

## REVIEW

# Interactions of glucocorticoids and $\beta_2$ -agonists

I.M. Adcock, D.A. Stevens, P.J. Barnes

*Interactions of glucocorticoids and  $\beta_2$ -agonists. I.M. Adcock, D.A. Stevens, P.J. Barnes. ©ERS Journals Ltd 1996.*

**ABSTRACT:** Beta<sub>2</sub>-adrenoreceptor agonists and glucocorticosteroids are the two most effective treatments for asthma and are often used in combination. Glucocorticoids mediate their anti-inflammatory effects through the action of activated glucocorticoid receptors (GRs). Many of the effects of GRs on the synthesis of cytokines and other inflammatory mediators are due to a direct interaction with other deoxyribonucleic acid (DNA)-binding proteins belonging to the basic leucine zipper (bZIP) group of transcription factors, such as activator protein-1 (AP-1) and nuclear factor-kappa B (NF- $\kappa$ B).

Beta<sub>2</sub>-agonists are potent bronchodilators at low doses and at high doses can activate gene transcription via a bZIP protein, cyclic adenosine monophosphate (cAMP) response element binding protein (CREB). Activated GRs and CREB can interact with each other within the nucleus to modulate both DNA-binding and gene transcription in either a positive or inhibitory manner, depending on cell type. In lung cells, high doses of  $\beta_2$ -agonists reduce the ability of GR to bind DNA, a process which is mediated by CREB activation.

Inhibition of GR DNA-binding by CREB raises the possibility that high-dose  $\beta_2$ -agonists could have functional antiglucocorticoid activity and may be a basis for the reported increase in asthma morbidity and mortality in industrialized countries, which have increasing *per capita*  $\beta_2$ -agonist use.

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Beta-adrenoreceptor agonists and glucocorticosteroids are the two most effective treatments for asthma and are often used in combination. In this review, we summarize the interactions between these drugs at a biochemical and molecular level and discuss the possible consequences for asthma therapy.

### Mechanism of steroid action

Glucocorticosteroids have been proposed to mediate their anti-inflammatory responses, not against the source of the stress itself, but by inhibiting the body's normal reactions to stress, preventing those reactions from overshooting and threatening homeostasis [1]. Thus, many of the physiological and pharmacological effects of glucocorticosteroids may be secondary to modulation of the action of numerous inter- and intracellular mediators, including other hormones, prostaglandins, lymphokines and bioactive peptides. Glucocorticosteroids act by influencing transcription of target genes [2]. Glucocorticoids freely diffuse into the cell from the surrounding milieu and bind to the 94 kDa glucocorticoid receptor (GR), which is held in an inactive form within the cytoplasm by the presence of two molecules of the molecular chaperone, hsp90. Upon ligand-binding the GR undergoes a conformational change resulting in dissociation of the hsp90 proteins and unmasking of a nuclear localization signal.

The steroid-GR complex translocates into the nucleus, where it may bind directly to glucocorticoid responsive elements (GREs) on deoxyribonucleic acid (DNA) in the promoter region of steroid sensitive genes. Alternatively, the steroid-receptor complex may form heterologous complexes with other DNA-binding proteins, hence modulating an effect that they would otherwise have on gene transcription [3]. An interaction between the pro-inflammatory transcription factors, activating protein-1 (AP-1) and nuclear factor-kappa B (NF- $\kappa$ B) and GR has been demonstrated in cultured cells [4, 5], in human peripheral blood cells [6], and in human lung [7, 8], and may be an important aspect of the anti-inflammatory effect of steroids [9] (fig. 1). Glucocorticoids exert effects on expression of a wide variety of genes, including those for cytokines, receptors and enzymes, but the absolute and relative importance of any of these to the therapeutic response in asthma is not yet clear.

### Mechanism of $\beta$ -agonist action

Beta-adrenoceptors ( $\beta$ R) in human airway smooth muscle are of the  $\beta_2$ -receptor subtype [10], and thus selective  $\beta_2$ -agonists are used to counteract airway constriction in asthmatic patients. Following ligand-binding to the receptor, receptor-associated stimulating G proteins (Gs) are activated by the exchange of bound guanosine diphosphate

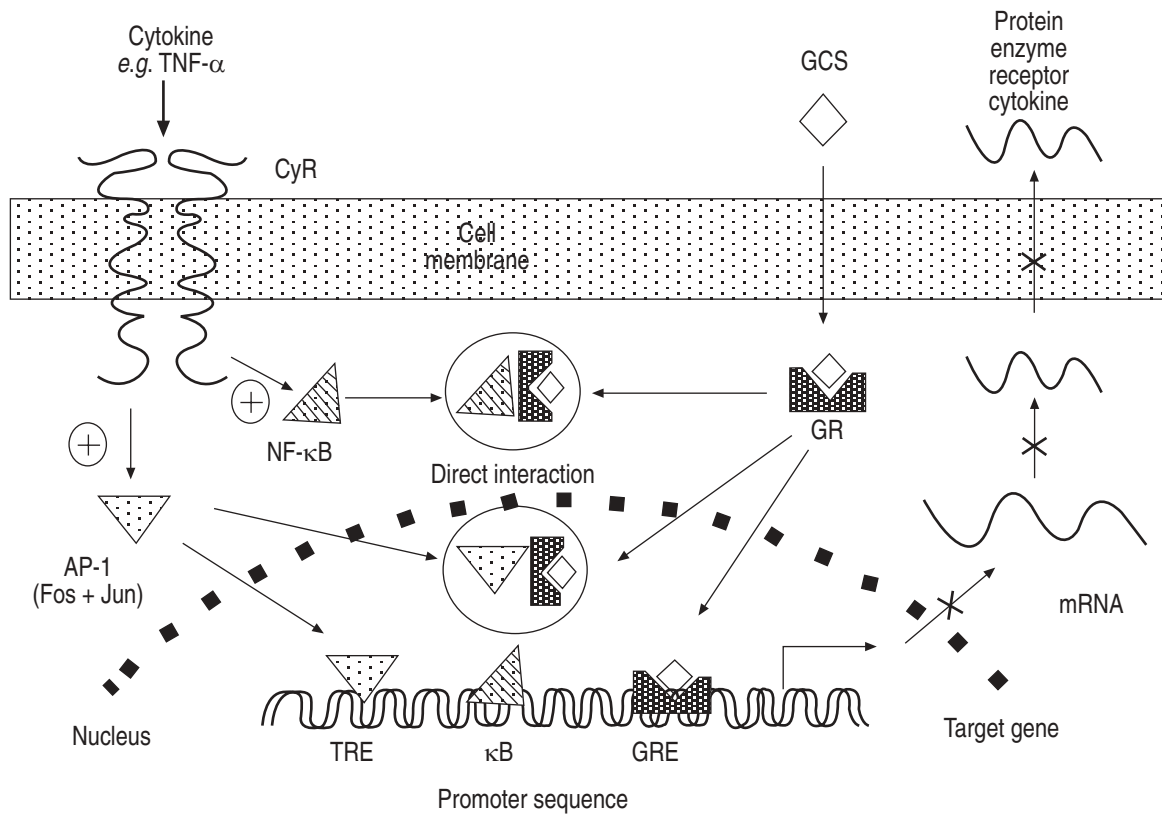


Fig. 1. – The mechanism of glucocorticoid action. Glucocorticosteroids (GCS) freely diffuse from the circulation across cell membranes where they interact with a glucocorticoid receptor (GR). Upon ligand-binding, the receptor is activated and translocates to the nucleus where it can bind to specific DNA sequences upstream of the start site of transcription. This causes bending of the DNA and interaction of the DNA-bound GR with the basal transcription complex and modulation of target gene transcription. Alternatively, GCS may act by inhibiting the ability of other transcription factors to bind to DNA. AP-1 and NF-κB activated by cytokines or other inflammatory mediators are prevented from binding to DNA by interaction with the GR causing mutual repression of DNA-binding. TNF-α: tumour necrosis factor-α; NF-κB: nuclear factor kappa B (binding protein); AP-1: activating protein-1; mRNA: messenger ribonucleic acid; DNA: deoxyribonucleic acid; GRE: glucocorticoid responsive element. CyR: cytokine receptor; TRE: TPA-response element.

(GDP) for guanosine triphosphate (GTP), and these then couple with adenylyl cyclase (AC) [11]. The coupling of activated G<sub>s</sub> and AC leads to enhanced production of cyclic 3'-5'-adenosine monophosphate (cAMP) and subsequent activation of cAMP-dependent protein kinase A (PKA). PKA then phosphorylates and, thus, inactivates myosin light chain kinase (MLCK), preventing myosin phosphorylation. At the same time, Ca<sup>2+</sup> is decreased through activation of Ca<sup>2+</sup>-Mg<sup>2+</sup>-adenosine triphosphatases (ATPases) in the endoplasmic reticulum and plasma membrane [12], reducing Ca<sup>2+</sup>-dependent actin-myosin interactions and leading to relaxation of airway smooth muscle. Beta-agonists may also cause airway smooth muscle relaxation through a cAMP-independent mechanism *via* direct coupling between the receptor-activated G<sub>s</sub> (α<sub>s</sub>) protein and a large conductance Ca<sup>2+</sup>-activated potassium channel, leading to cell hyperpolarization and, hence, smooth muscle relaxation [13]. Thus, low doses of β-adrenoceptor agonists may cause airway bronchodilation without elevation of intracellular cAMP.

Beta-adrenoceptor agonists may also influence gene transcription through elevation of cAMP and activation of PKA [14]. cAMP mediates the hormonal stimulation of a variety of eukaryotic genes through a conserved cAMP response element (CRE) [15]. Transcriptional induction by cAMP is rapid, peaking at 30 min and

declining gradually over 24 h [16]. This burst in transcription is resistant to inhibitors of protein synthesis, suggesting that cAMP may stimulate gene expression by inducing the covalent modification rather than *de novo* synthesis of specific nuclear factors. Since all the known cellular effects of cAMP occur *via* the catalytic subunit (C-subunit) of PKA, it appears that this enzyme mediates the phosphorylation of factors that are critical for the transcriptional response. Treatment of cells with cAMP causes translocation of the C-subunit to the nucleus [17]. Within the nucleus, PKA phosphorylates serine-133 on the cAMP response element binding (CREB) protein, enhancing its DNA-binding and transactivating activity [15]. Activated CREB may persist for prolonged periods within the nucleus and, therefore, even a brief exposure to β-adrenoceptor agonist may lead to a prolonged effect on transcription. cAMP, through activation of CREB, may interact directly with activator protein-1 (AP-1) and may also interfere with the effects of PKC activation through inhibition of mitogen-activated protein kinase (MAPK) [18–21] (fig. 2).

The rate of transcription of the β<sub>2</sub>R gene is increased in response to β-adrenergic agonist stimulation of the receptor at the cell surface. This effect is mediated by stimulation of AC, elevation of intracellular cAMP levels, and activation of CREB [22]. Thus, positive

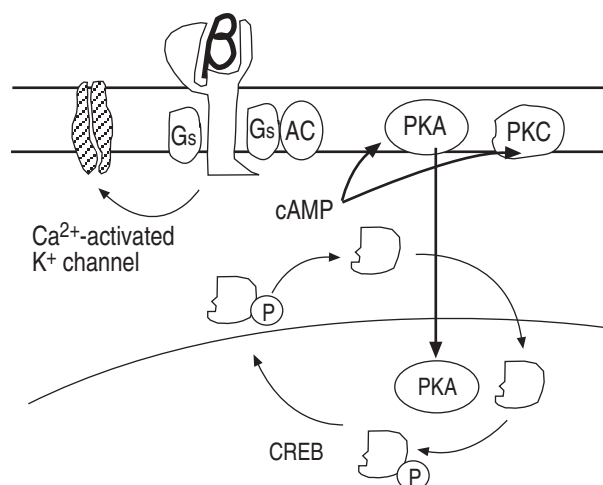


Fig. 2. – The mechanism of  $\beta_2$ -agonist action. Following ligand binding to the receptor,  $\beta$ -agonists may cause airway smooth muscle relaxation *via* a direct coupling between the receptor-activated Gs (*as*) protein and a large conductance  $\text{Ca}^{2+}$ -activated potassium channel, leading to cell hyperpolarization and smooth muscle relaxation. Alternatively, higher doses of  $\beta$ -agonists cause receptor-associated Gs proteins to be activated and couple with adenylyl cyclase (AC). The coupling of activated Gs and AC leads to enhanced production of cAMP and subsequent activation of cAMP-dependent protein kinase A (PKA). Activation of PKA leads to translocation of the C-subunit of the cAMP response element binding protein (CREB) enhancing its DNA-binding and transactivating activity. Gs: stimulatory G protein; PKC: protein kinase C; cAMP: cyclic 3'5'-adenosine monophosphate.

autoregulation of the  $\beta_2$ -adrenergic receptor gene appears to occur through receptor-mediated stimulation of AC, with consequent activation of CREB and stimulation of  $\beta_2$ -adrenergic receptor gene transcription. These results show that the  $\beta_2$ R can exert positive feedback regulation on its own expression [23]. However, most long-term exposure to  $\beta$ -agonists results in decreased messenger ribonucleic acid (mRNA) in cell lines and in lung *in vivo* [24–26]. This reduced expression of  $\beta_2$ -receptors is due to reduced gene transcription and is associated with a reduction in CREB activity [26].

### Effect of glucocorticosteroids on $\beta$ -receptors

#### Upregulation of receptor number

Glucocorticoids are known to have the ability to increase the number of  $\beta_2$ R in human lung measured by radioligand-binding [27]. Several putative GREs have been identified in the promoter sequence of the human  $\beta_2$ R gene [28]. Dexamethasone was found to increase the rate of  $\beta_2$ R gene transcription through a GRE in the 5'-flanking region of the gene [29] in human lung tissue in a time- and dose-dependent manner, which was consistent with the later induction of receptor-binding activity [25] (fig. 3). This confirmed earlier reports of a similar action of dexamethasone on  $\beta_2$ R in cultured cell lines [24]. The mRNA half-life and stability is tissue- and cell-specific and is determined to some extent by the level of ribonuclease (RNase) activity in the cytoplasm of each particular cell type. However, dexamethasone

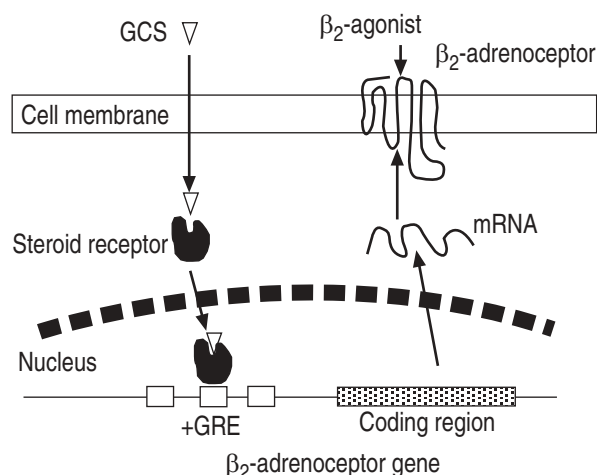


Fig. 3. – Effect of glucocorticosteroids (GCS) on  $\beta$ -receptor expression. Glucocorticoids induce an upregulation of  $\beta_2$ -receptor mRNA and protein through a direct action of the activated glucocorticoid receptor on positive GREs within the promoter region of the  $\beta_2$ -receptor gene. mRNA: messenger ribonucleic acid; GRE: glucocorticoid responsive element.

has not been found to alter the half-life of  $\beta_2$ R message [25]. The efficiency of coupling between the  $\beta_2$ R and Gs (the G protein that mediates stimulation of AC) has also been reported to be modulated by glucocorticoids [30]. As a result,  $\beta_2$ R-stimulated AC activity and cAMP accumulation increase following glucocorticoid treatment. Animals that have been depleted of glucocorticoids by adrenalectomy, in contrast, lose their ability to maintain the sensitivity of the  $\beta$ R-coupled AC system [24].

#### Inhibition of down-regulation

There is a downregulation of  $\beta_2$ R in the lung after chronic administration of  $\beta_2$ -agonists in animals *in vivo*, although this is less marked in airway smooth muscle than in lung parenchyma [26]. Agonist promoted downregulation of  $\beta_2$ R may be reversed by treatment with glucocorticoids *in vitro* [27]. Glucocorticoids induce an increase in the synthesis of  $\beta_2$ R in human and rat lung and restoration of desensitization of  $\beta_2$ R in human neutrophils and lymphocytes [27]. The prevention of agonist-induced downregulation of the  $\beta_2$ R by glucocorticoids has been reported in rat lung *in vivo* [31], and in cultured vas deferens smooth muscle cells (DDT1-MF2) *in vitro* at the levels of radioligand-binding and of mRNA [32]. Autoradiographic mapping studies indicate that glucocorticoids upregulate  $\beta_2$ -receptors and prevent downregulation of  $\beta_2$ -receptors in all cell types, including airway smooth muscle cells [31]. Such an effect may have clinical implications for preventing the development of tolerance to  $\beta_2$ -agonists in asthmatic patients treated with  $\beta$ -agonists.

Chronic antagonist therapy in asthmatic subjects results in reduction in  $\beta$ R density in circulating polymorphonuclear leucocytes and lymphocytes [33], and the downregulated  $\beta_2$ R number is restored with oral prednisone. However, a difference in susceptibility to downregulation between lung and lymphoid tissue has been reported [34].

### Do inhaled $\beta$ -agonists have adverse effects on airways?

Asthma is due to a chronic inflammation of the airways and glucocorticosteroids are highly effective in controlling asthmatic inflammation; indeed, inhaled steroids have now become the mainstay of chronic asthma therapy [35]. Despite the more widespread use of inhaled steroids, the prevalence, morbidity and mortality from asthma are increasing in many countries [36–38], and may correlate to high doses of inhaled  $\beta_2$ -adrenoceptor agonists [39–41].

There is, at present, controversy as to whether regular treatment with  $\beta$ -agonists reduces the overall control of asthma (see [42]). Most studies which have examined this problem suffer from methodological problems, using no or poor controls. However, there is evidence to suggest that both asthma control and lung function may worsen with regular treatment with  $\beta$ -agonists [43, 44]. Furthermore, in other studies, symptom scores, exacerbations and treatment failures were notably highest in those receiving high-dose  $\beta$ -agonists for longer periods [45–47] (table 1).

It is important to distinguish between acute bronchodilator effects (*e.g.* daytime peak expiratory flow (PEF) rates), and long-term changes (*e.g.* prebronchodilator morning PEF or prebronchodilator forced expiratory volume in one second (FEV<sub>1</sub>)) when assessing the effects of  $\beta$ -agonist drugs on lung function. Several investigations have found an improvement in lung function after the use of high-dose  $\beta$ -agonist therapy, while a substantial number have shown no improvement or evidence of deterioration in lung function consistent with the view that regular  $\beta$ -agonist treatment may be deleterious [42]. Downward trends in baseline lung function during regular treatment with  $\beta$ -agonists are also evident from other studies [49, 51–54].

There has been concern that regular use of inhaled  $\beta_2$ -agonists may result in tolerance to their beneficial effects in asthma. Although there is no loss of bronchodilator response to  $\beta_2$ -agonists, several studies have demonstrated loss of protection against various bronchoconstrictor challenges [50, 51, 55, 56], and this may be relevant to the reduced asthma control seen with the regular use of inhaled  $\beta_2$ -agonists [43, 49].

Table 1. – Comparative effects of high-dose as opposed to low-dose or on-demand  $\beta_2$ -agonist use on lung function and symptom scores

Decreased asthma control on regular <i>vs</i> on-demand inhaled fenoterol [46]
Increased airway responsiveness after regular <i>vs</i> on-demand inhaled salbutamol [44]
Increased annual decline in FEV <sub>1</sub> over 2 yrs on regular <i>vs</i> on-demand inhaled salbutamol [48]
Increased treatment failures and decreased asthma control with 3 months high-dose as compared to low-dose fenoterol [45]
Downward trend in baseline lung function with 22 months regular $\beta$ -agonist [49]
Decreased protective effect against direct and indirect challenge with regular terbutaline [50]

FEV<sub>1</sub>: forced expiratory volume in one second.

In most countries, sales of inhaled  $\beta_2$ -selective adrenoceptor agonists have risen steadily since the 1960s at a rate which has exceeded the increase in the prevalence of asthma, suggesting that the dose of  $\beta_2$ -adrenoceptor agonist per asthmatic patient may have increased. One possible explanation for a worsening of asthma control would be a negative interaction between  $\beta_2$ -adrenoceptor agonists and corticosteroids. There is preliminary evidence in asthmatic patients to support this. Regular treatment with the inhaled corticosteroid improves airway function and protects against bronchoconstriction induced by allergen inhalation, but when the  $\beta_2$ -adrenoceptor agonist terbutaline is given together with the inhaled steroid the protection against allergen-induced bronchoconstriction is lost [57].

Recently, several lines of research have converged to explain the asthma "paradox" and to indicate that the long-term use of  $\beta$ -agonists for control of chronic asthma is inappropriate, and may indeed be harmful. Clinical data, in spite of their limitations, show no clear evidence of symptomatic or functional improvement during long-term therapy, and in some instances show adverse effects. None of these data exclude  $\beta_2$ -agonists from their proper role in the management of asthma. Inhaled  $\beta_2$ -agonists are appropriate, indeed essential, for the treatment of acute severe attacks, and provide temporary relief for breakthrough and exercise-induced symptoms. However, regular or frequent use of inhaled  $\beta_2$ -agonists for maintenance treatment of chronic asthma is not to be recommended. The strength of present evidence indicates the need for a radical revision of our approach to the use of these drugs; alternative and more effective anti-inflammatory therapy, with appropriate but minimal as-needed bronchodilator use, appears at present to be the preferred treatment.

### GR interactions with other transcription factors

Recent studies have provided increasing evidence for functional interactions between nuclear receptors and other transcription factors not involving a natural promoter [2]. This is, in particular, the case with glucocorticoid-inducible genes, which frequently show GREs contiguous with DNA regulatory sequences for other transcription factors in the promoter region. These include binding sites for stimulatory protein-1 (SP-1), nuclear factor-1 (NF-1), CACCC and CCAAT box-binding proteins [58, 59], CREB [60–62] and AP-1 factors [4, 8], or a second GRE [58, 59]. An interaction between the glucocorticoid receptor and these accessory factors can lead either to enhancement or repression of gene transcription. More recently, the glucocorticoid receptor has been shown to repress gene expression by functional interference with several different transcriptional activators [4–6, 60–62]. For instance, repression of osteocalcin, proliferin and collagenase gene expression by glucocorticoids is due to inhibition at an AP-1 site by a different mechanism involving a direct protein-protein interaction between c-jun and the glucocorticoid receptor, leading to a mutual inhibition of their DNA-binding activities [63, 64].

### Actions of cAMP elevating agents on steroid actions

At the molecular level, several types of interaction have been described between the glucocorticoid receptor and either  $\beta$ -agonists or drugs that increase cAMP or CREB levels. These effects are both cell and context specific and also depend on the dose of  $\beta_2$ -agonist/cAMP elevating agent used.

#### Synergy

Synergy between the glucocorticoid receptor and other transcription factors was first reported for NF-1, SP-1 and CACCC binding proteins in genes encoding tyrosine aminotransferase and rat tryptophan oxygenase. The level of functional co-operation in these instances was shown to be critically dependent on the spacing between the regulatory elements but not their orientation, and is mediated by protein-protein interaction rather than co-operative DNA-binding [58, 59]. This suggests a basic model of glucocorticoid-induced transcriptional activation requiring multiple GREs or a combination of a GRE with other transcription factor-binding sites for constitution of a hormone-inducible enhancer.

Neurotensin (NT) production from rat hypothalamic neurones in primary culture is modified by dexamethasone and forskolin. Treatment with dexamethasone induced a dose-dependent increase in NT content, with a maximum of 100% at 1  $\mu$ M. In contrast, forskolin affected neither the content nor the number of immunoreactive NT cells. Co-treatment with dexamethasone and forskolin gave a 285% increase in NT content and a 430% increase in cell number compared to the effect of dexamethasone alone. This increase in NT content correlated with a similar increase in the amount of NT mRNA. Therefore, dexamethasone and forskolin can act synergistically to enhance NT production in hypothalamic neurones [65].

Tissue-type plasminogen activator (tPA) gene expression is also modulated by glucocorticoids and cAMP in rat hepatoma cells (HTC). Incubation with dexamethasone transiently increases tPA mRNA accumulation twofold, whereas incubation with the cAMP analogue, 8Br-cAMP, alone results in a sustained twofold increase. In combination, however, dexamethasone and 8Br-cAMP act synergistically to induce tPA mRNA levels 10–15 fold. This synergistic induction of tPA gene transcription requires concomitant protein synthesis. Furthermore, the action of dexamethasone must precede that of 8Br-cAMP and requires ongoing protein synthesis, whereas the action of 8Br-cAMP has no such requirement [66].

#### Addition

Glucocorticoids and elevations of intracellular cAMP may affect lymphocyte activation, proliferation and some effector functions in similar ways. Treatment of resting human lymphocytes with dexamethasone, sensitized prostaglandin E<sub>2</sub> stimulated cAMP accumulation in a

time- and concentration-dependent manner, and an enhanced inhibition of concanavalin A-induced Ca<sup>2+</sup> elevation. In contrast,  $\beta_2$ -adrenoceptor density, immunodetectable  $\alpha$ -subunits of the G-proteins, G<sub>s</sub> and inhibitory G-protein (G<sub>i</sub>), and pertussis toxin substrates were not significantly altered by dexamethasone treatment. From these studies, it was demonstrated that dexamethasone treatment sensitizes cAMP formation in resting human lymphocytes by altering AC rather than G-proteins or hormone receptors. This results in an enhanced capability of cAMP generating agonists to inhibit early steps of lymphocyte activation [67].

The somatostatin gene (SS) is transcriptionally regulated *via* a CRE located in its promoter region. cAMP and forskolin stimulated SS gene transcription 4–5 fold in PC12 cells, whilst dexamethasone induced a dose-dependent twofold stimulation of SS gene transcription. Dexamethasone exerted an additive effect on cAMP-induced SS gene transcription. The stimulatory effects of dexamethasone were removed upon deletion of the promoter fragment 250 to -71 (a region that does not contain a GRE) but did not affect cAMP action. Glucocorticoid-induced transactivation shows dependence on PKA activity, and may be mediated *via* protein-protein interactions between the GR and CRE binding proteins. The action of glucocorticoids was dependent on a functional interaction with CRE/CREB and related binding proteins [68].

cAMP caused a significant increase in the levels both of [<sup>3</sup>H]dexamethasone-binding capacity and GR mRNA in rat HTC. This increase in GR mRNA did not require ongoing translation or transcription and was due to an increase in mRNA half-life and on the phosphorylation state of the receptor. Furthermore, forskolin inhibited the GR-induced downregulation of GR protein in these cells [69].

In a human breast carcinoma-derived cell line, T47D(A1-2), containing a hormone-responsive luciferase reporter gene, manipulation of cell growth conditions or cellular signal transduction in a variety of ways can enhance or impair glucocorticoid-mediated induction of a target gene [70]. The fourfold induction by dexamethasone could be further enhanced 2–3 fold by co-addition of 8Br-cAMP. Treatment with forskolin, a direct activator of AC, also enhanced the hormone-induced level of luciferase approximately twofold in a dose-dependent manner. Forskolin had no effect in the absence of hormone. In contrast, activating PKA by inhibiting cAMP turnover with the phosphodiesterase inhibitors isobutylmethylxanthine (IBMX) or Ro20-1724 inhibited the hormone response rather than potentiated it.

Further evidence for an additive interaction between GR and CREB, whilst bound to DNA, was obtained by YAMAMOTO and co-workers [71], whilst investigating the expression of the PEPCK gene, which encodes the rate-limiting enzyme in gluconeogenesis. PEPCK mRNA in H4IIE rat HTC was induced fivefold by 0.1 mM cAMP, which was similar to the induction seen with 0.5  $\mu$ M dexamethasone. PEPCK mRNA was induced to a level 9.3 fold higher than in controls when cells were co-incubated with cAMP and dexamethasone.

### Antagonism

Inducible nitric oxide synthase (iNOS) is expressed in renal mesangial cells in response to two principal classes of activating signals that interact in a synergistic fashion. These two groups of activators comprise inflammatory cytokines, such as interleukin (IL)-1 $\beta$ , and agents that elevate cellular levels of cAMP. Bt<sub>2</sub>cAMP increases iNOS gene transcription and mRNA half-life in renal mesangial cells and in vascular smooth muscle cells. A nanomolar concentration of dexamethasone suppresses Bt<sub>2</sub>cAMP-induced iNOS protein and mRNA expression and production of nitrite. Furthermore, in these cells dexamethasone failed to inhibit IL-1 $\beta$ -stimulated iNOS synthesis, although this is not the case in pulmonary epithelial cells [72]. This suggests that dexamethasone acts at different levels, depending on the stimulus used, to suppress iNOS induction [73].

The  $\beta$ -adrenergic agonist, isoprenaline, and the cAMP analogue, 8Br-cAMP, increased PEPCK mRNA threefold in adipocytes. Dexamethasone decreased PEPCK mRNA by 80% and also counteracted the inductive effects of isoprenaline and 8Br-cAMP with a half maximal inhibition at 1 nM. Run-on experiments showed that the isoprenaline and dexamethasone actions were, at least in part, exerted at the level of gene transcription [74]. The different effects of dexamethasone on PEPCK gene expression in liver and adipocytes are of particular interest, as the PEPCK gene is a single copy gene under the regulation of a single promoter [75]. The effects of glucocorticoids and cAMP on PEPCK gene expression in adipocytes are, therefore, opposite to that found in the liver and in HTC, suggesting the requirement for other cell specific factors in mediating these effects.

Recent studies have provided evidence of cross-talk between steroid receptors and cAMP-signalling pathways in the regulation of gene expression. A synergism between intracellular phosphorylation inducers and glucocorticoids has been shown to occur during activation of the murine mammary tumour virus (MMTV) promoter. 8Br-cAMP synergizes significantly with glucocorticoids in activating the transiently transfected MMTV template. In contrast, 8Br-cAMP is antagonistic to hormone-induced activation of the stably replicating MMTV template. Nuclear run-on experiments demonstrate that this is a transcriptional effect both on hormone-induced transcription and basal transcription. Surprisingly, 8Br-cAMP does not inhibit glucocorticoid-induced changes in restriction enzyme access and NF-1 binding. However, association of a complex with the TATA box region is inhibited in the presence of 8Br-cAMP. This cAMP treatment interferes with the initiation process but does not inhibit the interaction of the receptor with the template. Since the replicated ordered MMTV templates and the transfected, disorganized templates show opposite responses to 8Br-cAMP treatment, we conclude that chromatin structure can influence the response of a promoter to activation of the cAMP signalling pathway [76].

Tissue-type plasminogen activator (tPA) gene expression is regulated by glucocorticoids and cyclic nucleotides in rat HTC. Incubation of HTC with the dexamethasone

transiently increases tPA mRNA accumulation twofold, whereas incubation with 8Br-cAMP alone results in a sustained twofold increase. Nuclear run-on studies indicate that these effects occur at the level of gene transcription. In combination, however, dexamethasone and 8Br-cAMP act synergistically to induce tPA mRNA levels 10–15 fold. This synergistic induction is, at least in part, transcriptional. The synergistic induction of tPA gene transcription requires concomitant protein synthesis. Furthermore, the action of dexamethasone must precede that of 8Br-cAMP, and the action of dexamethasone requires ongoing protein synthesis, whereas the action of 8Br-cAMP has no such requirement [66].

Transcription of the glycoprotein hormone alpha-subunit gene is repressed by glucocorticoids in a cell and tissue type manner, an effect that is mediated through the glucocorticoid receptor. Mutation of the previously identified GR-binding sites in the alpha-subunit promoter fails to abolish repression, indicating that specific DNA-binding to the alpha-subunit is not important for repression. Inhibition by GR is only effective when the alpha-subunit promoter is activated by CREB, implicating CREB as the target for GR-mediated repression. Reciprocally, overexpression of CREB interferes with GR-mediated transcriptional activation of MMTV (fig. 4). This activity is not affected by the phosphorylation state of CREB. In these studies, despite the mutual cross-interference with activation of gene expression, GR and CREB do not appear to have a high affinity protein: protein interaction *in vitro*. Nonetheless, GR and CREB may interact directly *in vivo* possibly through a third protein or, more likely, may sequester a mutually required target protein [60].

In human and rat lung [61, 77], human epithelial cells and human T-cells [78, 79], the  $\beta_2$ -agonists, salbutamol and fenoterol, decrease the binding of GR to GRE although they have no effect on the affinity or number of

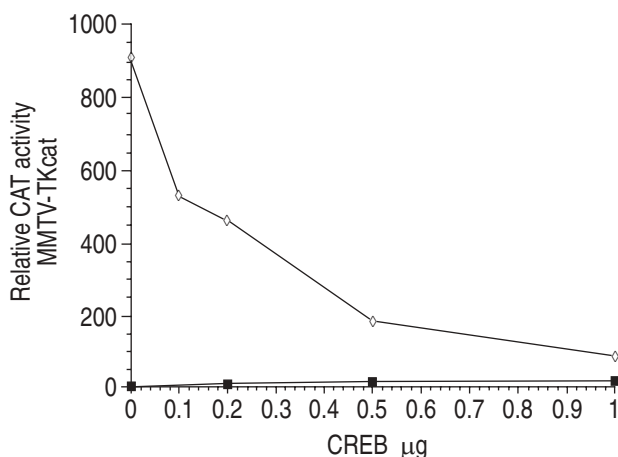


Fig. 4. — Overexpression of CREB protein on glucocorticoid-responsive gene transcription. Addition of increasing concentrations of recombinant CREB protein cells causes a dose-dependent inhibition of dexamethasone-stimulated gene transcription in JEG-3 cells. CREB: cAMP response element binding protein; MMTV: murine mammary tumour virus. —■—: control; —◇—: plus dexamethasone. (From [60], with permission). CAT: chloramphenicol acetyltransferase; MMTV-TKcat: mouse mammary tumour virus-thymidine kinase controlled CAT reporter gene.

glucocorticoid receptors. The inhibition of GRE-binding by salbutamol is concentration-dependent, can be blocked by propranolol and is seen following forskolin treatment. This effect appears to be due to an interaction between the GR and CREB, which is activated by high concentrations of  $\beta_2$ -agonists. By this mechanism, in these important cell types, high doses of inhaled  $\beta_2$ -agonists may inhibit the anti-inflammatory effects of endogenous glucocorticoids and exogenous corticosteroids used for asthma therapy [61].

These data support the concept that glucocorticoids and  $\beta_2$ -agonists may regulate gene transcription in a cell- and gene-specific manner at both the transcriptional and post-transcriptional level. For this transcription factor interaction to be of importance in asthma, it must occur in a cell (or cells) which are key targets for steroids and which also express surface  $\beta_2$ -receptors and respond to  $\beta_2$ -agonist stimulation with elevation of cAMP. Many pulmonary cells express  $\beta_2$ -receptors [10, 80], and these same cells express GR [81]. Airway, epithelial cells, T-lymphocytes and macrophages are target cells on which this effect may be of therapeutic relevance, particularly as all produce cytokines which may orchestrate or perpetuate the allergic inflammatory response [82]. The doses of drugs used are within the theoretical concentrations expected within the epithelial lining fluid after inhaled therapy, and so this interaction is likely to occur within the lung.

Our findings raise the possibility that  $\beta_2$ -agonists may have functional anticorticosteroid activity in the lung and in epithelial cells. By reducing the anti-inflammatory effects of endogenous steroids, asthma may be aggravated in asthmatics not taking inhaled corticosteroids. This adverse effect may be reversed by additional exogenous corticosteroids but the therapeutic effect of inhaled corticosteroid therapy may be diminished by the concurrent use of high doses of inhaled  $\beta_2$ -agonist. We believe that our results may provide a plausible explanation for the increase in morbidity and mortality which has been reported in industrialized countries which have an increasing *per capita* increase in inhaled  $\beta_2$ -agonist use.

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