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Cell-specific regulation of apoptosis by glucocorticoids: implication to their anti-inflammatory action

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Abstract

Glucocorticoids play a major role in attenuation of the inflammatory response. These steroid hormones are able to induce apoptosis in cells of the hematopoietic system such as monocytes, macrophages, and T lymphocytes that are involved in the inflammation reaction. In contrast, it was discovered recently that in glandular cells such as the mammary gland epithelia, hepatocytes, ovarian follicular cells, and in fibroblasts glucocorticoids protect against apoptotic signals evoked by cytokines, cAMP, tumor suppressors, and death genes. The anti-apoptotic effect of glucocorticoids is exerted by modulation of several survival genes such as *Bcl-2*, *Bcl-x_L*, and *NFkB*, in a cell-specific manner. Moreover, upregulation or downregulation of the same gene product can occur in a cell-dependent manner following stimulation by glucocorticoids. This phenomenon is probably due to composite regulatory cross-talk among multiple nuclear coactivators or corepressors, which mediate the transcription regulation of the genes, by their interaction with the glucocorticoid receptor. These observations suggest that the anti-inflammatory action of glucocorticoids is exerted by two complementary mechanisms: on one hand, they induce death of the cells that provoke the inflammation, and on the other hand they protect the resident cells of the inflamed tissue by arresting apoptotic signals. Moreover, the complementary action of glucocorticoids provides a new insight to the therapeutic potential of these hormones.

Keywords: Dexamethasone; Chemotherapeutic anticancer drugs; Ovary; Bcl-2

1. Introduction

Glucocorticoids exert a dramatic effect on a variety of mammalian cells. This class of steroids alters both cellular metabolism and gene expression and has long been known to display cytotoxic effects on certain cell types, such as lymphocytes (reviewed in [1]). Glucocorticoids released from the adrenal gland during periods of stress lead to the catabolism of proteins, lipids, and carbohydrates, and result in an increase in blood sugar [2,3]. Glucocrticoids repress or activate gene transcription through interaction with the glucocorticoid receptor (GR) (reviewed in [4]). The immune system is exquisitely sensitive to the lytic action of glucocorticoids as well as to their metabolic and genetic actions (reviewed in [4]). Since the complex physiological processes involved in mammalian immune and inflammatory responses are critical for the homeostasis and ultimate survival of an organism, their coordinate regulation must assure an appropriate and timely immune reaction without an overreaction that might damage the host (reviewed in [5,6]).

It has been known for the last decade that glucocorticoids induce apoptosis in most nucleated cells of the vascular system, such as thymocytes, myeloma cells, and peripheral blood monocytes (Table 1 and [7–9]), thus playing a central anti-inflammatory role. Recently there is increasing evidence for a complementary action of glucocorticoids in protecting the cells, tissues, and organs in which the inflammation takes place. This phenomenon raise intriguing questions:

- What is the impact of the cell origin (lineage) and final phenotype on the glucocorticoid effect on apoptosis?
- Does the anti-apoptotic action of glucocorticoids involve expression or attenuation of the same or different gene

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Abbreviations: Act D, actinomycin D; CHX, cycloheximide; Dex, dexamethasone; DAP3, death-associated protein 3; HSP 90, 90-kDa heat shock protein; 11βHSD 1, 11β-hydroxysteroid dehydrogenase type 1; GR, glucocorticoid receptor; GRα, α isoform of glucocorticoid receptor; GRE, glucocorticoid response element; TNFα, tumor necrosis factor α; TGFβ, tumor growth factor β.

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Table 1
Typical effects of glucocorticoids on apoptosis

Cell system	Mechanism involved	Inhibition of apoptosis induced by	Reference
Induction of apoptosis by dexamethasone			
Rat thymocytes	Interference between AP1 and GR in the binding to GRE		[7]
Acute myeloid leukemia cell lines[a]	Not completely understood. Bcl-2 and c-*myc* were unchanged		[82]
Myeloma cells	Downregulation of the activities of MAPK and $p70^{s6k}$		[8]
Human peripheral blood mononuclear cells (PBMCs)	Suppression of mitogen-induced PBMC-blastogenesis		[83]
T lymphocyte	Mediated by c-*fos*		[84]
B lymphocyte	Not fully characterized		[85]
Human monocytes[a,b]	Not fully characterized		[86]
Human eosinophils	Activation of JNK and p38 MAPK		[87]
Rat placenta	Not fully characterized		[88]
Thymocytes	Altering the plasma membrane potential		[20]
Inhibition of apoptosis by dexamethasone			
Mammary gland	An induction of protein kinase A, AP1 DNA binding activity, and elevated c-*fos*, jun B, and jun D mRNA levels	Spontaneous	[50]
Human neutrophil	Not fully characterized	Spontaneous	[89]
Human gastric cancer cell line TMK-1	Modulation of *Bcl-x* gene expression: suppression of $Bcl\text{-}x_S$ and increasing the basal level of $Bcl\text{-}x_L$	Transcription and translation inhibitors (CHX and ActD)	[32]
Lung epithelial cell	cIAP-2	IFNγ and FAS	[90]
Hepatoma cell lines	Induction of $Bcl\text{-}x_L$	Spontaneous and by TGFβ1	[71]
T lymphocyte overexpressing Bcl-2	Overexpression of Bcl-2	Serum deprivation	[27]
Osteoblasts (MC3T3E1)	Blocking JNK/SAPK activation	TNFα	[91]
Bovine glomerular endothelial cells[b,c]	Inhibition of caspase-3-like proteases activation	TNFα and lipopolysaccharide	[92]
Rat hepatoma cells (HTC)	Activation of NF*k*B	Serum deprivation	[41]
Granulosa cells[b]	Elevation in Bcl-2	Serum deprivation, cAMP accumulation, and p53 activation	[26]

[a] Also by prednisolone.
[b] Also by hydrocortisone.
[c] Also by fluocinolone, prednisolone, and corticosterone.

products which are involved in their proapoptotic action?
- Is the anti-apoptotic effect of glucocorticoids mediated by the same receptor as their proapoptotic effect?

2. The GR and apoptosis

Most of the effects of glucocorticoids in controlling the apoptotic process in various cell types are believed to be mediated by the GR (reviewed in [10]). However, additional points should be considered:

I. Two GR isoforms were characterized recently in human cells, both in health and disease ([11] and reviewed in [4]). However, all receptor activity was attributed to the α isoform of GR (GRα). It is not yet clear whether GRβ which is able to inhibit the activity of GRα is involved in glucocorticoid-induced modulation of apoptosis ([12] and reviewed in [4]). Recently, it was found that *GR*α gene is subject to alternative translation initiation from a downstream, in-frame of ATG codon. The longer protein from the first ATG codon (Met1) was termed as hGR-A and a shorter protein (751 amino acids) as hGR-B [13]. Both receptors exhibit similar subcellular localization and nuclear translocation after ligand activation. Functional analyses of hGR-A and hGR-B under various glucocorticoid-responsive promoters reveal the shorter hGR-B to be nearly twice as effective as the longer hGR-A species in gene transactivation, but not in transrepression [13].

II. Prior to activation of the GR by interaction with a cytoplasmic ligand, it is associated with the 90-kDa heat shock protein (HSP90) ([14,15] and Fig. 1). Upon hormone binding, a conformational change occurs whereby HSP90 dissociates from the receptor molecule and allows the receptor–hormone complex to migrate to the nucleus, where it can transactivate a wide spectrum of genes, some of them are involved in apoptosis (reviewed in [4,5,16]).

III. Activation of the receptor involves its phosphorylation on serine residues, which increases its transactivation potency, but it decreases its half-life ([17], Fig. 1 and reviewed in [18]). However, correlation between phosphorylation of the GR and the modulation of apoptosis has not yet been completely established.

IV. DNA binding of GR was found to be not essential for survival in $GR^{-/-}$ mice, which suggests the existence of DNA binding independent activities of GR [19].

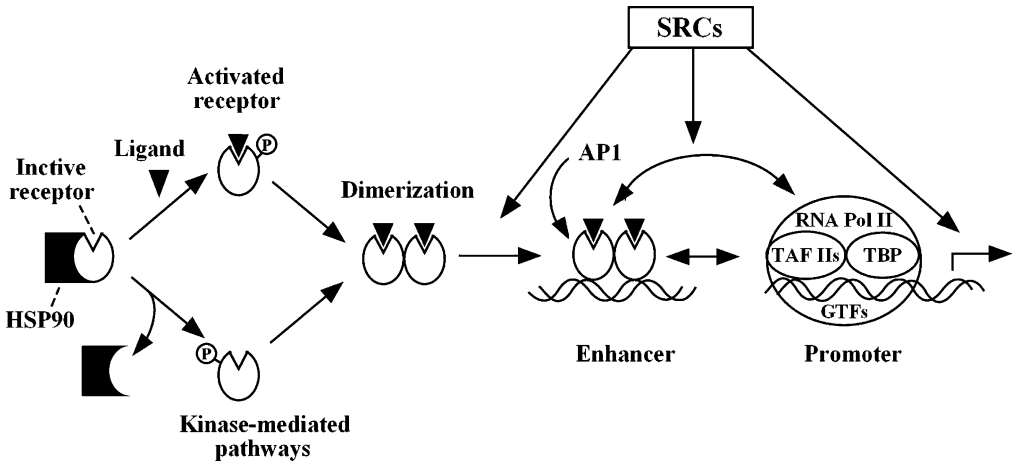

Fig. 1. Overview of events initiated by binding of ligand to nuclear receptor and culminating in transcriptional activation of a hormone-regulated gene. 'Activation' of receptor occurs either through hormone binding or by catalytic phosphorylation by kinases of specific residues on the receptor. Receptor phosphorylation may occur either in a ligand-dependent or -independent pathways [75–77]. Specific conformational alterations result in dimerization, nuclear translocation, and apposition to a specific *cis*-acting hormone response element. The precise mechanism of assembly of a stable preinitiation complex composed of basal transcription factors and other promoter-specific factors is unknown. While receptor can directly contact general transcription factors, many coactivators are known to mediate these interactions. In addition, they are likely to play multiple role in both processes which precede DNA binding and those which follow formation of the transcriptional preinitiation complex. Abbreviations: TBP, TATA box-binding protein; TAFs IIs, TBP-associated factors; GTFs, general transcription factors; RNA Pol II, RNA polymerase II; HSP90, heat shock protein; SRCs, steroid receptor coactivators [78–81]. For details see [15].

V. It was discovered recently that glucocorticoids exert some of their effects *via* a membrane receptor, distinct from the classical intracellular GR (reviewed in [20]) working by a nongenomic mechanism. However, there is no evidence that glucocorticoids affect apoptosis by a nongenomic pathway.
VI. Most recently, it was demonstrated that glucocorticoids could alter the membrane potential, leading to specific changes in gene expression, suggesting a novel mechanism for the induction of apoptosis by glucocorticoid hormones [21].

3. Activation of genes involved in apoptosis

Some of the most active genes, involved in both induction and prevention of apoptosis are the members of the Bcl-2 family (reviewed in [22,23]). The family consists of proteins, which are proapoptotic, such as Bcl-x_S, Bad, Bax, Bid and anti-apoptotic, such as Bcl-2, Mcl-1, and Bcl-x_L (reviewed in [24]). The various Bcl-2 family members can dimerize with homolog monomers or with heterolog monomers enhancing or antagonizing the function of the other. In this way, the ratio of inhibitors to activators in a cell may determine the propensity of the cell to undergo apoptosis [25]. However, despite intense efforts, the complete mechanism by which Bcl-2 family of proteins regulate cell death remains obscure. Exerting a proapoptotic activity, glucocorticoid can suppress Bcl-2 levels in LTR-6 myeloid leukemia [26]. On the other hand, Bcl-2 protects against induction of apoptosis subsequent to its enhanced expression induced by glucocorticoids in T lymphocytes and ovarian granulosa cells (Table 1 and [26,27]). Bcl-x_L regulates the membrane potential and volume homeostasis of mitochondria [28]. In addition, Bcl-x_L was able to form an ion channel in synthetic lipid membranes [29]. Bcl-2, present in the outer mitochondrial membrane, has been suggested to block apoptosis by inhibiting the release of apoptosis-inducing factors from mitochondria, such as cytochrome *c* (reviewed in [21]). Cytochrome *c* serves as an important cofactor for the activation of Apaf-1 which is located in the cytosol and is able to activate caspase 9 [25].

It is not yet clear why in different cell types glucocorticoids can affect the expression of the same gene in an opposite way. One possibility is that different patterns of comodulators existing in different cell types can affect the promoter of the *Bcl-2* gene in an opposite manner [30,31]. Another mechanism for Bcl-2 regulation of glucocorticoid-mediated apoptosis is by affecting the stability of Bcl-2 family proteins, as was demonstrated for Bcl-x_L and Bcl-x_S [32]. Dexamethasone (Dex) not only suppressed the apoptosis-associated upregulation of Bcl-x_S, but also enhanced the basal level of Bcl-x_L in TMK-1 human gastric cancer cells. At the level of posttranscriptional modulation, Bcl-x mRNA stability was significantly extended in the presence of Dex [32]. Posttransitionally, Bcl-2 can be phosphorylated by PKCα at serine 70 [33]. Ito *et al.* [34] found that Bcl-2 phosphorylation is required for full Bcl-2 death suppression activity in IL-3-dependent myeloid cell line. Whether phosphorylation of Bcl-2 plays a role in apoptosis in other cell systems has yet to be determined.

The possibility also exist that glucocorticoid induction of apoptosis may be mediated by interference with transcription factors, such as AP1, required for cell survival, rather than direct induction of 'death genes' [35,36]. It was demonstrated that T cell leukemia cell lines expressing mutants to GR, defective in gene activation, were fully sensitive to glucocorticoids inhibiting IL-2 production, repressing AP1 activity, and inducing apoptosis [36].

Gene regulation by glucocorticoids can be positive or negative, depending on the cell type [26]. This bipolarity is probably due to composite regulatory cross-talk among multiple nuclear comodulators or by interactions with transcription factors from other families (reviewed in [37,38]). It was shown that the cell specificity of GR action on the composite response element of proliferin (PifG) is determined by the composition of AP1; specifically the ratio of c-*jun* to c-*fos* [39]. F9 cells, which lack AP1 activity, under appropriate culture conditions, displayed no hormonal regulation of PifG-linked CAT sequences. Hela cells, which express AP1 predominantly as c-*jun* homodimers, showed enhanced reporter expression in response to Dex, while CV-1 cells, in which AP1 is composed mainly of c-*jun*/c-*fos* heterodimers, showed repressed reporter gene expression upon Dex addition [39].

Another route related to modulation of apoptosis by glucocorticoid is *via* NF*k*B, a transcription factor induced by proinflammatory cytokines (reviewed in [40]). NF*k*B plays a crucial role in immunological and inflammatory processes by directing transcription of chemoattractants, cytokines such as tumor necrosis factor α (TNFα) and IL-2, cytokine receptors and cell adhesion molecules (reviewed in [38]). In hepatoma cells, Dex increased the nuclear translocation of NF*k*B [41]. Although, there is some controversy about the role of this transcription factor in apoptosis, most evidence supports an anti-apoptotic action on TNF-induced apoptosis [42]. In addition, it was shown that cells that are naturally resistant to TNF-induced apoptosis became sensitized when they are transfected with an expression vector for I*k*B, which inactivates NF*k*B [43]. Interestingly, a functional link between NF*k*B and Bcl-2 may exist since oxidative stress of cardiac transplantation was associated with decreased Bcl-2 expression and increased NF*k*B activity [44]. Additional factor that can modulate induction of apoptosis by glucocorticoid is the proapoptotic death-associated protein, DAP3. It is suggested that dissociation of GR–HSP90 complex is a prerequisite for migration of receptor–hormone complex and transactivation of specific genes that control apoptosis or survival (see Fig. 1 and [45]).

Glucocorticoids have been shown to inhibit the production of many cytokines but they also act synergistically with various cytokines on several types of cells. Suprisingly, glucocorticoids have been shown to upregulate the expression of various cytokine receptors, which is in apparent contrast to the other 'restraining' effects of these hormones This would suggest that endogenous glucocorticoids not only suppress but also enhance immune functions [46,47]. It should be noted that high constitutive GR β in human neutrophils enable them to reduce their spontaneous rate of cell death in response to corticoids [48].

DNA microarray studies revealed gene expression profiles of proliferating vs G1/G0 arrested human leukemia cells suggesting that glucocorticoid-induced apoptosis results from positive GR autoregulation entailing persistent downregulation of metabolic pathways critical for survival. Performing a kinetic of glucocorticoid-dependent gene regulation suggested that the subsequent continuous repression of various metabolic pathways such as downregulation of the lactate dehydrogenase might have a role in cell cycle arrest and ultimately lead to cell death [49].

4. The anti-apoptotic effect of glucocorticoids in specific tissues

4.1. Mammary gland

Dex, a stable and potent glucocorticoid hormone analog, inhibited involution and programmed cell death in the mouse mammary gland [50]. Injection of Dex led to milk accumulation and was accompanied by an induction of protein kinase A, elevated c-*fos*, jun B, and jun D mRNA levels and AP1 DNA binding activity. Potential target genes of AP1 such as *stromelysin-1*, *c-jun*, and *SGP-2*, induced during normal involution, were strongly inhibited in Dex-treated animals [50]. These results suggest that the cross-talk between steroid hormone receptors and AP1 leads to an impairment of AP1 activity and to an inhibition of involution in the mammary gland, implying that apoptosis in the postlactational mammary gland depends on functional AP1 [50]. Moreover, these experiments provide evidence that the anti-apoptotic activity of glucocorticoids enhances the release of products of differentiation, such as milk from an exocrine gland. These *in vivo* studies demonstrated the potential role of the glucocorticoids in protecting against apoptosis under physiological conditions. In addition, it was shown that Dex activates potent survival pathways in the human mammary epithelial cell line MCF10A independently of the anti-apoptotic PI-3-K and Akt/protein kinase B signaling pathways [51].

4.2. Ovary

It has been proposed that locally activated glucocorticoid production play a role in limiting ovarian tissue damage and mediating repair/remodeling after human ovulation (reviewed in [52,53]). Support for this proposal comes from the finding that soon before follicular rupture there is an elevation of total cortisol concentration in follicular fluid relative to that in serum [53]. Additionally, the proinflammatory cytokines, TNFα and interleukin-1β, were recently reported to stimulate the expression of 11β-hydroxysteroid

dehydrogenase type 1 (11βHSD1) mRNA in glomerular mesangial cells [54] and rat granulosa cells [55] *in vitro*. Because 11βHSD1 promotes reversible formation of cortisol from cortisone, it was suggested that the proinflammatory cascade that leads to ovulation induce a compensatory anti-inflammatory response in the ovary, which includes upregulation of 11βHSD1 and increased local availability of cortisol [56]. It was, therefore, proposed that developmental increase of 11βHSD1 expression before follicular rupture serve to raise the intrafollicular cortisol level. Since ovulation is an inflammatory process and glucocorticoids are anti-inflammatory [57,58], the locally increased cortisol level may serve to minimize inflammatory tissue damage and encourage rapid healing of the ovarian surface in anticipation of the next ovulatory cycle (reviewed in [52]).

The role of glucocorticoids on ovarian steroidogenesis and apoptosis in primary and immortalized human granulosa cells was recently studied in detail [26,59]. Dex and hydrocortisone synergies with gonadotropin to increase progesterone production. In addition, these glucocorticoids almost completely inhibited apoptosis exerted by serum deprivation, activation of the tumor suppressor gene *p53* or *TNFα* [26,59]. This protective effect coincided with increased expression of cadherins and connexins, the major building blocks of the adherence and gap junctions. A mechanistic approach to the involvement of glucocorticoids in preventing p53-induced apoptosis in endothelial and neroblastoma cells was recently proposed. Dex inhibits the transactivation of Bax and p21 WAF1/CIP1 and downregulation of Bcl-2, following a formation of a trimeric complex with Hdm2, which lead to enhancement of their degradation by Hdm2 [60,61] This would suggest that GR and p53 acting as an opposite forces in the decision between cell death and survival. Glucocorticoids also increased in granulosa cells, the network of the actin cytoskeleton, without affecting the *de novo* synthesis of actin [59]. Most pertinently, glucocorticoids increased the intracellular level of the anti-apoptotic gene protein Bcl-2 [26,59]. It was, therefore, suggested that protection of granulosa cells from apoptosis by glucocorticoids is achieved by enhancement of cell contacts and intercellular communication, stabilization of the actin cytoskeleton, and by upregulation of Bcl-2 expression. This protection of granulosa cells may contribute to the anti-inflammatory reaction, suggested to take place following follicular rupture (reviewed in [52,59]), by minimizing the damage to the follicular tissue as well as by stimulation of postovulatory steroidogenesis [59]. Moreover, we suggest that glucocorticoids could serve as a potential drug to overcome specific cases of ovarian failure.

4.3. Liver and fibroblasts

A clear example of the anti-apoptotic effect of glucocorticoids on resident cells was demonstrated in hepatocytes and fibroblast cells (Table 1, [41,62]). The cellular resistance to TNF of most cell types has been attributed to both a protective pathway induced by this cytokine and the preexistence of protective factors in the target cell [62]. NF*k*B has been postulated as one of the principal preexisting factors, which protect against apoptosis [62]. However, the recent demonstration that glucocorticoids protect the naturally TNF-sensitive L-929 fibroblast cells from apoptosis revealed that glucocorticoids protected against TNF-induced apoptosis at the same ratio at either normal or low levels of NF*k*B induction [62]. Thus, although glucocorticoids inhibit NF*k*B transactivation in these cells, this is not required for their protection from TNF-induced apoptosis [62]. Most relevantly, glucocorticoids suppressed apoptosis induced by serum starvation of HTC rat hepatoma cells in a GR-dependent manner. This effect is specific for glucocorticoids; progesterone, estrogen, and thyroid hormone did not have a similar effect [41]. Glucocorticoids effectively protected against apoptosis by inhibiting the loss of mitochondrial membrane potential and by activation of the transcription factor NF*k*B, which also serves in hepatoma cells as an anti-apoptotic factor [41]. In parallel, it was found that glucocorticoids protect against TNFα-induced apoptosis in ovarian granulosa cells and in immortalized human granulosa cells [26,63]. This protection coincided with elevation of intracellular levels of Bcl-2, with no change in the levels of NF*k*B [64].

5. Anti-apoptotic effect of glucocorticoids: implication in health and disease

Glucocorticoids, among the most widely prescribed compounds in current medical practice, are invaluable in the treatment of inflammatory disorders and pulmonary insufficiency during the prenatal period [65]. These hormones induce apoptosis in lymphocytes and are, therefore, used as chemotherapeutic agents against many leukemias [66,67]. In contrast, glucocorticoids suppress spontaneous apoptosis of neutrophils, TNFα-mediated apoptosis of mouse L929 fibroblasts, and involution of the mouse mammary gland [50,68,69]. In addition, they have profound protective effects against apoptosis in human and rat hepatocytes, and in HTC rat hepatoma cells [70–72]. Early research on internucleosomal cleavage of DNA during apoptosis showed that administration of the Dex to rats reduced the amount of endonuclease activity in liver cells [73]. Glucocorticoids also protect normal rat hepatocytes from undergoing apoptosis induced by inhibition of electron transport and suppress apoptosis of K2 hepatoma cells, induced by tamoxifen or TGFβ, as well as, spontaneous and TGFβ-induced apoptosis of McA-RH7777 and McA-RH8994 rat hepatoma cells [41,71,72]. Since glucocorticoids, which prevent apoptosis in hepatoma cells and gastric cancer cells, are administered in conjunction with chemotherapeutic anticancer drugs, which induce apoptosis

[74], the timing of glucocorticoid administration to patients should be evaluated in order to avoid reduction of the efficiency of anticancer drugs.

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