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Activation of NF-κB by cAMP in human myeloid cells

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Nuclear factor kappa B (NF-κB) is a DNA-binding regulatory factor that controls the transcription of a number of genes. Various agents are known to activate this factor. We have studied the ability of cAMP to stimulate NF-κB activity in human myeloid cells. Electrophoretic mobility assay revealed that structural cAMP analogs and agents elevating intracellular cAMP levels induced NF-κB DNA-binding activity. The inducibility was dependent on the maturation stage of myeloid cells. In promyelocytic HL-60 cells cAMP induced higher NF-κB activity than in more differential THP-1 cells and in human monocytes. By transfecting HL-60 and THP-1 cells with reporter constructs containing NF-κB DNA-binding sites, we observed that cAMP-induced NF-κB was transcriptionally active.

Protein kinase C; Cyclic AMP; NF-κB; Myeloid cell; Human

1. INTRODUCTION

Transcription of eukaryotic genes is regulated by a number of nuclear factors that bind to specific DNA sequences. Some nuclear factors are present in all tissues as a part of the general transcription machinery. On the other hand, another group of transcription factors are induced after specific stimuli in a tissue-specific manner. Nuclear factor kappa B (NF-κB) is one of the transcription factors which is inducible by a variety of stimuli in different cell types [1,2]. NF-κB is typically described as a complex of two proteins with molecular sizes of 50 and 65 kDa. It has now been shown that the subunits of NF-κB share significant homology with the proto-oncogene c-*rel* [3–5]. Both p50 and p65 can interact with related DNA sequences as a homo- or heterodimer, and in addition the product of proto-oncogene c-*rel* can bind to similar DNA motifs by itself or as a heterodimer with p50 or p65 [6–8]. In unstimulated cells a cytoplasmic inhibitor protein (IκB) interacts with NF-κB, thereby inhibiting its activity [9]. After cellular activation NF-κB is released from IκB and translocated to the nucleus, where it binds to the κB motif (5′-GGGACTTTCC-3′) or related DNA sequences. Recently several IκB molecules have been described [10–13]. These molecules specifically interact with p50, p65 or *rel*-related proteins.

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Abbreviations: CAT, chloramphenicol acetyltransferase (EC 2.3.1.28); BrcAMP, 8-bromoadenosine 3′:5′-cyclic monophosphate; Bt_2cAMP, N_6-2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate; EMSA, electrophoretic shift assay; IBMX, 3-isobutyl-1-methyl-xanthine; LPS, bacterial lipopolysaccharide; PKA, protein kinase A; PKC, protein kinase C.

Various agents are known to activate NF-κB, including mitogens, cytokines, viruses and agents provoking oxidative stress [2]. In the present report, we have evaluated the ability of cAMP to stimulate NF-κB activity in human myeloid cells. HL-60 cells are from the promyelocytic stage of differentiation and THP-1 cells resemble human monocytes. Our findings show that in human promyelocytic HL-60 cells, the cAMP analogs and agents elevating intracellular cAMP levels induce a significant NF-κB activity, but that in more mature THP-1 cells and human monocytes NF-κB activity is poorly induced.

2. MATERIALS AND METHODS

2.1. *Cell cultures*

Leucocyte-rich buffy coats were obtained from the Finnish Red Cross Blood Transfusion Service (Helsinki, Finland). Mononuclear cells were isolated from buffy coats by Ficoll-Isopaque centrifugation (Pharmacia, Uppsala, Sweden) and resuspended in RPMI-1640 medium (Flow Laboratories, Irvine, Scotland, UK) containing 10% human AB serum (Finnish Red Cross Blood Transfusion Service), 10 mM HEPES, 2 mM L-glutamine and antibiotics. After 1 h of culture in Petri dishes (Nunc, Roskilde, Denmark) non-adherent cells were removed by vigorous pipetting with warm RPMI-1640 medium containing 20 mM HEPES. To avoid inter-individual variations between the buffy coats, each experimental group consisted of cells from 5 buffy coats.

The HL-60 and THP-1 cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in RPMI 1640 medium containing 10 mM HEPES, 2 mM L-glutamine, antibiotics and 10% fetal calf serum (Gibco Life Technologies Ltd., Paisley, Scotland, UK). THP-1 medium also contained 70 μM 2-mercaptoethanol. The cell cultures were stimulated with N_6-2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (Bt_2cAMP), 8-bromoadenosine 3′:5′-cyclic monophosphate (BrcAMP), forskolin or 3-isobutyl-1-methyl-xanthine (IBMX), which were all from Sigma Chemical Co. (St. Louis, MO, USA) or with bacterial lipopolysaccharide (LPS, *E. coli* 026:B6; Difco, Detroit, MI, USA).

2.2. *Electrophoretic mobility shift assay*

Nuclear extracts were prepared as previously described [14,15]. Nuclear protein–DNA-binding reactions were carried out in a volume of 20 μl containing 5 μg nuclear extract protein, 10 mM Tris-HCl (pH 7.5), 40 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 2 μg of Poly(dI-dC) as a non-specific competitor and 10 fmol of 5′-labelled double-stranded 22-mer oligonucleotide containing the NF-κB DNA-binding site (Promega, Madison, WI). The binding reaction was performed at room temperature for 30 min. Samples were analyzed by electrophoresis on 4% non-denaturing low-ionic strength polyacrylamide gels in 0.25 × TBE (22 mM Tris-borate, 22 mM boric acid, 0.5 mM EDTA). Gels were dried, visualized by autoradiography and analyzed by laser densitometry.

2.3. *Transfections and assay of CAT activity*

pκB/TK5-CAT and pκB/TK-10-CAT were a generous gift of Dr. C.V. Jongeneel, Ludwig Institute, Epalinges, Switzerland [16]. Plasmid DNA was introduced into HL-60 and THP-1 cells by electroporation (960 μF, 300 V) as previously described [17]. Immediately after transfection, the cells were stimulated. 16 h after stimulation, the cells were harvested for CAT assay. The cells were washed twice in PBS and resuspended in 0.25 M Tris-HCl, pH 7.8. Cell extracts were prepared by four cycles of freezing and thawing. The CAT reaction was performed and the samples were developed on a silica gel 60 thin-layer chromatography (TLC) plate (Merck, Darmstadt, Germany), visualized by autoradiography and analyzed by laser densitometry.

3. RESULTS

3.1. *Activation of NF-κB DNA-binding activity by Bt_2cAMP*

To determine whether the membrane-permeable structural cAMP analog, Bt_2cAMP, has an effect of NF-κB DNA-binding activity in human myeloid cells, nuclear proteins were extracted from untreated cells and cells treated with 1 mM Bt_2cAMP and DNA-binding activity was studied by EMSA (Fig. 1). In promyelocytic HL-60 cells NF-κB DNA-binding activity increased time-dependently (Fig. 1A). The increased NF-κB DNA-binding activity becomes detectable in nuclear extracts of HL-60 cells after 3 h exposure to 1 mM Bt_2cAMP. An increase of approximately 3-fold was seen within 6 h of stimulation. In contrast, in the more mature monocytic THP-1 cells and human mon-

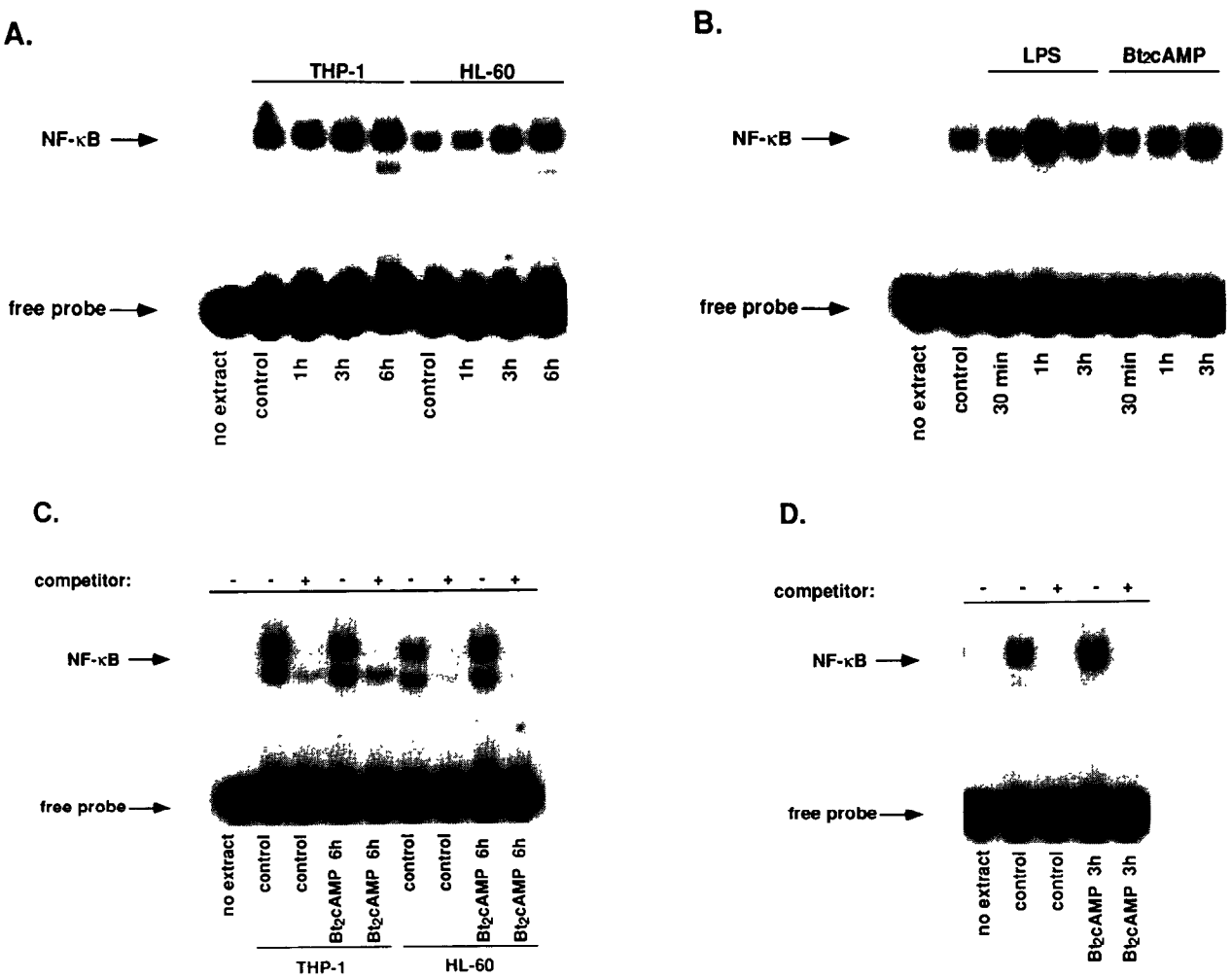

Fig. 1. Time-course for the induction of NF-κB binding activity in myeloid cells. Nuclear extracts were prepared from THP-1 cells (A), HL-60 cells (A) or human monocytes (B) treated with culture medium alone (referred to as control), Bt_2cAMP (1 mM) or LPS (10 μg/ml) for indicated periods. The specificity of binding in THP-1 and HL-60 cells (C) and in human monocytes (D) was determined by competition with 200-fold molar excess of unlabelled double-stranded oligonucleotide.

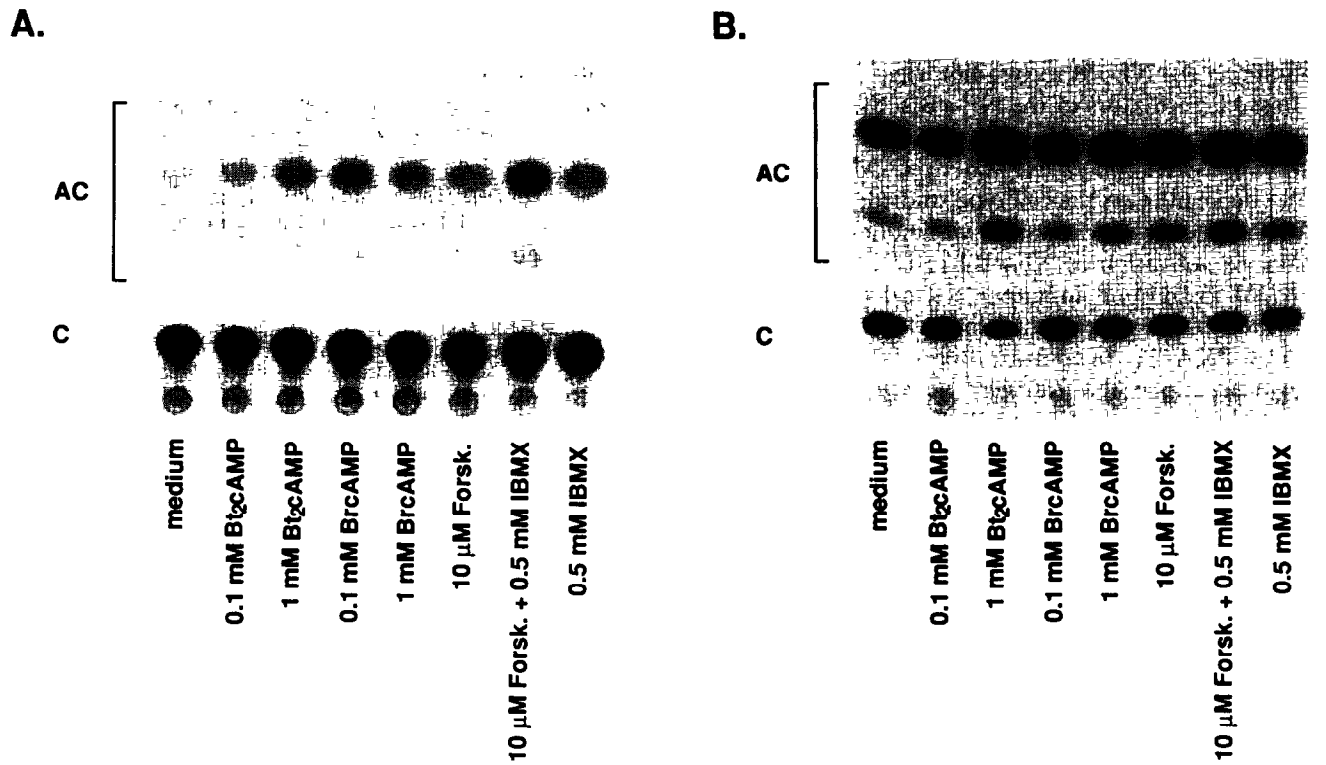

Fig. 2. Activation of the NF-κB enhancer. THP-1 (A) and HL-60 cells (B) were transfected with pκB/TK5 plasmid by electroporation. After transfection cells were cultured with or without Bt_2cAMP (0.1 mM/1 mM), BrcAMP (0.1 mM/1 mM), forskolin (Forsk.) (10 μM) or IBMX (0.5 mM) for 16 h. CAT activity was assayed by thin-layer chromatography. Representative data of three independent experiments are shown. The unreacted substrate is labeled as C for chloramphenicol and the products are labeled as AC for acetylated chloramphenicols.

ocytes 1 mM Bt_2cAMP induced only a slight increase in NF-κB DNA-binding activity (Fig. 1A and B). Our studies are in accordance with previous studies showing that the basal activity level of NF-κB is high in mature monocytic cells and in adherent human monocytes [18,19]. We also found some basal activity in HL-60 cells but it was lower compared to the more mature cells. The appearance of the increased NF-κB DNA-binding activity in the nuclei of human monocytes after Bt_2cAMP treatment was slower compared to LPS-induced NF-κB activity, which was detectable already after 1 h treatment (Fig. 1B). The specificity of Bt_2cAMP-induced NF-κB DNA-binding activity was confirmed by competion of the DNA binding with a 200 molar excess of unlabelled NF-κB oligonucleotide (Fig. 1C and D).

3.2. *Effect of Bt_2cAMP on transcription mediated by NF-κB*

To investigate the role of elevated intracellular cAMP levels on transcriptional responses mediated by NF-κB, we used CAT reporter constructs, pκB/TK10 and pκB/TK5 contains two and six copies of NF-κB binding sites of mouse TNF-α κB enhancer, respectively, inserted in the pBLCAT2 expression vector [16]. The pκB/TK5 construct was transfected into HL-60 and THP-1 cells by electroporation. Immediately after transfection the cells were treated with agents increasing the intracellular cAMP levels (Fig. 2). In HL-60 cells a 4-fold increase in NF-κB enhancer activity was seen after 1 mM Bt_2cAMP treatment (Fig. 2A), whereas in THP-1 cells the increase was 2-fold (Fig. 2B). A similar, although weaker, effect was also seen with another cAMP analog, BrcAMP, or when the endogenous cAMP levels was increased with 10 μM forskolin, which directly stimulates adenylate/cyclase. Moreover, the forskolin-induced NF-κB enhancer activity was enhanced by 0.5 mM IBMX, which indirectly elevates cAMP levels by inhibiting the cAMP-directed phosphodiesterase. In addition, in HL-60 cells IBMX alone was able to induce some NF-κB enhancer activity. These results are in accordance with the EMSA data showing that in immature myeloid cells NF-κB activity is more inducible than in more mature cells. The number of NF-κB binding sites did not have any effect on the NF-κB enhancer activity, since the same cAMP effect was seen with the reporter construct, pκB/TK10, which contains two NF-κB binding sites (data not shown).

4. DISCUSSION

In the present study we have shown that the increased intracellular cAMP levels stimulate NF-κB DNA-binding activity in human myeloid cells. In addition, tran-

sient transfection indicated that the enhanced binding activity leads to functional activation of a promoter containing the NF-κB recognition site.

Possible mediators of the NF-κB activation are protein kinases, protein phosphatases and oxygen radicals [10]. The activation of NF-κB by cAMP appears to differ depending on the cell type studied. In pre-B-cells cAMP induced the synthesis of κ immunoglobulin light-chain via activation of NF-κB-like DNA-binding protein [20]. In addition, in human NK-cells NF-κB can be activated by treating cytoplasmic extracts with either PKC or PKA [21]. In contrast, Bomsztyk et al. [22] have previously shown that transient elevation of intracellular cAMP by Bt_2cAMP and PGE_2 is not sufficient to activate NF-κB in the murine pre-B-cell line.

The findings presented in this study show that the inducibility of NF-κB DNA-binding activity by cAMP is dependent on the maturation stage of myeloid cells. In the human promyelocytic HL-60 cells the cAMP analogs and the agents elevating the intracellular cAMP levels induced a significant NF-κB activity, but in more mature THP-1 cells and in human monocytes, NF-κB activity was poorly induced. Previously Griffin et al. [18] have shown that NF-κB DNA-binding activity is developmentally regulated in the monocyte lineage. In the immature cell lines NF-κB was inducible by phorbol esters, which causes the differentiation into mature monocytes and macrophages, whereas in the mature monocytic cell lines and in human monocytes NF-κB was constitutively expressed.

The different kinetics of DNA-binding observed after LPS and Bt_2cAMP treatments in human monocytes indicated that, despite the ubiquitous nature of NF-κB, its activity is differentally regulated depending on the activation pathway. A crucial step in the activation of NF-κB is the inactivation of IκB, possibly by phosphorylation. Recently Mufson et al. [19] have demonstrated that a basal PKC activity is needed for a constitutive NF-κB activity. Phorbol ester treatment leads to the down-regulation of PKC and to the disappearance of constitutive NF-κB activity in immature and mature myeloid cells. The findings presented in this study suggest that the cAMP-dependent activation of NF-κB differs from that induced by PKC, since the prolonged elevation of intracellular cAMP caused an increase in NF-κB activity, even in mature monocytes.

IκB has been shown to be a substrate of PKC, whereas PKA does not phosphorylate IκB [23]. The members of the NF-κB family have a potential phosphorylation site (Arg-Arg-X-Ser) in a region containing a DNA-binding and a dimerization domain [24]. Rel protein is partly induced to migrate to the nucleus by phosphorylation at the conserved PKA site [25]. According to our studies and those of others it can be proposed that PKA mediates the activation of NF-κB by phosphorylating the components of NF-κB.

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REFERENCES

[1] Lenardo, M.J. and Baltimore, D. (1989) Cell 58, 227–229.
[2] Baeuerle, P.A. (1991) Biochim. Biophys. Acta 1072, 63–80.
[3] Bours, V., Villalobos, J., Burd, P.R., Kelly, K. and Siebenlist, U. (1990) Nature 348, 76–80.
[4] Ghosh, S., Gifford, A.M., Riviere, L.R., Tempst, P., Nolan, G.P. and Baltimore, D. (1990) Cell 62, 1019–1029.
[5] Kieran, M., Blank, V., Logeat, F., Vandekerckhove, J., Lottspeich, F., Le Bail, O., Urban, M.B., Kourilsky, P., Baeuerle, P.A. and Israël, A. (1990) Cell 62, 1007–1018.
[6] Nolan, G.P., Ghosh, S., Liou, H.-C., Tempst, P. and Baltimore, D. (1991) Cell 64, 961–969.
[7] Ruben, S.M., Dillon, P.J., Schreck, R., Henkel, T., Chen, C.-H., Maher, M., Baeuerle, P.A. and Rosen, C.A. (1991) Science 251, 1490–1493.
[8] Urban, M.B., Schreck, R. and Baeuerle, P.A. (1991) EMBO J. 10, 1817–1825.
[9] Baeuerle, P.A. and Baltimore, D. (1988) Science 242, 540–546.
[10] Schmitz, M.L., Henkel, T. and Baeuerle, P.A. (1991) Trends Cell Biol. 1, 130–137.
[11] Beg, A.A., Ruben, S.M., Scheinman, R.I., Haskill, S., Rosen, C.A. and Baldwin Jr., A.S. (1992) Genes Dev. 6, 1899–1913.
[12] Naumann, M., Wulczyn, F.G. and Scheidereit, C. (1993) EMBO J. 12, 213–222.
[13] Zabel, U., Henkel, T., dos Santos Silva, M. and Baeuerle, P.A. (1993) EMBO J. 12, 201–211.
[14] Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11, 1475–1489.
[15] Prywes, R. and Roeder, G.R. (1986) Cell 47, 777–784.
[16] Shakhov, A.N., Collart, M.A., Vassalli, P., Nedospasov, S.A. and Jongeneel, C.V. (1990) J. Exp. Med. 171, 35–47.
[17] Pahl, H.L., Burn, T.C. and Tenen, D.G. (1991) Exp. Hematol. 19, 1038–1041.
[18] Griffin, G.E., Leung, K., Folks, T.M., Kunkel, S. and Nabel, G.J. (1989) Nature 339, 70–73.
[19] Mufson, R.A., Myers, C., Turpin, J.A. and Meltzer, M. (1992) J. Leuk. Biol 52, 637–644.
[20] Shirakawa, F., Chedid, M., Suttles, J., Pollok, B.A. and Mizel, S.B. (1989) Mol. Cell. Biol. 9, 959–964.
[21] Shirakawa, F. and Mizel, S.B. (1989) Mol. Cell. Biol. 9, 2424–2430.
[22] Bomsztyk, K., Toivola, B., Emery, D.W., Rooney, J.W., Dower, S.K., Rachie, N.A. and Hopkins-Sibley, C. (1990) J. Biol. Chem. 265, 9413–9417.
[23] Ghosh, S. and Baltimore, D. (1990) Nature 344, 678–682.
[24] Blank, V., Kourilsky, P. and Israël, A. (1992) Trends Biochem. Sci. 17, 135–140.
[25] Mosialos, G., Hamer, P., Capobianco, A.J., Laursen, R.A. and Gilmore, T.D. (1991) Mol. Cell. Biol. 11, 5867–5877.