Modulation of HIV-1 enhancer activity and virus production by cAMP

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Abstract The effect of cAMP on the transcriptional activity of the HIV-1 long terminal repeat/enhancer was investigated and compared to the effect of cAMP on virus replication. In culture cAMP repressed virus replication in vivo using different cell types. Transient transfection studies with HIV-1 enhancer-derived luciferase reporter gene constructs identified the minimal DNA sequence mediating the negative regulatory effect of cAMP on HIV-1 transcription. A single nuclear factor κB element from the HIV-1 enhancer mediates the repressive effect on transcription. AP-2 is not involved in cAMP repression. Stable transfection of Jurkat T cells with the co-activators CREB binding protein (CBP) and p300 completely abolished the cAMP repressive effect, supporting the hypothesis that elevation of intracellular cAMP increases phosphorylation of CREB, which then competes with phosphorylated p65 and Ets-1 for limiting amounts of CBP/p300 thereby mediating the observed repressive effect on transcription. These findings suggest an important role of cAMP on HIV-1 transcription.

Key words: HIV-1 enhancer; cAMP; Negative regulation; Transient transfection; Virus production

1. Introduction

The human immunodeficiency viruses HIV-1 and HIV-2 are the etiological agents of AIDS [1–3]. Viral transcription is controlled by cellular factors that interact with sequences located in the HIV long terminal repeat (LTR) [4–6]. The HIV-1 core enhancer, which extends from position −109 to −79, represents the major regulatory domain and contains overlapping nuclear factor κB (NF-κB), Ets and AP-2 binding sites [7–13].

The transcription factor NF-κB plays a major role in the regulation of HIV-1 gene expression [9]. The predominant form of this transcription factor is a heterodimer consisting of 50-kDa (p50) and 65-kDa (p65 or RelA) subunits [14,15]. Upon stimulation, cytoplasmic NF-κB bound to inhibitory IκB proteins is released, translocates to the nucleus, binds to its DNA binding sites and up-regulates gene expression [16,17]. NF-κB plays a central role in the up-regulation of HIV LTR reporter constructs or of proviral sequences in chronically infected cell lines [18–20].

The transcription factor AP-2 is a sequence-specific DNA binding protein involved in cAMP induction and shows no similarity to other known transcription factors. AP-2 contains a proline-rich transcriptional activation domain and a helix-span-helix structure [21]. Perkins et al. demonstrated a mutually exclusive binding of AP-2 and NF-κB to the HIV-1 enhancer region [10]. The role of AP-2 in HIV gene regulation remains to be defined.

Another important and ubiquitous regulatory molecule is cAMP. It coordinates diverse metabolic processes, and induces the expression of numerous genes through protein kinase A (PKA)-mediated phosphorylation of cAMP response element (CRE) binding factors, including CREB [22]. Many genes are regulated and their rate of transcription is rapidly altered by cAMP [23–28]. In transformed T cells, NF-κB activation was inhibited by elevated cAMP levels [29,30], while the nuclear translocation of NF-κB complexes was not affected [31]. As cAMP can inhibit proinflammatory cytokines, agents that raise intracellular cAMP levels such as pentoxyphilline or rolipram have been tested for potentially beneficial effects [32]. However, the effects of cAMP on HIV transcription remain controversial [33,34].

We studied the mechanism of cAMP action on the HIV-1 LTR. Virus production assays showed a negative effect of cAMP on the production of HIV-1, both in primary human lymphocytes and in T cell lines. We also found a repressive effect of cAMP on HIV-1 LTR transcription in primary blood lymphocytes and human T cell lines. In transient transfection experiments, this inhibitory effect was mediated by NF-κB binding sites in the HIV-1 enhancer region. The overlapping AP-2 binding site is not involved in cAMP-mediated repression of the HIV-1 enhancer. Finally we show that a stable transfection of Jurkat T cells with expression constructs of the co-activators CREB binding protein (CBP) or p300 completely abolished the cAMP repressive effect.

2. Materials and methods

2.1. Reagents

Phorbol 12-myristate 13-acetate (PMA), phytohemagglutinin and sodium metavanadate were purchased from Sigma-Aldrich. All enzymes used for cloning as well as the non-radioactive reverse transcriptase assay were bought from Boehringer Mannheim/Roche.

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Abbreviations: CRE, cAMP response element; CREB, CRE binding protein; CBP, CREB binding protein; HIV, human immunodeficiency virus; LTR, long terminal repeat; PBLs, peripheral blood lymphocytes; PKA, protein kinase A; RT, reverse transcriptase

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8-Bromoadenosine-3′,5′-monophosphate sodium salt (8-Br-cAMP) was obtained from BioLog Life Science, forskolin from BIOMOL.

2.2. Cell culture conditions

Human Jurkat T cells and human HUT-78 lymphoma cells were obtained from the American Type Culture Collection. Both cell lines and peripheral blood lymphocytes (PBLs) were grown in RPMI 1640 with GlutaMax-I (Life Technologies) supplemented with 10% fetal calf serum (FCS, BioWhittaker). For transfection cells were seeded at a density of 1×10^5 cells/ml and grown at 37°C in an atmosphere of 95% air and 5% CO_2.

2.3. HIV-1 virus production assay

For virus production assays three different cell types were used: clones of HUT-78 and Jurkat T cells permanently producing HIV-1_{LAI} and human PBLs prepared from the blood of a HIV-negative donor which had been freshly infected with HIV-1_{LAI} [37]. 2×10^5 cells per well were treated with various concentrations of 8-Br-cAMP (1 mM, 0.5 mM or 0.25 mM). At various time points reverse transcriptase (RT) activity in cell culture supernates was measured using a commercial non-radioactive RT assay (Roche) [38]. All experiments were performed in duplicate. Cell growth and viability (by trypan blue exclusion) were checked by microscopic examination at least three times per week.

2.4. Isolation of peripheral blood leukocytes

Peripheral blood leukocytes were isolated from 300 ml buffy coats (leukocyte concentrates) which were obtained by a 4 h apharesis from healthy volunteers. The leukocyte concentrate was diluted 1:1 with 0.9% NaCl solution and layered on top of a Ficoll cushion. After a 30 min centrifugation step the lymphocyte/monocyte layer was collected from the interphase. After washing three times in phosphate-buffered saline (PBS), the cells were cultured and used for transient transfection experiments and viral production assays as described below.

2.5. Plasmids

The construct containing the full-length HIV-1 LTR sequence upstream of a luciferase reporter gene and the tat expression construct (pSV2tat72) were kindly provided by R. Brack-Werner (GSF Munich, Germany). The pCMV-CBP and the pCMV-p300 expression plasmids were a kind gift of Dr. C. Glass (University of California, San Diego, CA, USA) and have been described previously [35]. The plasmid pRc/CMV (Invitrogen) contains as a dominant selectable marker the neo gene under control of the SV40 early promoter allowing selection of stably transfected cells with the neomycin analogue Geneticin (G418). Additional luciferase plasmids were generated by inserting double-stranded oligonucleotides with *Bgl*II/*Bam*HI compatible ends into the *Bgl*II site of the pGL3 promoter vector (Promega) in sense and antisense orientation. The HIV-A double-stranded oligonucleotide was inserted into a *Sma*I-digested pGL3 promoter vector (Promega). For the HIV-AB trimer and HIV-AB trimer/AP-2m luciferase constructs three copies of the corresponding oligonucleotides were cloned in a head to tail orientation into the *Bgl*II site of the pGL3 promoter vector (Promega). The following oligonucleotides were used (only the sequences of the sense strands are shown):

HIV-A: 5′-CCAAGGGACTTTCCGCTG-3′

HIV-AB monomer/*Bgl*II/*Bam*HI: 5′-GATCCCAAGGGACTTTC-CGCTGGGGACTTTCCAGGA-3′

HIV-AB monomer/AP-2m/*Bgl*II/*Bam*HI: 5′-GATCCCAAGGGA-CTTTCCATATGGGACTTTCCAGGA-3′.

2.6. Transient transfections and luciferase assays

For transient transfection experiments, aliquots consisting of 5×10^6 proliferating Jurkat T cells or PBLs were pelleted (5 min at 850 rpm), resuspended in 10 ml transfection medium [36] containing 10 μg plasmid DNA of the corresponding luciferase construct and incubated for 1 h at 37°C. For co-transfection experiments with tat, the transfection mix was supplemented with 5 μg pSV2tat72 plasmid DNA. All plasmid DNAs used for transfection experiments were prepared by alkaline lysis and purified by two consecutive CsCl gradients. To increase transfection efficiency, a dimethylsulfoxide (DMSO) shock was performed after the DEAE dextran treatment (5 min room temperature, medium with 10% DMSO). Cells were washed twice with PBS, cultured in RPMI 1640 with GlutaMax-I (Life Technologies) and FCS and stimulated with PMA (25 ng/ml) and/or 8-Br-cAMP (1 mM) for 12 h. Cells were harvested, and washed three times with PBS before they were lysed for 10 min on ice by addition of 250 μl lysis buffer (1% Triton X-100, 25 mM glycyl-glycine pH 7.8, 15 mM $MgSO_4$, 4 mM EGTA pH 8.0, 1 mM dithiothreitol (DTT)). Lysates were cleared by centrifugation (5 min, 13 000 rpm, 4°C). Luciferase enzyme activity was measured as relative light units (RLU) in a luminometer (Berthold Lumat LB9501) by injecting 100 μl luciferase assay substrate (Promega) to a tube containing 50 μl cleared lysate and 300 μl of reaction buffer (25 mM glycyl-glycine pH 7.8, 15 mM $MgSO_4$, 4 mM EGTA, 15 mM K_2PO_4 pH 7.8, 1 mM DTT, 2 mM ATP). A suitable aliquot of the lysate was used to determine the protein concentration with a commercial Bradford assay. RLUs were corrected for 10 μg of protein after subtraction of the luciferase background value.

2.7. Generation of stably transfected cells

For the generation of CBP or p300 overexpressing cell lines, Jurkat

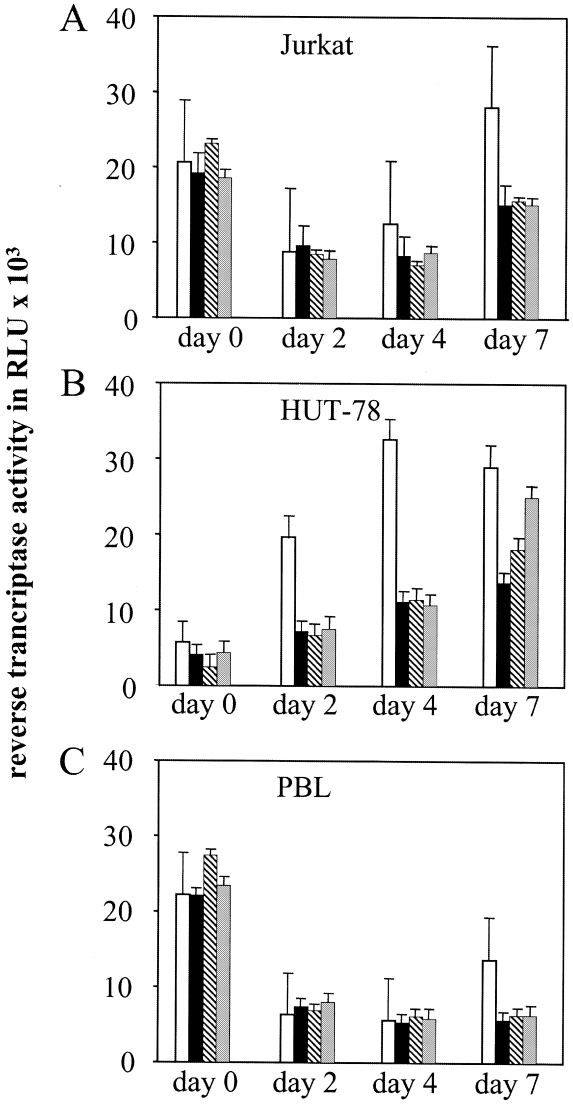

Fig. 1. Influence of 8-Br-cAMP on HIV-1 virus production. The time course of HIV-1 virus production with and without cAMP is shown. A: Results obtained with a permanently producing HIV-1_{LAI} Jurkat T cell clone. Similar results were obtained using a HIV-1_{LAI} HUT-78 cell clone (B) or freshly infected PBLs from a healthy volunteer (infection at day −3) (C). On day 0 aliquots of 2×10^5 cells were seeded in the wells of a 96-well plate. After 2, 4 and 7 days HIV-1 RT activity was measured in supernates. The diagrams show the RT activity of untreated cells (white bars) or cells treated with 1 mM (black bars), 0.5 mM (hatched bars) or 0.25 mM (gray bars) 8-Br-cAMP. All experiments were performed in duplicate.

Table 1
Influence of 8-Br-cAMP on cell viability

	Percent living cells		
Control	100	100	97.8
cAMP 1 day	100	99	100
cAMP 2 days	98.9	100	98.9
cAMP 4 days	97.7	98.7	98.8
cAMP 7 days	98.6	97.2	100

Jurkat cells were grown in 24 well plates in the presence or absence of 1 mM 8-Br-cAMP for 1, 2, 4 or 7 days as indicated. Cell viability was determined by trypan blue exclusion and the percentage of living cells is given.

T cells were stably co-transfected with the plasmids pCMV-CBP or pCMV-p300 and pRc/CMV. For transfection, 5 μg of pCMV-CBP or pCMV-p300 digested with *Pvu*I and 0.5 μg of pRc/CMV linearized with *Xba*I were combined with approximately 5×10^6 Jurkat T cells in 400 μl PBS. This mixture was transferred to a GenePulser cuvette with a 4 mm electrode gap. Electroporation was performed at room temperature using a GenePulser apparatus (Bio-Rad Laboratories) set to 250 V and 960 μF. One day later, stably transfected cells were selected by adding G418 (Life Technologies) in a concentration of 800 μg/ml to the cell culture medium. Previous dose–response experiments had shown that this concentration was lethal for untransfected Jurkat T cells. After 15 days of mass culture, limited dilution cloning was performed to obtain monoclonal cell lines. Three clones of each line were selected and used for additional experiments.

3. Results

3.1. HIV-1 virus production is repressed by cAMP

To address the question if cAMP has an effect on HIV-1 replication, virus production assays were performed. Fig. 1 shows the results obtained with two human T cell lines and primary human PBLs. A Jurkat T cell clone stably infected with HIV-1 was treated with 8-Br-cAMP in three different concentrations. Seven days treatment with cAMP reduced viral replication as determined by RT activity in cell culture supernates by approximately 40% (Fig. 1A). In HUT-78 cells stably infected with HIV-1, cAMP was found to repress virus production up to 60% after 2–4 days of cAMP treatment (Fig. 1B). Comparable results were obtained in primary human PBLs prepared from an HIV-1-negative donor. Incubation

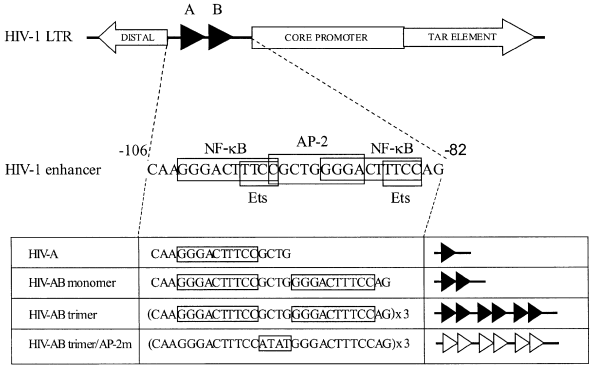

Fig. 2. Schematic representation of the HIV-1 LTR- and HIV-1 LTR-derived sequences used in luciferase constructs. The nucleotide sequence around the two NF-κB binding sites of the HIV-1 enhancer is shown. The NF-κB, Ets and AP-2 binding sites are boxed. The various regions of the HIV-1 LTR included in luciferase constructs are depicted in the lower part of the figure together with their names and the corresponding symbols. Wild-type oligonucleotides are represented by black triangles, whereas oligonucleotides with wild-type NF-κB sites and a mutated AP-2 site are indicated by white triangles.

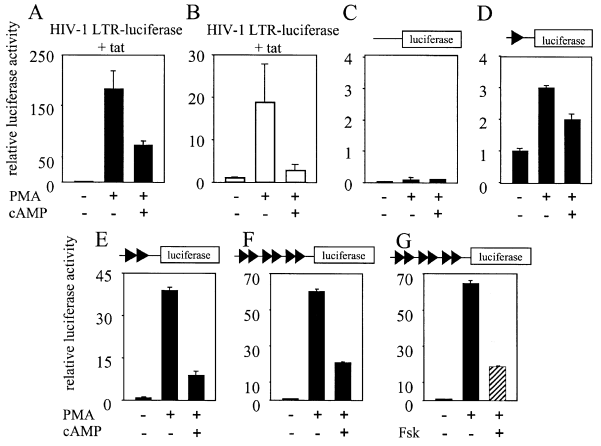

Fig. 3. Effect of cAMP on HIV-1 LTR-derived luciferase constructs. Jurkat T cells (A) and PBLs (B) were transiently co-transfected with a full-length HIV-1 LTR luciferase and a tat expression construct. After transfection cells were treated with PMA and/or 8-Br-cAMP. Luciferase activity was measured in cellular extracts 12 h after transfection and results are expressed as relative luciferase activities with the uninduced level set at 1. One relative unit corresponds to 10 181 RLU in A and to 1236 RLU in B. Different reporter gene constructs (pGL3-promoter luciferase (C), HIV-A luciferase (D), HIV-AB monomer luciferase (E), HIV-AB trimer luciferase (F and G) (see also Fig. 2)), were transiently transfected into Jurkat T cells. Cells were treated with PMA and/or 8-Br-cAMP (C–F) and/or forskolin (G). Results are expressed as relative luciferase activity with the uninduced level set at 1, except in C. One relative unit corresponds to 29 901 RLU for C, 43 796 RLU for D, 3509 RLU for E, 91 662 RLU for F and 248 573 RLU for G. The experiments shown were repeated two times with comparable results. Every transfection experiment was performed in triplicate.

of freshly HIV-1-infected PBLs with cAMP reduced viral production by 50% after 7 days treatment (Fig. 1C). An inhibitory effect of cAMP on cell viability or proliferation as an explanation for these results was ruled out in control experiments (Table 1).

3.2. HIV-1 LTR activity is repressed by cAMP

In order to analyze the effect of cAMP on HIV-1 LTR transcription (see Fig. 2), transient transfection experiments were performed. As T cells are a major target of HIV-1, human PBL preparations and the human Jurkat T cell line were used for these experiments. Cells were co-transfected with a luciferase reporter gene construct containing the full-length LTR sequence of HIV-1 and an expression plasmid encoding tat to achieve maximal LTR activity. After transfection the cells were treated for 12 h with PMA either alone or in combination with 8-Br-cAMP prior to determination of luciferase activity. PMA stimulation led to a more than 180-fold induction of the HIV-1 LTR in Jurkat T cells and to a more than 19-fold induction in PBLs. Co-incubation with 8-Br-cAMP resulted in a marked repressive effect (approx. 60% repression in Jurkat T cells (Fig. 3A) and more than 80% repression in PBLs (Fig. 3B). As different PBL preparations showed a high degree of variability in transient transfection experiments, all subsequent experiments were performed in the Jurkat T cell line.

3.3. The cAMP repressive effect requires only the NF-κB binding sites from the HIV-1 LTR

After demonstrating a repressive effect of cAMP on HIV-1

LTR activity, we sought to determine which sequences within the LTR were necessary for this repression. The two adjacent NF-κB binding sites within the HIV-1 enhancer were considered the most likely candidate sequences and our analysis focused on that region. Luciferase constructs containing the complete LTR sequence, one HIV-1 NF-κB binding site (HIV-A) or both naturally occurring NF-κB binding sites (HIV-AB monomer) upstream of the herpes simplex virus (HSV) thymidine kinase (TK) promoter were generated (Fig. 2). To further enhance the effects obtained with the HIV-AB monomer luciferase construct, three copies of HIV-AB monomer oligonucleotide were inserted in a head to tail fashion upstream of the HSV TK promoter in pGL3 (HIV-AB trimer) (see Fig. 2). Transfection of cells with pGL3 promoter alone revealed only a very low basal luciferase activity (Fig. 3C). PMA stimulation led to an approximately three-fold induction of the HIV-A luciferase construct (Fig. 3D) and to an approximately 40-fold induction of the HIV-AB monomer luciferase construct (Fig. 3E). The HIV-AB trimer luciferase construct showed an approximately 60-fold induction after stimulation with PMA (Fig. 3F). Co-incubation of cells with PMA and 8-Br-cAMP resulted in 40% repression (HIV-A luciferase) or 80% repression (HIV-AB monomer luciferase) of the luciferase activity compared to cells stimulated with PMA alone (Fig. 3D,E). With the HIV-AB trimer construct 65% repression was observed after addition of 8-Br-cAMP (Fig. 3F). Treatment of cells with cAMP alone did not lead to significant changes in luciferase activity in comparison to untreated cells (data not shown). The cAMP repressive effect did not depend on the method used to increase the intracellular cAMP levels. When 8-Br-cAMP was replaced by forskolin, an adenylate cyclase activator, a comparable level of repression was achieved (compare Fig. 3F and G).

The HIV-1 enhancer luciferase reporter gene constructs did not contain the TAR element required for tat binding. In transient transfection experiments comparable results were obtained with or without co-transfection of the tat expression plasmid (data not shown). Therefore, tat was omitted in all transfection experiments using minimal constructs.

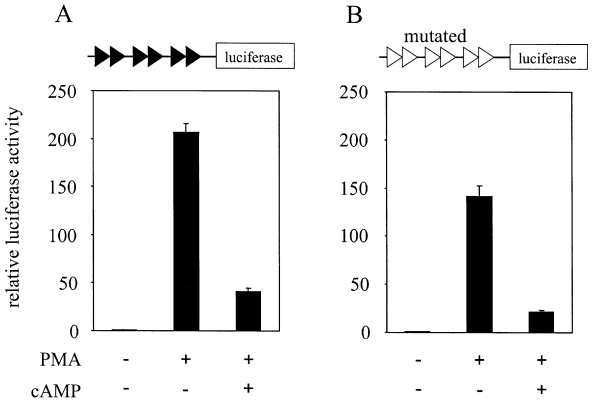

Fig. 4. Effect of cAMP on a HIV-1 enhancer luciferase construct with a mutated AP-2 site. A: Transient transfection experiment in Jurkat T cells with the HIV-AB trimer luciferase construct containing the wild-type AP-2 site. B: Results of a transfection experiment with the HIV-AB trimer/AP-2 mutated luciferase construct (see also Fig. 2). The cells were treated with PMA and/or 8-Br-cAMP immediately after transfection. One relative unit of luciferase activity corresponds to 20 563 RLU in A and 7401 RLU in B. Comparable results were obtained in three separate experiments.

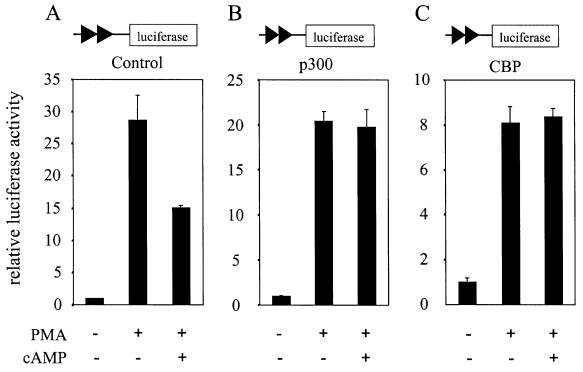

Fig. 5. Effect of CBP/p300 on cAMP-mediated repression. A: Normal Jurkat T cells were transiently transfected with the HIV-AB monomer luciferase construct and the luciferase activity was measured in untreated, PMA- and/or 8-Br-cAMP-treated cells. B: Transient transfection experiment with the HIV-AB monomer luciferase construct using Jurkat T cells which were stably transfected with a p300 expression construct. The cells were treated with PMA and/or 8-Br-cAMP. C: Transient transfection experiment using Jurkat T cells, which were stably transfected with a CBP expression construct. The cells were treated with PMA and/or 8-Br-cAMP and luciferase activity was measured in cellular extracts 12 h later. Results are expressed as relative luciferase activities. One relative unit corresponds to 5781 RLU in A, 2831 RLU in B and 21 948 RLU in C. The figure shows one representative of three independent experiments. For each of the two stable cell lines three independent clones were tested with comparable results. The transient transfection experiments were performed in triplicate.

3.4. The AP-2 binding site is not involved in cAMP-mediated repression

The HIV enhancer contains overlapping binding sites for the transcription factors NF-κB, AP-2 and Ets (see Fig. 2). Cyclic AMP can induce AP-2 transcription [39,40], and it has been reported that the binding of NF-κB and AP-2 to the HIV-1 enhancer is mutually exclusive [10]. To test whether the AP-2 site is functionally involved in cAMP repression, transient transfection experiments were performed with a luciferase construct in which the AP-2 site had been selectively destroyed by point mutations [10] (see Fig. 2). The construct contained three copies of the HIV-AB monomer/AP-2m oligonucleotide upstream of the HSV TK promoter in pGL3. Transient transfection experiments with the HIV-AB trimer and HIV-AB trimer/AP-2m luciferase constructs showed a comparable induction of luciferase activity after PMA stimulation. Co-incubation with PMA and 8-Br-cAMP showed the same degree of repression for the wild-type (80%) and the AP-2-mutated construct (85%) (Fig. 4).

3.5. CBP and p300 play an important role in the regulation of the HIV-1 enhancer region by cAMP

The two closely related proteins CBP and p300 represent important co-activators connecting transcription factors with the basal transcription machinery [41–44]. It has been reported that CBP and p300 can interact with the p65 subunit of NF-κB and also with Ets-1 [45–47]. Since PKA-phosphorylated CREB binds to CBP, Parry and Mackman hypothesized that elevated cAMP levels may inhibit NF-κB-mediated transcription by a competition between CREB and NF-κB for limiting amounts of CBP/p300 [48]. To test whether CBP or p300 is the limiting factor in our model system, we attempted to generate T cell lines overexpressing either human CBP or human p300. For that purpose Jurkat T cells were stably

transfected with expression constructs encoding either CBP or p300 [35]. Three independent clones from both CBP and p300 stable cell lines were selected and further expanded. Subsequently, transient transfection experiments were performed with these clones and wild-type Jurkat T cells using the HIV-AB monomer luciferase construct. PMA stimulation was not affected in the stably transfected CBP or p300 cell lines. In contrast, in both cell lines the suppressive effect of cAMP was completely abolished (Fig. 5). Taken together these results suggest that overexpression of CBP or p300 overcomes the inhibitory effect of cAMP. These results are consistent with the hypothesis that cAMP elevation in PMA-stimulated cells leads to a competition of phosphorylated CREB and phosphorylated NF-κB for binding to CBP or p300 which represent the limiting factors in transcriptional activation.

4. Discussion

HIV-1 transcription is mainly regulated by the LTR region. In this study the repressive effect of cAMP on the transcriptional activity of the HIV-1 LTR was investigated and compared to effects of cAMP on virus replication.

The production of HIV-1 was studied both in primary cells and in immortalized human T cell lines. In Jurkat and HUT-78 T cell clones stably infected with HIV-1 the virus production rates were decreased up to 60% by cAMP (Fig. 1). This effect was dependent on the incubation time. No clear cAMP concentration dependence was observed. A cytotoxic effect of cAMP was ruled out using a cell viability assay (Table 1). To confirm these data in native human T cells, primary PBLs were infected with HIV-1, and again cAMP reduced HIV-1 replication. Reports on the regulation of the HIV-1 LTR activity by cAMP have yielded controversial results [32,34]. In latently infected cells of the monocyte/macrophage lineage cAMP interacting with phorbol esters causes an increase in HIV-1 LTR activity [34]. In contrast, a repressive effect of cAMP on the HIV-1 LTR was demonstrated in primary T cells by Navarro and colleagues [33]. Our data obtained in primary PBLs and human T cell lines confirm the results of Navarro et al. Thus, cAMP effects may be different in monocytes and T cells. In human PBLs and Jurkat T cells a negative effect of cAMP on a full-length HIV-1 LTR-controlled luciferase reporter construct was seen (Fig. 3A,B). The negative regulatory effect was obtained with 8-Br-cAMP, which is a membrane-permeable and non-hydrolyzable cAMP analogue. The same effect was also seen with forskolin, which activates the adenylate cyclase and thereby elevates intracellular cAMP levels (Fig. 3G). These data support an inhibitory effect of cAMP on the HIV-1 LTR activity in T cells, as also reported for dB-cAMP, another cAMP analogue, and the phosphodiesterase inhibitor rolipram [33].

The transcriptional activity of the HIV-1 LTR is critically dependent on the activation of NF-κB [49]. The HIV-1 enhancer contains overlapping binding sites for the transcription factors NF-κB, AP-2 and Ets (Fig. 2). Various minimal constructs containing the HIV-1 NF-κB sequence upstream of an HSV TK luciferase reporter gene were generated and used in transient transfection experiments (Fig. 2). Our reporter assay results show that the NF-κB sites are required not only for the activation of the HIV-1 LTR, but also for the repression by cAMP. A single HIV-1 NF-κB sequence is sufficient to mediate the stimulatory effect of PMA, as well as the repressive effect of cAMP (Fig. 3D).

Perkins et al. demonstrated mutually exclusive in vitro binding of NF-κB and AP-2 to the HIV-1 enhancer region [10]. They explained this by overlapping recognition sites for these transcription factors (see Fig. 2). Interestingly, the transcription factor AP-2 can be induced in response to both cAMP and phorbol esters [39,40]. Therefore the negative regulatory effect of cAMP on the HIV-1 LTR could potentially result from competitive binding of AP-2 (activated by cAMP) instead of NF-κB (as a downstream signalling factor of PMA) to the HIV-1 enhancer region. According to this hypothesis HIV-1 LTR activity should decrease with increased binding of AP-2 instead of NF-κB. To examine this hypothesis an HIV-AB trimer/AP-2m luciferase construct was generated (according to [10]; Fig. 2) and compared with an HIV-AB trimer construct. Surprisingly, the mutation of the AP-2 binding site did not abolish the repressive effect of cAMP. Comparable results were obtained using an HIV-AB monomer/AP-2m luciferase construct (Fig. 4). These data suggest that the core AP-2 site between the two κ elements may be important for the full transcriptional activity of the HIV-1 enhancer, but is not required for the repressive mechanism of cAMP, at least in this model system.

Parry and Mackman proposed that activation of cAMP-dependent PKA may inhibit NF-κB-mediated transcription by phosphorylation of proteins, e.g. CREB, which can bind to CBP only after PKA phosphorylation and which then competes with phosphorylated p65 for limiting amounts of the co-activator CBP [48]. CBP is a ubiquitously expressed nuclear phosphoprotein that functions as a co-activator by linking several transcriptional activators, each activated by different agonists, to the basal transcriptional machinery [35,41,42]. In this context it is of interest that a protein–protein interaction between Ets-1 and CBP has been reported [47]. To confirm this 'CBP competition model', Jurkat T cell lines were stably transfected with expression constructs for CBP or p300. With overexpression of CBP or p300 these proteins should no longer be limiting and hence the cAMP repressive effect should be lost. This is in fact what the experiments show (Fig. 5).

In summary, a negative effect of cAMP on HIV-1 replication and on the transcriptional activity of the HIV-1 LTR could be shown for primary human PBLs and human T cell lines. The transcription factor AP-2 does not appear to be involved in repression of HIV-1 enhancer activity by cAMP. The observed inhibitory effect of cAMP may be explained by activation of CREB protein which competes with phosphorylated NF-κB and Ets-1 proteins for limiting amounts of CBP/p300.

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