitor (serpin) superfamily. It has retained the stressed native structure typical of the inhibitor members of the family, and the transition from the stressed to the relaxed conformation of the protein has been adapted to allow altered hormone delivery at inflammatory sites [6]. CBG acts as a substrate for neutrophil elastase. However, CBG does not alter the activity of this enzyme but is cleaved by it at a single location close to its carboxyl-terminus; this reduces its molecular size by 5 kDa, with concomitant release of more than 80% of CBG-bound cortisol. It has been shown that granulocytes from septic patients, but not from control individuals, reduced the molecular weight of CBG by about 5 kDa and destroyed its steroid-binding activity. These findings suggest that CBG-elastase release of cortisol allows for localized delivery of cortisol to sites of inflammation, avoiding systemic side effects [7].

CBG may also directly modulate cortisol concentration in response to a given production rate. Indeed, in dexametha-

sone-suppressed adults, cortisol concentrations correlated with exogenous cortisol infusion rate only when adjusted for CBG levels [44]. In addition, CBG levels inversely correlated with the cortisol disappearance rate, suggesting that CBG actively modulates the disposition of cortisol in humans [44]. Sepsis following trauma and burns is characterized by reduced activity and amount of CBG [45–47], which may be related to circulating IL-6 levels. In addition, reports in burned patients have shown that low-fat diet was associated with a significant increase in serum CBG concentrations, suggesting that dietary manipulations may modulate circulating CBG levels [46]. The decreased circulating CBG levels eventually result in decreased cortisol distribution and delivery to the site of inflammation and to immune cells, although the fraction of serum free cortisol is increased. In addition, at the tissue level elastase is crucial for CBG cleavage and thus for cortisol release. Therefore, drugs that inhibit elastase will prevent cortisol release from CBG and cortisol access to the tissue.

Tissue levels of cortisol are also regulated by enzymatic conversion of cortisol to its inactive form, cortisone, by the 11β-HSD type 2. Sepsis is usually characterized by an increase in the cortisol/cortisone ratio that is proportional to the increase in acute phase protein concentration, suggesting a pivotal role for 11β-HSD isoenzyme 1 in the modulation of systemically available cortisol [48]. In addition, it has been shown that IL-1β and TNF-α upregulate 11β-HSD type 1 activity [49], and TNF-α decreases 11β-HSD type 2 activity [50]. Thus, in the early phase of the inflammatory process, mediators derived from the recruitment of T-helper-1 cells increase the conversion of cortisone to cortisol. Cortisone serves as an additional source for cortisol at the site of inflammation. In a second phase, cortisol enhances the recruitment of T-helper-2 cells, and subsequently released cytokines such as IL-2, IL-4 and IL-13 stimulate 11β-HSD type 2 activity, converting cortisol to cortisone [51]. Thus, at the site of inflammation, the tight crosstalk between immune cells and cortisol allows local cortisol levels to increase in the early phase of the inflammatory process, thus counteracting the effects of proinflammatory mediators. Afterward, it allows cortisol levels to decrease, avoiding local immunosuppression. Because cytokine-regulated cortisol–cortisone shuttle plays such a pivotal role in the regulation of tissue glucocorticoid activity, the ratio of tissue cortisol/cortisone concentrations is the best marker of glucocorticoid activity.

Decreased glucocorticoid receptor number/affinity

When cortisol is delivered to target cells, it freely crosses the cell's membrane and then it interacts in the cytosol with specific receptors. Glucocorticoids mediate their effects on target immune tissues via two distinct receptor subtypes: the mineralocorticoid receptor and the glucocorticoid receptor (GR). Although the mineralocorticoid receptor has a higher affinity for circulating glucocorticoids than the GR, the GR is expressed in much higher amounts in immune tissues [52].

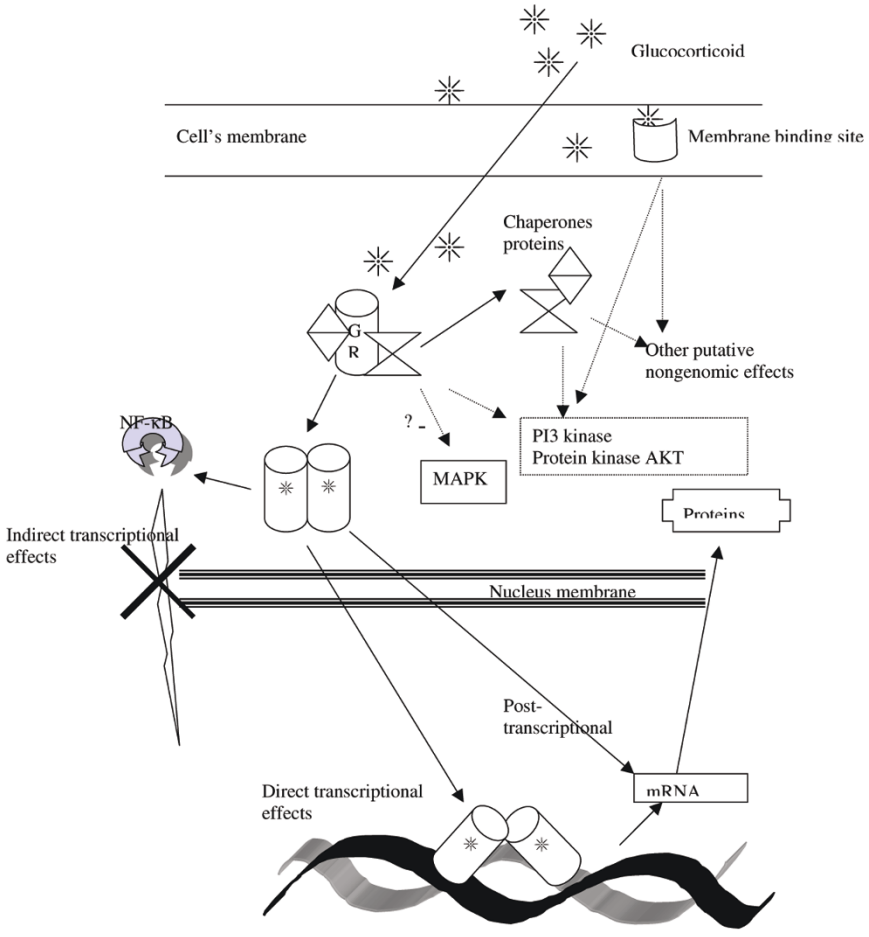
There are no data suggesting that sepsis or other diseases may be associated with impaired cortisol entry into the cells. Both endotoxin and lipopolysaccaride (LPS) have been shown to decrease GR affinity for ligand, mainly by inducing cytokine expression [53]. Studies have shown that cytokines may alter the GR function in various cell types, including T cells [54], monocytes/macrophages [55], bronchial lung [53] and liver [55] cells. A similar reduction in GR function and affinity for ligand can be demonstrated on peripheral cells and tissues from patients with inflammatory diseases such as asthma, ulcerative colitis, AIDS, rheumatoid arthritis, acute respiratory distress syndrome and sepsis [56–64]. Investigations into GR expression yielded heterogeneous findings. Some studies found downregulation of GR [53,65–67] and others found upregulation [68–70]. These discrepancies may result from the use of different types of cells and tissues, as well as different treatments (IL-1α or IL-1β, or IL inducers such as endotoxin). In addition, studies conducted in cells treated with IL-1 for 24–48 hours or in tissues from animals with chronic sepsis or patients with chronic inflammation consistently showed GR upregulation [61,70,71], whereas experiments with shorter treatments with IL-1 inducers or conducted in the early phase of human sepsis showed GR downregulation [53,66,67]. Most of the studies showing GR downregulation also found decreased cytosolic GR binding, which may result from compartmentalization of the GR during the acute response to cytokines. The hypothesis of GR compartmentalization may be supported by the fact that LPS and IL-1β induced GR upregulation without increasing GR mRNA [69].

Potential mechanisms for cytokine-induced reduction in GR function and affinity may include inhibition of GR translocation from cytoplasm to nucleus and reduction in GR-mediated gene transcription [68]. In addition, FLICE-associated huge protein – a transducer of TNF-α and Fas ligand signals – may participate in TNF-α-induced blockade of GR transactivation by binding to nuclear receptor binding domain of GR-interacting protein 1. Thus, TNF-α may induce glucocorticoid resistance acting upstream and independently of nuclear factor-κB (NF-κB) [72].

Molecular action of glucocorticoids

Glucocorticoids act by binding to a specific GR. A 94 kDa protein, the GR is a member of the nuclear receptor family. Upon activation it dissociates from a multiprotein complex, dimerizes, enters the nucleus and binds to specific DNA regions termed glucocorticoid responsive elements (Fig. 2). The GR contains three domains. The amino-terminal domain harbours transactivation functions (τ1 region) and regulates many biological effects. The DNA-binding domain is well conserved among the nuclear hormone receptors. The carboxyl-terminal domain, called the ligand-binding domain, also contains a transactivation region (τ2). At homeostasis the GR forms a multiprotein complex with numerous members of the heat shock protein (hsp) family (hsp90,

Figure 2



Molecular action of glucocorticoids. GR, glucocorticoid receptor; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PI3 kinase, phosphatidylinositol 3-kinase.

hsp70, hsp56 and hsp40), immunophilins (FKBP51 and FKBP52), P23 and potentially other proteins that are as yet unknown [73]. The transactivation regions τ1 and τ2 probably constitute major areas for interaction with coactivator and corepressor on nuclear receptor transcriptional activities [74].

Upon activation, subsequent to ligand binding, the GR undergoes conformational changes, dissociation from other proteins (particularly shedding from hsps), dimerization, translocation to the nucleus and contact with general transcription factors, adapter proteins and various co-activators. Then, transcriptional activation or repression of specific target genes occurs and subsequently levels of regulated proteins change. In addition, post-transcriptional effects such as on mRNA may occur. GR interactions with the other proteins of the complex are still poorly understood. However, it is thought that these interactions may account for a number of rapid nongenomic biological effects of glucocorticoids (e.g. phosphorylation/dephosphorylation of GR, calcium signalling-related effects, and effects due to membrane events) [75]. Indeed, these effects are too rapid to allow time for transcriptional and translational events to take place, and they are insensitive to appropriate inhibitors. One must distinguish glucocorticoid-induced genomic and non-genomic effects.

Genomic effects

The GR directly activates or represses target genes by binding to hormone response elements in promoter or enhancer regions and by binding to other DNA sequence specific activators, and it can inhibit the transcriptional activities of other classes of transcription factors by transrepression. Regulation of gene trascription by nuclear receptors requires the recruitment of coregulators. Their number do not allow direct ineraction, suggesting that they act in combination or in a sequential manner [76]. Among these coregulators, the p160 steroid receptor coactivator (SRC) gene family contains three homologous members

(SRC-1, SRC-2 and SRC-3). These coactivators are crucial in facilitating chromatin remodelling, assembly of general transcription factors, and transcription of target genes by the recruitment of histone acetyltransferases and methyltransferases to specific enhancer/promotor regions [77]. The GR-induced transrepression occurs through DNA-dependent mechanisms (i.e. displacement of an activator, overlapping binding sites, or binding to continuous negative glucocorticoid responsive element) and via DNA independent mechanisms (without direct contact between the GR and DNA). The latter includes binding of GR to a DNA-bound activator (tethering mechanism) or formation of abortive complex between GR and another transcription factor (squelching mechanism) [78].

Studies using DNA microarray analysis combined with quantitative TaqMan polymerase chain reaction and flow cytometry showed the complex transcriptional effects of glucocorticoids. They transactivated genes for chemokines, cytokines, complement family members and newly discovered innate immune-related genes, including scavenger and Toll-like receptors. Glucocorticoids also transrepressed adaptive immune-related genes. Finally, glucocorticoids may simultaneously transactivate and repress inflammatory T-helper subsets and apoptosis-related gene clusters [79]. Development of GR agonists that may favour transrepression over transactivation represent an exciting new field of research [80].

The NF-κB protein family includes p65 and p50, which form a complex that is maintained in its inactive form by a specific inhibitor – IκB-α – in the cytosol [81]. The interaction between glucocorticoids, NF-κB and activator protein-1 represents the main GR-induced, DNA-independent mode of transrepression and is reviewed elsewhere [82] Briefly, GR prevents activator protein-1 from interacting with its binding site within the promoters. *In vitro* inhibition of NF-κB activation has been reported in various types of cells, although an enhanced expression of the p65 component of NF-κB has been reported in response to glucocorticoids. In addition, the induction of IκB-α by glucocorticoids further inhibits NF-κB-dependent gene transcription.

Glucocorticoids may also regulate inflammatory mediators by acting at the post-transcriptional level, on mRNA or on proteins. For example, via post-transcriptional mechanisms, dexamethasone inhibits IL-8 mRNA and protein expression in cultured airway epithelial cells [83], inhibits inducible NOS expression and activity in C6 glioma cells [84], increases macrophage migrating inhibitory factor in rat tissues [85], and increases angiotensin-converting enzyme in primary culture of adult cardiac fibroblasts [86].

Nongenomic effects

Membrane-bound receptors are thought to mediate specific nongenomic effects of glucocorticoids [87]. Indeed, membrane-binding sites for different glucocorticoids have been described in many tissues and cells, including liver plasma membranes and neuronal synaptic membranes, with evidence for both nonclassic receptors and a membrane form of classic GR [88]. Conversely, nonspecific nongenomic effects are thought to result from physicochemical membrane interactions, and to occur within seconds to minutes but only at high doses of glucocorticoid [89].

Thus far, rapid glucocorticoid action has been intensively investigated mainly in the central nervous system, and includes effects on neuronal excitability, neuroendocrine responses and behavioural tasks [90]. Some of these effects might be important in the host response to sepsis.

Nonspecific nongenomic effects

Direct membrane effects of glucocorticoids in the hypothalamic synaptosomes have been suggested as the cellular mechanism for plasma cortisol-induced negative feedback [91]. The loss of this effect may partly explain the disruption in circadian rhythm of cortisol synthesis during sepsis. Acetylcholine-induced current in pheochromocytoma cell line PC12 is inhibited by extracellular but not intracellular application of corticosterone [92]. These effects are not inhibited by the transcription inhibitors, and allow glucocorticoids to control immediate catecholamine release from sympathetic cells. This may explain the rapid restoration of the sympathetic modulation of heart rate and vasomotor tone [93], as well as the potentiation of exogenous catecholamine action that can be seen within minutes after a 50 mg bolus of hydrocortisone in septic shock [94,95].

Specific nongenomic effects

Some of these effects may be relevant to sepsis treatment because they may account for glucocorticoid-induced rapid anti-inflammatory and cardiovascular effects.

The p38 mitogen-activated protein kinase (MAPK) participates in intracellular signalling cascades resulting in inflammatory responses. Studies in healthy volunteers challenged with LPS showed that p38 MAPK is a determinant of LPS-induced cytokine production, leucocyte responses [96], neutrophil activation and chemotaxis [97], and of LPS-induced coagulation activation, fibrinolysis inhibition and endothelial cell activation [98]. The classic GR may interfere directly with Raf-1, which is downstream of Ras in MAPK cascade, or via 14-3-3 (an adapter protein that is known to interplay with proteins such as protein kinase C and Raf-1) [99]. In addition, the GR may inhibit Raf/MAPK extracellular signal-regulated kinase activation through protein–protein interactions [100]. Whether the interaction between GR and p38 MAPK accounts for nongenomic anti-inflammatory effects of glucocorticoids remains to be investigated.

Membrane GRs that are present in normal and in cancerous lymphoid cells may be involved in disruption of the

mitochondrial membrane potential and in decreased ATP availability, and subsequently may lead to apoptosis [101].

It has recently been shown that glucocorticoids, through non-nuclear activation of phosphatidylinositol 3-kinase and the proteine kinase Akt, could exert perfusion-independent protective effects in a model of ischaemic brain injury [102]. Similarly, binding of glucocorticoids to the GR-stimulated phosphatidylinositol 3-kinase and protein kinase Akt, leading to endothelial NOS activation and nitric oxide dependent vasorelaxation, is the mechanism by which glucocorticoids decreased vascular inflammation and reduced myocardial infarct size following ischaemia/reperfusion injury in mice [103].

Competing interests

None declared.

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