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Glucocorticoids suppress human immunodeficiency virus type-1 long terminal repeat activity in a cell type-specific, glucocorticoid receptor-mediated fashion: direct protective effects at variance with clinical phenomenology

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Abstract

Glucocorticoid administration and/or excess secretion have been associated with increased Human Immunodeficiency Virus Type-1 (HIV-1) replication and AIDS progression. The HIV-1 long terminal repeat (LTR) promoter contains glucocorticoid-responsive element (GRE)-like sequences that could mediate a positive effect of glucocorticoids on HIV-1. In addition, we recently demonstrated that the HIV-1 accessory protein Vpr is a potent coactivator of the glucocorticoid receptor, which, like the host coactivator p300, potentiates the effect of glucocorticoids on GRE-containing, glucocorticoid-responsive genes. Such an effect may increase the sensitivity of several host target tissues to glucocorticoids by several fold, and may, thus, contribute to a positive effect of glucocorticoids on the HIV-1-LTR in infected host cells. In this study, we determined the direct effect of glucocorticoids on HIV-1-LTR by examining the ability of dexamethasone to modulate the activity of this promoter coupled to the luciferase reporter gene in human cell lines. Dexamethasone markedly inhibited Tat-stimulated, p300- or Vpr-enhanced luciferase activities in a cell-type specific, dose-dependent, and glucocorticoid receptor-mediated fashion. This effect of dexamethasone was not potentiated by Vpr, was antagonized by the glucocorticoid receptor antagonist RU 486 and required the DNA-binding domain of the receptor. These data suggest that the inhibitory effect of glucocorticoids on the HIV-1-LTR may be exerted via non-GRE-dependent inhibition of the strongly positive host transcription factor NF-κB, which interacts with the DNA- and ligand-binding domains of the receptor. Alternatively, it is also possible that dexamethasone-activated glucocorticoid receptor competes with other transcription factors for their binding sites on the promoter region or squelches transcription factors shared by HIV-1-LTR and glucocorticoid-responsive promoters. We conclude that glucocorticoids suppress, rather than stimulate, the HIV-1 promoter, thus acting, protectively for the host. Their apparent negative clinical association with AIDS is most likely due to immunosuppression of the host. Published by Elsevier Science Ltd.

Keywords: Glucocorticoid receptor; Glucocorticoids; Human immunodeficiency virus; Protective effects; Type-1 long terminal repeat activity

1. Introduction

Glucocorticoids exert their effects through the ubiquitously expressed glucocorticoid receptor (GR) [1]. Upon binding with glucocorticoids, the GR dissociates from a hetero-oligomer of cytoplasmic heat shock proteins and translocates into the nucleus to exert its effects by binding either to glucocorticoid-responsive elements (GREs) in the promoter region of glucocorticoid-responsive genes or to other transcription factors, such as the NF-κB [2–4], AP-1 [5,6], and STAT proteins [7–9], through direct protein–protein interactions. Gene promoter-associated GRs modulate the stability of transcription initiation complexes, which

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contain RNA polymerase II and general transcription factors, through coactivators/corepressors with 'bridging' and enzymatic activities, mostly histone acetylation, deacetylation, respectively, necessary for loosening or strengthening promoter DNA winding around nucleosomes [10–13]. The GR also interacts with other chromatin-modulating protein complexes, such as the SWI/SNF and BRG proteins, which remodel chromatin structure and allow access to, and mobility of, the transcription complex on the DNA [14].

Glucocorticoids are frequently used in the treatment of various disorders associated with Human Immunodeficiency Virus Type-1 (HIV-1) infections, including *Pneumocystis carinii* pneumonia [15–17], space occupying lesions due to toxoplasmosis [18] or tuberculosis [19,20], HIV-associated nephropathy [21], and when the patients develop lymphoma [22]. Glucocorticoids are also given in short (7 day) bursts during interleukin-2 therapy cycles to minimize the toxicity of the cytokine (NIH protocol). Furthermore, we recently demonstrated that the HIV-1 accessory protein, Vpr, acts as a potent coactivator of the glucocorticoid receptor, probably increasing the sensitivity of certain host target tissues to glucocorticoids by three- to 20-fold [23]. A direct positive effect of glucocorticoids on HIV-1 replication would be detrimental to the patients [24,25]. This was examined experimentally over a decade ago, and a mild positive effect of glucocorticoids on the replication of HIV-1 was shown [26,27]. Subsequent studies on the role of these hormones on the HIV-1 life cycle, however, have been controversial, showing both a mild stimulatory and a moderate inhibitory effect of glucocorticoids on the HIV-1 5′ long terminal repeat (LTR) promoter [28–33].

HIV-1 gene expression is controlled in large part by its LTR promoter, a complex, 'modular' structure containing multiple protein-binding sites [34]. Viral *trans*-activating protein Tat is a major positive regulator of this promoter [35–37], cooperating with several host transcription factors, such as NF-κB [38], AP1 [39], Sp1 and others [40–42], some of which may share part of the same transcription machinery with the glucocorticoid receptor [12]. Although the presence of a half-perfect GRE that bound dimerized, ligand-activated glucocorticoid receptor was shown in the HIV-1-LTR [43], its functional significance has remained unclear. To clarify the direct role(s) of glucocorticoids on HIV replication, we examined the effect of dexamethasone on the HIV-1-LTR in human rhabdomyosarcoma-derived A204, monocytoid U937, T-cell lymphoma-derived Jurkat and cervical carcinoma-derived HeLa cells, as well as in African green monkey-derived CV-1 cells, which are devoid of functional endogenous glucocorticoid receptors.

2. Materials and methods

2.1. Cell lines

Human rhabdomyosarcoma A204, monocytoid U937, T-cell lymphoma Jurkat E6-1 and African green monkey kidney CV-1 cells were purchased from the American Type Culture Collection (American Type Cell Culture) (Rockville, MD). Human cervical carcinoma HeLa cells were a kind gift from Dr S.S. Simons Jr (National Institutes of Health, Bethesda, MD).

2.2. Plasmids

L3-Tat expresses the 1–72 amino acid polypeptide corresponding to the first exon Tat protein from the NL_{4-3} HIV-1 strain under the control of the HIV-1-LTR. L3-Luc contains the luciferase gene under the control of full-length wild-type HIV-1-LTR. These two plasmids were generous gifts from Dr G.N. Pavlakis (National Cancer Institute, Frederick, MD). pCDNA3-VPR was described elsewhere [23]. pMMTV-luc, which contains the murine mammary tumor virus LTR-driven luciferase cDNA, was a kind gift from Dr G. Hager (National Institutes of Health, Bethesda, MD). pCMVβ-p300-CHA was a kind gift from Dr D. Livingston (Harvard Medical School, MA). pRShGRα, a human glucocorticoid receptor α expression vector under the control of the Rous Sarcoma Virus promoter, its mutants (G-gal-G, RGR, Δ262-404, Δ9-385, Δ428-490, 550* and 515*) and pRSV-erbA^{-1}, which possesses the thyroid hormone receptor cDNA in the reverse orientation instead of hGRα cDNA, but is otherwise the same as pRShGRα, were generous gifts from Dr R.M. Evans (Salk Institute, La Jolla, CA). pSV40-β-Gal was purchased from Promega (Madison, WI).

2.3. Cell cultures and transfections

A204 rhabdomyosarcoma cells were cultured in DMEM/F-12 supplemented with 10% FBS, 50 μg/ml of streptomycin, and 50 units/ml of penicillin. HeLa and CV-1 cells were kept in DMEM containing 10% FBS, 50 μg/ml of streptomycin and 50 units/ml of penicillin. U937 and Jurkat E6-1 cells were cultured in RPMI1640 supplemented with 10% FBS, 3 mM L-glutamine, 10 mM HEPES, 1.5 g/l of sodium bicarbonate, 50 μg/ml of streptomycin, and 50 units/ml of penicillin. A204 and HeLa cells were plated in 35 mm well plates at 3×10^5 cells/well, and CV-1 cells were seeded at 2×10^5 cells/well in the same plates. The cells were allowed to incubate for 24 h and transfected with the appropriate plasmids by the lipofectin (Gibco GRL, Geithesberg, MD) method. Specifically, A204 cells, HeLa and CV-1

cells were incubated with opti-MEM medium containing 5 μg/ml of lipofectin and 0.5–2.0 μg/ml of DNA for 18–24 h, depending on the cell line. After these treatments, the media were replaced with DMEM/F-12 containing 10% FBS for A204 cells and DMEM containing 10% FBS for HeLa and CV-1 cells and cultured for an additional 24 h. The cells were stimulated with 1×10^{-6} M dexamethasone or vehicle and were lysed in 1 × Promega cell lysis buffer for the luciferase and β-galactosidase assays after an additional 24 h. 1×10^{7} cells of U937 or Jurkat cells were transfected with 50 μg/cuvette of DNA by an electroporation method (960 μF, 250 mV) and cultured in 7 ml of RPMI1640 containing 10% FBS. After 24 h, the cells were stimulated with 1×10^{-6} M of dexamethasone or vehicle. After an additional 24 h incubation, cells were lysed in 1 × Promega cell lysis buffer for the luciferase and β-galactosidase assays.

To achieve equal total DNA content, pCDNA3 or Bluescript SK(+) (Stratagene, La Jolla, CA) were used in lieu of pCDNA3-VPR and pCMVβ-p300-CHA, or L3-Tat, respectively. For the experiments examining the effect of GR chimeras or deletion mutants (Figs. 3 and 4), pRSV-erbA^{-1} was used as a negative control since this plasmid possesses the same plasmid comformation without expressing GR-related proteins.

2.4. Luciferase and β-galactosidase assay

For the luciferase activity assay, 350 μl of assay buffer solution, consisting of 25 mM Gly-Gly, 10 mM ATP, 25 mM $MgSO_4$, and 1% Triton-X 100 (pH 8.0), were added to 50 μl of cell lysate sample. Luciferase activity was integrated on a Monolight 2010 Luminometer (Analytical Luminescence Laboratory, Ann Arbor, MI) for 10 s after the addition of 100 μl of 1 mM D-luciferin sodium salt solution. β-galactosidase was determined by using Galacto-Light™ (Tropix, Bedford, MA), following the manufacturer's protocol on the same 2010 luminometer. Luciferase activity was corrected by β-galactosidase activity. All experiments were performed in triplicate.

2.5. Statistical analyses

Statistical analysis was carried out by ANOVA, followed by Student's *t*-test with a Bonferroni correction for multiple comparisons.

3. Results

Tat has a strong stimulatory effect on the transcriptional activity of the HIV-1-LTR in A204, U937, Jurkat and HeLa cells (Fig. 1A). This effect was greater than 20-fold over the baseline in all cell lines tested. Dexamethasone suppressed Tat-stimulated HIV-1-LTR by about 60% in A204 (a) and U937 (b) cells, while it had no effect in Jurkat (c) and HeLa (d) cells (Fig. 1A). In A204 cells, dexamethasone suppressed Tat-activated HIV-1-LTR dose-dependently; the addition of 10^{-5} M of the glucocorticoid receptor antagonist RU 486 shifted the dose–response curve to the right (Fig. 1B).

Since p300, a cointegrator of several transcription factors, acts as a coactivator of both the GR and Tat [12,44,45], and Vpr functions as a coactivator of the GR as well as of Tat's activity on the HIV-1-LTR [23,46], we examined their involvement in the dexamethasone suppression of Tat-activated HIV-1-LTR in A204 cells. Transfection of p300 or Vpr potentiated the activity of Tat; however, neither changed the suppressive effect of dexamethasone upon the promoter (Fig. 2A and B).

To further analyze how dexamethasone modulates Tat-activated HIV-1-LTR in A204 cells, we employed several GR mutants that affect GR-induced transactivation at different steps (Fig. 3A). We employed the non-protein-expressing erbA^{-1} as a negative control for all mutants employed.

First, we conducted experiments in CV-1 cells, which do not contain functional GR, by co-transfecting GR mutants with the strongly glucocorticoid-responsive MMTV-LTR, a well-characterized GRE-containing system. In Fig. 3B, ligand-dependent transcriptional activation of the MMTV-LTR was clearly observed in GR and GRΔ262-404 transfected CV-1 cells. The GRΔ9-385 mutant showed a weak but significant ligand-dependent transactivation on this promoter, while the GR550* and 515* mutants exhibited constitutive transactivation activities; erbA^{-1}, G-gal-G and RGR did not display any activities.

Next, we examined the activities of the above mutants on the MMTV-LTR in A204 cells (Fig. 4A). GR, GRΔ262-404 and GRΔ9-385 showed dexamethasone-dependent enhancement of MMTV-LTR-driven transcription; erbA^{-1} and G-gal-G had no effect. RGR suppressed dexamethasone-induced transactivation; GR550* and 515* caused constitutive activation of the MMTV-LTR.

Finally, we examined the effects of the above GR mutants on the dexamethasone modulation of the Tat-activated HIV-1-LTR in A204 cells (Fig. 4B). Transfection with erbA^{-1}, GR, G-gal-G, GRΔ262-404 or GRΔ9-385 did not change the suppressive effect of dexamethasone on Tat-stimulated HIV-1-LTR activity, while transfection of RGR, 550* or 515* reduced the activities of Tat-stimulated HIV-1 promoter but had no effect on the dexamethasone-induced suppression of this promoter.

4. Discussion

Dexamethasone suppressed Tat-activated HIV-1-LTR in a cell-type-specific fashion. This effect was antagonized by RU 486, suggesting that it was mediated by the GR. To further characterize the suppressive effect of dexamethasone on the HIV-1-LTR, we employed several GR mutants with different transactivation properties. Transfection of the GR mutants G-gal-G, which possess the immunogenic and ligand-binding domains but not the DNA-binding domain, did not influence the suppressive effect of dexamethasone on Tat-activated HIV-1-LTR in A204 cells. As would have been predicted, these receptors did not influence the effect of endogenous GR on the MMTV promoter either.

The transfection of the receptors Δ262–404 or Δ9–385, which lack parts of the immunogenic domain but retain transactivation activity on the MMTV-LTR, did not modulate the suppressive effect of dexamethasone on Tat-activated HIV-1-LTR in A204 cells, while transfection of the mutant receptors RGR550* and 515*, which can bind GREs and modulate transcription of MMTV-LTR in a dexamethasone-independent fashion, reduced Tat-stimulated HIV-1-LTR activity and abolished dexamethasone-induced suppression of Tat-stimulated HIV-1-LTR activity.

The above data suggest that the DNA-binding domain is necessary for the suppressive effect of dexamethasone on the HIV-1-LTR. It is thus possible that direct interaction of the GR with a GRE-like sequence in the HIV-1-LTR might interfere with the transcription induced by Tat. There are several reports showing GRE-like sites in the HIV-1 genome, including the LTR [29,43]. Findings similar to ours, describing a suppressive effect of glucocorticoids on the HIV-1-LTR, were reported elsewhere in similar cell lines such as monocytoid or promonocytic cells [30,47]. Some authors suggested that a GRE-like sequence downstream from the transcription start site was critical for the suppressive effect of glucocorticoids by competition of GR with a cellular transcription factor leader binding protein that is expressed in specific cell lines [33,48]. Others suggested that a cellular protein belonging to distinct signaling pathways regulated by glucocorticoids may regulate HIV-1-LTR activity in a cell-type-specific manner [47]. Indeed, the DNA-binding domain of the GR might interfere with other, positive transcription

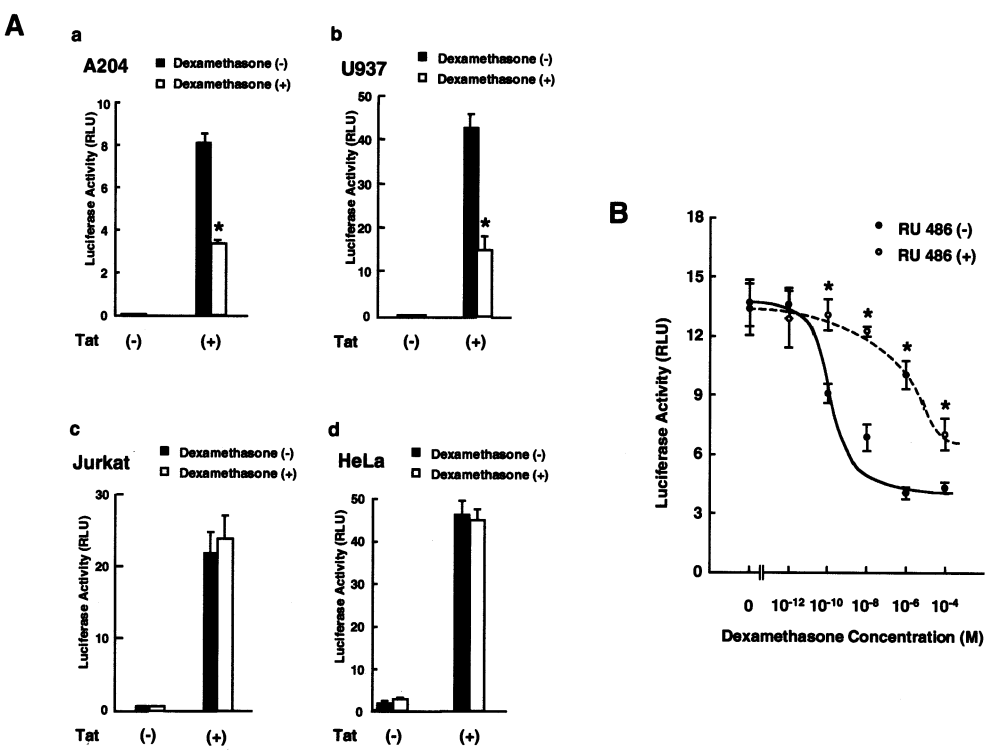

Fig. 1. (A) Effects of dexamethasone on the Tat-stimulated HIV-1 LTR in A204 (a), U937 (b), Jurkat (c) and HeLa cells (d). U937 cells (b) and Jurkat cells (c) were transfected with 10 μg/cuvette of L3-Tat together with 20 g/cuvette of L3-Luc and 10 μg/cuvette of pSV40-β-Gal. A204 cells (a) and HeLa cells (d) were transfected with 1.0 μg/well of L3-Tat together with 1.5 μg/well of L3-luc and 0.5 μg/well of pSV40-β-Gal. Bars show the mean ± S.E. obtained in the presence (solid) or absence (open) of 10^{-6} M dexamethasone. *$P < 0.01$, comparing the data with and without dexamethasone. (B) Dose-dependent effects of dexamethasone on Tat-stimulated HIV-1-LTR in A204 cells. A204 cells were transfected with 1.0 μg/well of L3-Tat, 1.5 μg/well L3-luc and 0.5 μg/well of pSV40-β-Gal. Solid and open circles show the mean ± S.E. obtained in the presence or absence of 10^{-5} M of RU 486. *$P < 0.01$, comparing the data with and without RU 486 at the same concentration of dexamethasone.

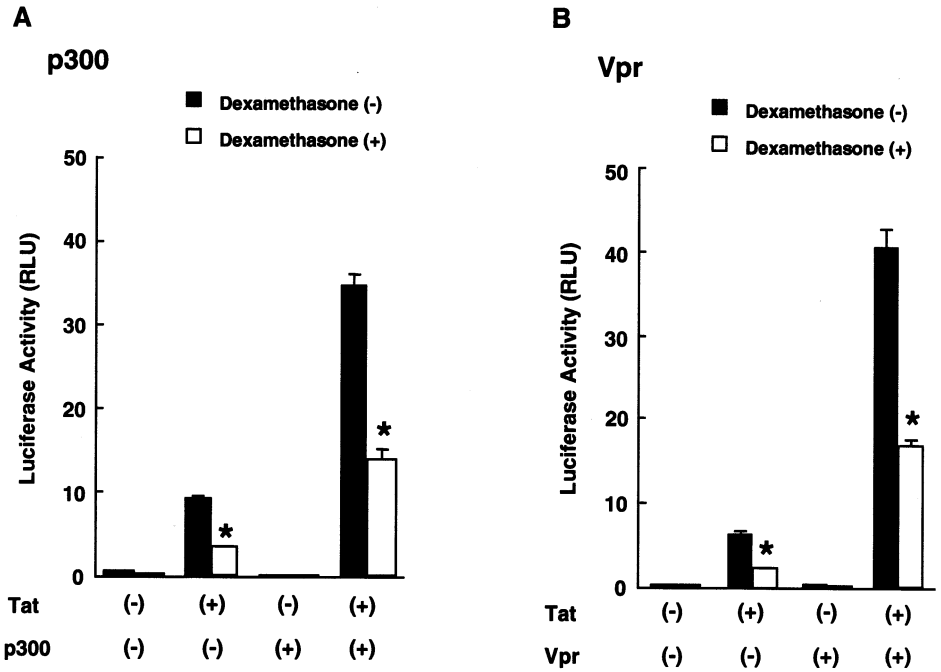

Fig. 2. Effect of p300 (A) or Vpr (B) transfection on the dexamethasone suppression of the Tat-activated HIV-1 LTR in A204 cells. A204 cells were transfected with 1.0 μg/well of L3-Tat, 1.5 μg/well of L3-Luc, 0.5 μg/well of pSV40-β-Gal and 2.0 μg/well of pCMVβ-p300-CHA (A) or 0.6 g/well of pCDNA3-VPR (B). Bars show the mean ± S.E. obtained in the presence (solid) or absence (open) of 10^{-6} M dexamethasone. *$P < 0.01$, comparing the data with and without dexamethasone in the same condition.

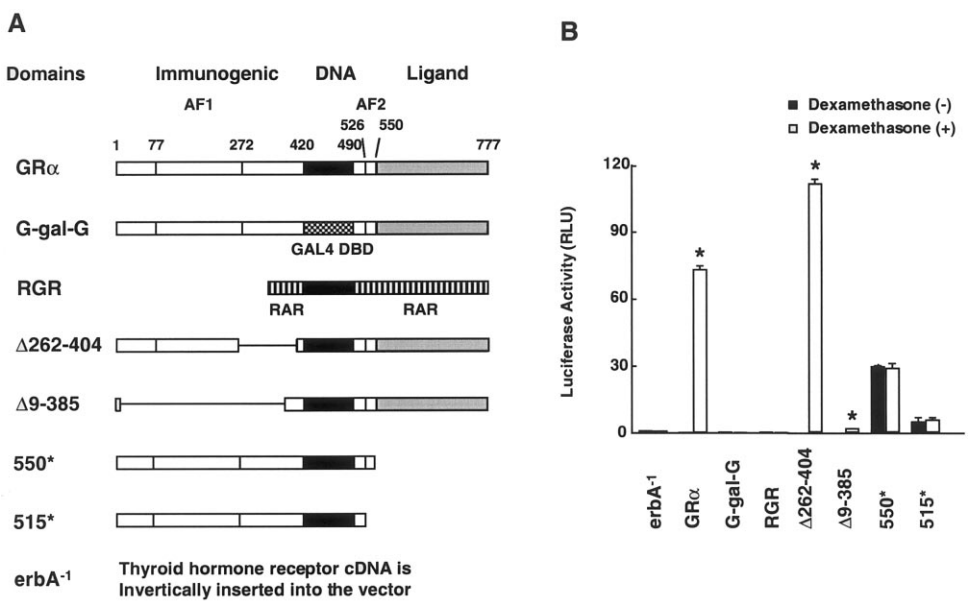

Fig. 3. (A) Schematic model of human glucocorticoid receptor and mutants employed. (B) Effects of glucocorticoid receptor and mutants on MMTV-LTR in CV-1 cells. CV-1 cells were transfected with 0.3 μg/well of pMMTV-luc, 0.1 μg/well of pSV40-β-Gal and 0.3 μg/well of each of pRShGRα or its mutants. Bars show the mean ± S.E. obtained in the presence (solid) or absence (open) of 10^{-6} M dexamethasone. *$P < 0.01$, comparing the data with and without dexamethasone.

factors to produce suppression of HIV-1 expression. Several studies demonstrated that glucocorticoids suppressed HIV-1-LTR activity stimulated by PMA, GM-CSF or TNFα, strongly suggesting such a possibility [47,49]. A good candidate GR partner affected by glucocorticoids is the NF-κB transcription factor, which interacts with the GR employing a protein interface lying in the DNA-binding and ligand-binding domains of the receptor. NF-κB is a major host transactivator of the HIV-1-LTR that cooperates with

both Tat [38,50] and the cointegrator p300 [51,52] to exert its effect. Indeed, the ligand-activated GR inhibits the activity of NF-κB without binding to the DNA of a NF-κB-responsive promoter, even though its DNA-binding domain is a sine qua non for this action for the protein–protein interaction with NF-κB [4]. Interestingly, we have demonstrated that Vpr has no coregulator effect on the ability of NF-κB to stimulate a κB-responsive element-containing promoter (Kino T., unpublished data).

Recently, the retinoic acid receptor was reported to enhance Tat-stimulated HIV-1-LTR activity in the neuroblastoma cell line SH-SY5Y, cooperating with the steroid receptor coactivator (SRC)-1[53]. As the retinoic acid and glucocorticoid receptors share several transcriptional components, including SRC-1 type coactivators [12], it is possible that ligand-bound GR might squelch such transcription compnents from the HIV-1-LTR. We suggest that this is unlikely, granted that in our studies, p300 and Vpr could not overcome the suppressive effect of dexamethasone on Tat-stimulated HIV-1-LTR promoter.

There are reports demonstrating a positive correlation between glucocorticoid administration [26,27] or levels of endogenous glucocorticoids and HIV-1 replication and/or disease progression [54–59]. As glucocorticoids do not seem to stimulate the HIV-1-LTR, but rather to suppress it in a cell-type-specific fashion, it is quite likely that the above correlation is not due to a direct positive action of glucocorticoids on the activity of HIV-1-LTR, but rather an indirect effect of glucocorticoid-induced host immunosuppression and unbridled viral replication. As expected, the glucocorticoid receptor coactivator activity of Vpr was not present in the interaction of the GR with the HIV-1-LTR. We suggest that Vpr may contribute to the HIV-1 replication indirectly by enhancing the sensitivity of host immune cells to glucocorticoids, thus potentiating the immunosuppressive and anti-inflammatory effects of the not necessarily elevated levels of endogenous glucocorticoids [23].

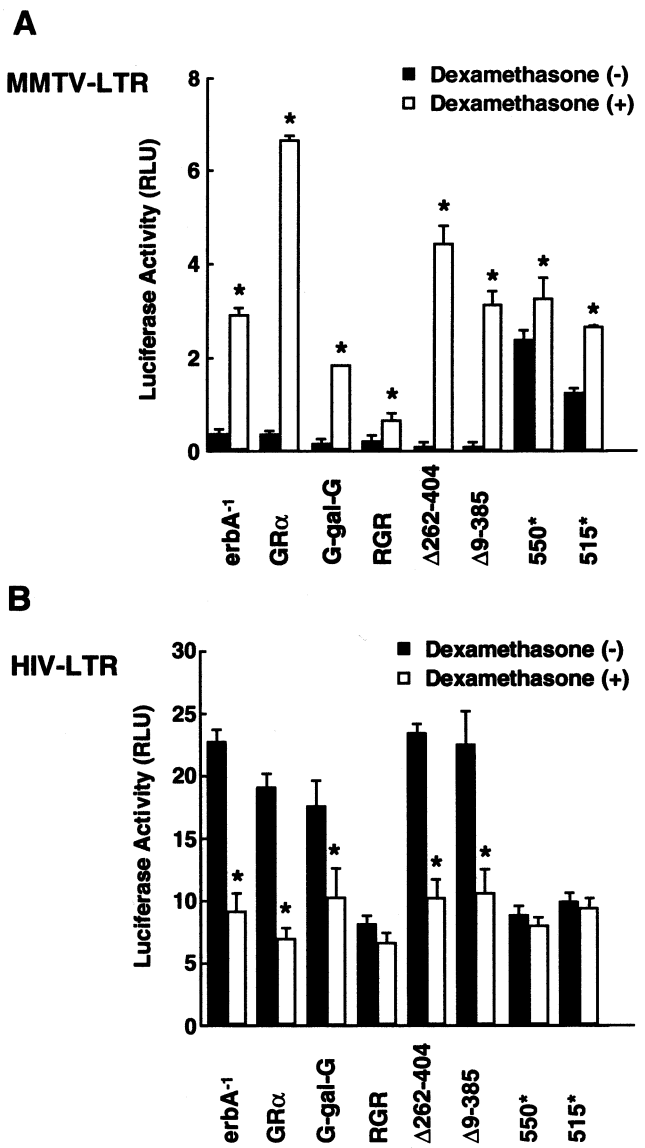

Fig. 4. Effects of the glucocorticoid receptor and its mutants on MMTV-LTR (A) and HIV-1-LTR (B) in A204 cells. (A) A204 cells were transfected with 1.5 μg/well of pMMTV-luc, 0.5 μg/well of pSV40-β-Gal and 1.0 μg/well of each of pRShGRα or its mutants. Bars show the mean ± S.E. obtained in the presence (solid) or absence (open) of 10^{-6} M dexamethasone. $^{*}P < 0.01$, comparing the data with and without dexamethasone. (B) A204 cells were transfected with 1.5 μg/well of L3-Luc, 0.5 μg/well of pSV40-β-Gal, 1.0 μg/well of L3-Tat and 0.3 μg/well of each of pRShGRα or its mutants. Bars show the mean ± S.E. obtained in the presence (solid) or absence (open) of 10^{-6} M dexamethasone. $^{*}P < 0.01$, comparing the data with and without dexamethasone.

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