Δ-9,11 Modification of Glucocorticoids Dissociates Nuclear Factor-κB Inhibitory Efficacy from Glucocorticoid Response Element-Associated Side Effects

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ABSTRACT

Glucocorticoids are standard of care for many inflammatory conditions, but chronic use is associated with a broad array of side effects. Accordingly, dissociative glucocorticoids—drugs able to retain or improve efficacy associated with transrepression [nuclear factor-κB (NF-κB) inhibition] but with the loss of side effects associated with transactivation (receptor-mediated transcriptional activation through glucocorticoid response element gene promoter elements) —are being investigated as a source of safer therapeutic agents for chronic inflammatory disorders. We investigated a glucocorticoid derivative with a Δ-9,11 modification as a dissociative steroid. The Δ-9,11 analog showed potent inhibition of tumor necrosis factor-α-induced NF-κB signaling in cell reporter assays, and this transrepression activity was blocked by 17β-hydroxy-11β-[4-dimethylamino phenyl]-17α-[1-propynyl]estra-4,9-dien-3-one (RU-486), showing the requirement for the glucocorticoid receptor (GR). The Δ-9,11 analog induced the nuclear translocation of GR but showed the loss of transactivation as assayed by GR-luciferase constructs as well as mRNA profiles of treated cells. The Δ-9,11 analog was tested for efficacy and side effects in two mouse models of muscular dystrophy: mdx (dystrophin deficiency), and SJL (dysferlin deficiency). Daily oral delivery of the Δ-9,11 analog showed a reduction of muscle inflammation and improvements in multiple muscle function assays yet no reduction in body weight or spleen size, suggesting the loss of key side effects. Our data demonstrate that a Δ-9,11 analog dissociates the GR-mediated transcriptional activities from anti-inflammatory activities. Accordingly, Δ-9,11 analogs may hold promise as a source of safer therapeutic agents for chronic inflammatory disorders.

Introduction

Glucocorticoids have been studied extensively for the past 60 years and are among the most prescribed drugs (Hillier, 2007). They exhibit potent anti-inflammatory properties and are standard of care for many chronic and acute inflammatory conditions, including lupus, myositis, asthma, rheumatoid arthritis, and muscular dystrophy (Baschant and Tuckermann, 2010). However, the side-effect profiles of pharmacological glucocorticoids are significant, including muscle atrophy, adrenal deficiency, osteoporosis, spleen atrophy, short stature, and mood and sleep disturbances, among others (Chrousos et al., 1993; DeBoscher, 2010). This has led to a search for dissociative steroids—drugs able to retain activities responsible for molecular and clinical efficacy with the loss of subactivities responsible for side effects (Newton and Holden, 2007).

The mechanism of action of glucocorticoids is through transrepression and transactivation properties. Transrepression...
involves ligand/receptor interactions with other cellular signaling proteins, such as the inhibition of nuclear factor-κB (NF-κB) complexes (Rhen and Cidlowski, 2005; Newton and Holden, 2007). Transcription has been associated with anti-inflammatory activity and clinical efficacy. Transactivation (also termed cis-regulation) is mediated by ligand/glucocorticoid receptor (GR) translocation from the cytoplasm to the nucleus, with ligand/receptor dimers binding directly to glucocorticoid response elements (GREs) in the promoters of target genes (Dostert and Heinzel, 2004). Transactivation has been associated with deleterious side effects (Newton and Holden, 2007).

The balance of efficacy and side effects is well illustrated in the effects of glucocorticoids on muscle tissue. Chronic administration of glucocorticoids leads to relatively rapid muscle loss via the Forkhead box O (FOXO)/atrogen signaling pathway, and can cause glucocorticoid myopathy (critical care myopathy) (Di Giovanni et al., 2004; Puthucheary et al., 2010). In contrast, chronic glucocorticoids are used in Duchenne muscular dystrophy to increase muscle strength and prolong ambulation, possibly through their anti-inflammatory effect (Bach et al., 2010; Hussein et al., 2010; Escolar et al., 2011). The beneficial properties of steroids are offset partly by side effects, including atrogen-mediated muscle wasting bone fragility, obesity, and short stature (Schara et al., 2001).

Lazaroid Δ9,11 analogs were developed originally as non-glucocorticoid steroids with effects on cell membranes and tested clinically for neuroprotection by the inhibition of lipid peroxidation (Taylor et al., 1996; Bracken et al., 1997; Vanaghan and Kam, 2001). More recent studies of lazarooids in muscle found the inhibition of calcium release in C2C12 muscle cells (Passaquin et al., 1998) and the attenuation of ischemia/reperfusion injury (Campo et al., 1997). Lazaroids have been found to protect against acute inflammation (endotoxinemia/reperfusion injury) (Campo et al., 1997). Lazaroids have been associated with deleterious side effects (Newton and Holden, 2007). Transrepression has been associated with clinical efficacy. Transactivation is mediated by ligand/glucocorticoid receptor (GR) translocation from the cytoplasm to the nucleus, with ligand/receptor dimers binding directly to glucocorticoid response elements (GREs) in the promoters of target genes (Dostert and Heinzel, 2004). Transactivation has been associated with deleterious side effects (Newton and Holden, 2007).

Materials and Methods

Mice. C57BL/6, SJL/J (dysferlin deficient), and C57BL/10ScSn-Dmd<sup>mdx</sup> mice were purchased from The Jackson Laboratory (Bar Harbor, ME). GR<sup>dim/dim</sup> mice (Reichardt et al., 1998) were obtained from Deutsches Krebsforschungszentrum (German Cancer Research Center, Heidelberg, Germany). All of the animal experiments were conducted in accordance with our Institutional Animal Care and Use Committee guidelines under approved protocols.

Synthesis of Δ9,11 Analogs. Analogs were synthesized by Bridge Organics (Kalamazoo, MI) (Supplemental Fig. 1). Anecortave is the prodrug for VBP1.

NF-κB Inhibition Assay. The in vitro drug screening assay for NF-κB inhibition in C<sub>2</sub>C<sub>12</sub> cells was done as described previously (Baudy et al., 2009). To measure the ability of NF-κB effects by the addition of a GR antagonist, reporter cells were pretreated for 1 h with the drug at a constant concentration and 17β-hydroxy-11β-[4-dimethylamino phenyl]-17α-[1-propynyl]estra-4,9-dien-3-one (RU-486) at increasing concentrations (1 nM to 10 μM). After the pretreatment, cells were stimulated with TNF-α (10 ng/ml) and assayed for luciferase activity 3 h later.

For studies using GR<sup>dim/dim</sup> mice, splenocytes were isolated from GR<sup>dim/dim</sup> and C57BL/6 mice. Splenocytes were treated with prednisolone (10 μM), anecortave (10 μM), or vehicle (dimethyl sulfoxide; DMSO) for 24 h. After the treatment, cells were stimulated with TNF-α (10 ng/ml) for an additional 24 h. A murine NF-κB-regulated cDNA plate array (Signosis, Inc., Sunnyvale, CA) was used to monitor gene expression.

Nuclear Translocation Assays. Translocation assays were performed by DiscoveRx (Fremont, CA) using GR, mineralocorticoid receptor (MR), and androgen receptor (AR) Nuclear Translocation PathHunter cells (DiscoveRx). Immunofluorescence. A549 cells were incubated with the drug for 24 h in serum-free media, fixed using formaldehyde, and then processed with primary antibody (rabbit anti-GR; 1:50; GHR H-300; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Alexa Fluor 568 goat anti-rabbit IgG1:100 (Invitrogen, Carlsbad, CA), and 4,6-diamidine-2-phenylindole were used for visualization on an LSM 510 confocal microscope (Carl Zeiss Inc., Thornwood, NY).

GRE Transcription Assay. HEK293 cells stably transfected with a luciferase reporter construct regulated under a GRE (Panomics, Fremont, CA) were grown according to the manufacturer’s instructions. Cells were treated with drugs for 6 h at 37°C, and luciferase activity was measured (Promega, Madison, WI) using a Centro LB 960 luminometer (Berthold Technologies, Bad Wildbad, Germany).

Myotube Microarrays. H-2K myoblasts were obtained as described previously (Morgan et al., 1994; Harris et al., 1999). Conditionally immortalized wild-type and mdx H-2K myoblast cell lines underwent differentiation to myotubes for 5 days and were exposed to prednisolone, anecortave, or DMSO vehicle for 4 h. RNA was isolated and analyzed on Affymetrix 430a 2.0 microarrays. Probe set signals were derived using the PLIER probe set algorithm. Thresholds used for the comparisons of drug-treated versus vehicle-treated were p value ≤0.01, fold change ≥1.2.

A549 Gene Transcription Assay. A549 cells were grown to confluence in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and exposed to prednisolone (1, 10, or 100 μM), anecortave (1, 10, or 100 μM), or vehicle control (DMSO) for 4 h at 37°C and for 24 h at 37°C. Real-time polymerase chain reaction was performed for FKBP5, GILZ, CCL2, and MRP-1 mRNAs, and 18S rRNA (housekeeping control) using TaqMan primers (Applied Biosystems, Foster City, CA).

Receptor Binding Assays. GR binding assays were performed by the State University of New York at Buffalo using methods published previously (Almon et al., 2008). Competitive binding assays were performed by Caliper Life Sciences (Hopkinton, MA), using radiolabeled <sup>3</sup>H ligands and partially purified full-length human receptors expressed from recombinant baculovirus-infected insect cells.

Preclinical Trials. In a short-term trial, prednisolone (1 mg/kg) and anecortave (5 mg/kg) were administered orally to 8-week-old female mdx mice daily via syrip drops for 3 weeks. The visualization of inflammation in vivo using the noninvasive imaging of cathepsin B caged near-infrared substrate ProSense 680 (PerkinElmer, Waltham, MA) was done as described previously (Baudy et al., 2011).

ABBREVIATIONS: NF-κB, nuclear factor-κB; AR, androgen receptor; FOXO, Forkhead box O; GR, glucocorticoid receptor; GRE, glucocorticoid response element; MR, mineralocorticoid receptor; TNF-α, tumor necrosis factor-α; VBP1, anecortave desacetate; RU-486, 17β-hydroxy-11β-[4-dimethylamino phenyl]-17α-[1-propynyl]estra-4,9-dien-3-one; DMSO, dimethyl sulfoxide; HEK, human embryonic kidney.
Membrane permeability was assessed using Cy5.5-labeled 10-kDa dextran beads injected intraperitoneally (150 μl/mouse). The forelimbs and hindlimbs were scanned using the eXplore Optix (GE Medical Systems, London, ON, Canada) optical scanner 24 h after injection and then quantified using Optiview software to calculate the total number of photon counts per square millimeter of scanned area.

For the 4-month trials, 8-week-old female mdx mice (n = 24, 8 per group) and 12-week-old SJL mice (n = 24, 8 per group) were separated into untreated, anecortave-treated, and prednisolone-treated groups. Drugs were administered to the treated mice via food for 4 months at a dose of 40 mg/kg anecortave and 5 mg/kg prednisolone. Evaluation of function, behavior, and histology using hematoxylin and eosin of formalin-fixed, paraffin-embedded muscle was performed as described previously (Spurney et al., 2009). Force measurements were conducted on the extensor digitorum longus muscle of the right hindlimb of the mdx mouse as described previously (Spurney et al., 2011).

Results

Δ-9,11 Analogs Show Retention of NF-κB Transrepression Activities. To determine whether Δ-9,11 analogs retained NF-κB inhibitory activity, we used a TNF-α-induced NF-κB luciferase construct stably transfected in C2C12 cells

![Diagram](image1)

**Fig. 1.** Δ-9,11 Glucocorticoid analogs retain transrepression activities. A, the Δ-9,11 double bond dissociates the transactivational activities of traditional steroids (VBP1, anecortave) (arrow). Lazaroids share the Δ-9,11 double bond but have larger moieties designed for membrane integration. B, Δ-9,11 analogs show potent inhibition of TNF-α-induced NF-κB activity, similar to that seen by prednisolone. Shown is a luciferase reporter assay in C2C12 cells. C, the inhibition of TNF-α-induced NF-κB activity is blocked by increasing concentrations of the GR antagonist RU-486, showing GR dependence of NF-κB inhibition (one-way analysis of variance with Dunnett’s multiple comparison test: *, p < 0.05; **, p < 0.01). Mean ± S.E.M. values are representative data from three independent experiments performed in triplicate and are expressed as the percentage of reporter inhibition compared with no-drug controls.
and studied drug effects on both undifferentiated myoblasts and multinucleated myotubes (Baudy et al., 2009). We synthesized anecortave, and the 21-hydroxy desacetate form, VBP1 (Fig. 1; Supplemental Fig. 1). Δ-9,11 Analogs showed NF-κB inhibitory activity in both cell differentiation stages at potencies comparable with that of prednisolone (Fig. 1B). The transrepression activities of prednisolone, anecortave, and VBP1 were blocked by increasing the concentrations of the receptor antagonist RU-486, suggesting that the NF-κB inhibitory activity of all three compounds is mediated by GR (Fig. 1C). RU-486 also antagonizes the progesterone receptor; however, we found no detectable binding of anecortave or VBP1 with progesterone receptor over a broad concentration range (see below).

To determine whether Δ-9,11 analogs induced nuclear translocation of GR, we studied dexamethasone and the two analogs using a β-galactosidase chemiluminescence binding partner assay in a CHO cell line. Both Δ-9,11 analogs induced nuclear translocation at approximately half the levels induced by dexamethasone (Fig. 2A). In addition, immunostaining of A549 cells after the incubation with VBP1 or dexamethasone for 30 min showed nuclear translocation of GR (Fig. 2B).

**Δ-9,11 Analogs Show the Loss of GRE-Mediated Transactivation Activities.** The ability of the Δ-9,11 analogs to mediate transcriptional activity using GREs was studied by measuring the response of a HEK293 cell line with GRE consensus sites coupled to a luciferase reporter. Prednisolone strongly induced GRE-mediated luciferase expression in a dose-dependent manner, whereas Δ-9,11 analogs showed no activity at 200 times prednisolone concentrations (Fig. 3A). These data suggested that Δ-9,11 analogs lack transactivation (GRE-mediated transcriptional) properties.

To more broadly test global transcriptional responses to prednisolone and Δ-9,11 analogs, wild-type and dystrophin-deficient mdx H-2K myotubes were treated with drugs followed by mRNA profiling using Affymetrix microarrays (Fig. 3B). We have shown previously that substantial transcriptional responses to corticosteroids occur in muscle approxi-
mately 4 to 8 h after a bolus of the drug in vivo (Almon et al., 2007; Yao et al., 2008) and selected the 4-h time point to enrich for direct transcriptional targets of ligand/receptor complexes. A total of 148 mRNA transcripts modulated by 10 μM prednisolone at 4 h in both wild-type and mdx cultures were seen (p < 0.01 and fold-change >1.2 in both experiments), with 75% transcriptionally activated by prednisolone (red color) and 25% transcriptionally repressed (green color) (Fig. 3B). The anecortave-treated cultures showed little overlap with prednisolone and instead appeared more similar to control (DMSO vehicle)-treated cultures.

The mRNA profiles were queried for molecular networks associated with GR (NRC31) (Supplemental Fig. 2A), and this showed prednisolone to cause the expected negative transcriptional regulation of GR (Burnstein et al., 1991), as well as the induction of a key muscle atrophy transcript, FOXO (Reed et al., 2012) (Supplemental Fig. 2, A and B). Comparing this to anecortave-regulated transcripts, there was little evidence of a shared transcriptional response with prednisolone, with no down-regulation of GR or transcriptional activation of FOXO1. In a different nonmuscle assay, A549 cells treated with prednisolone induced the expected transcriptional up-regulation of FKBP5, GILZ, and MKP-1 (Chivers et al., 2006), whereas exposure to Δ-9,11 analogs did not alter the expression of these target genes (Supplemental Fig. 2C). All of the data were consistent with the loss of GRE-mediated transcriptional response by Δ-9,11 analogs.

**Δ-9,11 Analogs Mediate Transrepression as Ligand/GR Monomers.** To test whether GR-mediated NF-κB inhibition was a dimer- or monomer-mediated event, we used GR(dim/dim) mice transgenic for a GR gene containing a point mutation preventing dimerization (Reichardt et al., 2001). Splenocytes were isolated from GR(dim/dim) and wild-type mice, pretreated with 10 μM anecortave or prednisolone, and then tested for the inhibition of TNF-α induced NF-κB transcriptional targets using a mouse NF-κB-regulated cDNA array. We found that both prednisolone and anecortave inhibited TNF-α-induced targets efficiently in GR(dim/dim) mice but less so in wild-type mice. In addition, some genes (IRF1, FASLI, IFNB1, COX2, IFNγ, and IL1α) were inhibited more strongly by anecortave than by prednisolone in wild-type splenocytes but not in GR(dim/dim) mice (Supplemental Table 1). This suggested that the NF-κB inhibitory activity was mediated by GR/ligand monomers.

**Δ-9,11 Glucocorticoid Analogs Show Differential Cross-Reactivity with Nuclear Hormone Receptors.** Competitive binding assays were carried out for Δ-9,11 analogs compared with known high-affinity ligands for the GR, MR, AR, and estrogen and progesterone receptors (Supplemental Fig. 3; Supplemental Table 2). VBP1 showed binding to GR at approximately 50-fold reduced affinity (triamcinolone IC50 = 1.34 × 10−7; VBP1 IC50 = 6.53 × 10−8). Neither anecortave nor VBP1 showed any detectable binding to the estrogen or progesterone receptors (Supplemental Fig. 3). For MR, binding affinity varied significantly between the different Δ-9,11 glucocorticoid analogs—VBP1 showed 11-fold higher affinity than spirolonactone, whereas anecortave had a 5-fold lower binding affinity (Supplemental Fig. 3; Supplemental Table 2). For AR, anecortave and VBP1 showed >100-fold reduced affinity compared with methyltrienolone. In contrast to GR, the Δ-9,11 analogs did not induce the nuclear translocation of MR or AR (E.K.M Reeves, unpublished observations).

Given the relatively high affinities of VBP1 and anecortave for MR and the known anti-inflammatory activities of MR complexes (Yagi and Sata, 2011), we tested the abilities of prednisolone and anecortave to induce MR gene targets in normal and mdx myotubes. The mRNA profiles used to analyze GR targets were queried for known MR gene targets, and the data were analyzed statistically, visualized by heat maps, and tested for MR-associated networks using Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA). We found no evidence of the regulation of MR targets by either prednisolone or anecortave (Z. Wang and E. P. Hoffman, unpublished observations).

**In Vivo Tests of Efficacy and Side Effects in Murine Models of Muscular Dystrophy.** We compared anecortave to prednisolone in three preclinical trials using mouse muscular dystrophy models: one short-term, 3-week daily oral bolus dosing study to assess effects on muscle inflammation in dystrophin-deficient mdx mice and two longer-term, 4-month studies with administration in food in both dystrophin-deficient mdx and dysferlin-deficient SJL mice.

In the 3-week acute trial, a daily oral bolus was given in syrup (1 mg/kg per day prednisolone or 5 mg/kg per day anecortave). A significant reduction of cathepsin B activity, an indicator of muscle inflammation and regeneration, in the forelimbs of both prednisone- and anecortave-treated mdx mice was observed in comparison to untreated mdx mice as shown by the caged near-infrared cathepsin B substrate Prosense 680 (Fig. 4A) (Baudy et al., 2011).

In the 4-month trials (separate mdx and SJL trials), we used chronic dosing in food at higher levels (mouse chow prepared with ~5 mg/kg per day prednisolone or ~40 mg/kg per day anecortave) (approaching the maximum tolerated doses for both drugs). At the study end point, prednisolone-treated mice in the mdx mouse trial showed significant losses of both body weight and spleen weight (Fig. 4, B and C), which is consistent with previous reports of deleterious side effects in rats (Orzechowski et al., 2002), whereas anecortave at higher doses showed none of these side effects. Likewise, in the dysferlin-deficient (SJL) trial, prednisolone, but not anecortave, induced a significant loss of spleen weight (control, 6.09 ± 0.34 g; prednisolone, 4.11 ± 0.23 g, *p < 0.05); anecortave, 6.52 ± 0.59 g). In the SJL trial, the body weights of prednisolone mice were similar to those of controls, whereas anecortave mice significantly increased body weight (control, 19.62 ± 0.62 g; prednisolone, 20.16 ± 0.41 g; anecortave, 21.83 ± 0.68 g). In the SJL trial, prednisolone induced significantly increased heart weight normalized to body weight, whereas anecortave did not induce this off-target effect (control, 4.98 ± 0.13 mg; prednisolone, 6.29 ± 0.27 mg; anecortave, 4.84 ± 0.20 mg).

In both mdx and SJL trials, multiple histological and functional end points reflective of drug efficacy showed improvement with both anecortave and prednisolone, and often greater efficacy was seen with anecortave. In the mdx trial, inflammatory infiltrates decreased, whereas muscle force increased significantly with anecortave (Supplemental Fig. 4). In the SJL trial, forelimb grip strength increased significantly in anecortave mice (control, 95 ± 3 g; prednisolone, 97 ± 1 g; anecortave, 108 ± 3 g), as did performance on a rotordor motor coordination test (control, 103.6 ± 4.76 s; pred-
Discussion

Existing models of glucocorticoid anti-inflammatory activities involve ligand binding to the GR cytoplasmic receptor. Previous reports using transgenic mice harboring GR mutations that prevent dimerization of GR (GR(dim/dim)) have shown that these mice retain the anti-inflammatory response to pharmacological glucocorticoids but do not transactivate genes with GRE cis-elements (Reichardt et al., 1998). These data suggest that neither dimerization nor GRE-mediated transcriptional activation (transactivation properties) are required for the efficacy of glucocorticoids. The GR(dim/dim) data set the stage for the efforts to develop and test dissociative steroidal compounds that bind and translocate GR but do not modulate GRE-mediated transcription.

In this article, we show that a Δ-9,11 analog of glucocorticoids [anecortave (prodrug), VBP1] is a dissociative steroid—it translocates GR and shows anti-inflammatory activities yet lacks GRE-mediated transcription. To our knowledge, we present the first data testing the transrepression and transactivation activities of Δ-9,11 analogs. We found that the Δ-9,11 analog was able to bind GR (albeit at a lower affinity compared with classic ligands) and retained activity in inducing translocation of GR from the cytoplasm to the nucleus (Fig. 2) but did not induce GRE-mediated transcription. It also retained potent NF-κB inhibitory activities, at levels similar to standard GR ligands, and this transactivation response was blocked by RU-486, showing a requirement for GR (Fig. 1C). Furthermore, this NF-κB inhibitory activity still was retained in cells unable to form GR dimers and undergo GRE-mediated transactivation [GR(dim/dim) transgenic mice], suggesting that transpression was mediated by ligand/GR monomers. The Δ-9,11 analog showed anti-inflammatory activity in vivo in acute (4 weeks) and long-term (4 month) studies in two animal models of muscular dystrophy (dystrophin-deficient mdx and dystrophin-deficient SJL). Efficacy of the Δ-9,11 analog using multiple histological and functional outcome measures was equal to or greater than that of prednisolone, although dosing regimens varied, and more extensive dose optimization is required (Fig. 4).

Many inflammatory genes are regulated by both direct GR/GRE action on their gene promoters as well as NF-κB DNA binding. Indeed, part of the complexity of GR action on anti-inflammatory genes includes GR/NF-κB cis-elements (Reichardt et al., 1998). These data suggest that neither dimerization nor GRE-mediated transcriptional activation (transactivation properties) are required for the efficacy of glucocorticoids. The GR(dim/dim) data set the stage for the efforts to develop and test dissociative steroidal compounds that bind and translocate GR but do not modulate GRE-mediated transcription.
tion of the expression of genes that have both GREs and NF-κB promoter elements (IRF1, FAS, IFNβ1, COX2, and IFNγ) versus those that have only NF-κB promoter elements (IL-6, NOS2, MMP1, IL-9, TNFα, TNFR, MYC, and IL-1α) (Supplemental Table 1). Consistent with this model, transcripts containing only NF-κB promoter elements were inhibited strongly by both prednisolone and anacortave in both wild-type and GR(dim/dim) mice. In contrast, inflammatory transcripts containing both GRE and NF-κB promoter elements were inhibited more strongly by anacortave compared with prednisolone in wild-type mice (85.5% inhibition by anacortave; 50.8% by prednisolone), and this differential activity was lost in the GR(dim/dim) mice (98% inhibition for both prednisolone and anacortave). These data suggest that Δ9,11 analogs may prove to be more effective anti-inflammatory drugs than traditional glucocorticoids, because they remove some or all of the steric occlusion between GRE and NF-κB promoter elements.

The dissociation of transrepression from transcription suggests that Δ9,11 analogs have the potential for replacing current glucocorticoid drugs. Our data suggest that the analogs bind GR and induce nuclear translocation and transrepressive activities independent of dimer formation but do not permit binding to GRE elements in gene promoters (transactivation). We suggest that future optimized lead compounds could be selected based upon GR translocation and NF-κB inhibition, with reduced cross-reaction to other nuclear hormone receptors.

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Authorship Contributions

Participated in research design: Baudy, Reeves, Damsker, Heier, McCall, Rose, Nagaraju, and Hoffman.


Contributed new reagents or analytic tools: McCall.

Performed data analysis: Baudy, Reeves, Damsker, and Heier.

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