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Involvement of Cyclic AMP-Dependent Protein Kinases in the Signal Transduction Pathway for Interleukin-1

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Expression of a highly specific protein inhibitor for cyclic AMP-dependent protein kinases in interleukin-1 (IL-1)-responsive cells blocked IL-1-induced gene transcription that was driven by the κ immunoglobulin enhancer or the human immunodeficiency virus long terminal repeat. This inhibitor did not affect protein kinase C-mediated gene transcription, suggesting that cyclic AMP-dependent protein kinases are involved in the signal transduction pathway for IL-1 in a number of responsive cell types.

Cyclic AMP (cAMP) is an important intracellular second messenger in the induction of gene transcription by several hormones (1, 7, 10, 11, 14, 16, 21–23). Mellon et al. (9) demonstrated that overexpression of the catalytic subunit of protein kinase A (PKA) can induce the expression of cAMP-responsive genes, supporting the concept that phosphorylation is a required event in cAMP-mediated gene expression. In several instances, the linkage between cAMP, PKA, and activation of specific genes is mediated via one or more DNA-binding proteins that recognize a conserved cAMP-responsive element (10, 11, 14, 22). For example, Montminy and Bilezikjian (10) have characterized a 43,000-molecular-weight phosphoprotein which binds with high affinity to the cAMP-responsive element of the rat somatostatin gene.

Whitehouse and Walsh (23) have characterized and purified a heat-stable skeletal muscle protein that specifically inhibits cAMP-dependent protein kinases but that has no effect on cyclic GMP-dependent protein kinases or protein kinase C (PKC). This inhibitory protein (termed PKI) is a high-affinity inhibitor that competes with substrates for the binding to the catalytic subunit of PKA. Based on the amino acid sequence of this inhibitor (16), Grove et al. (6) generated an expression plasmid containing the cDNA that encodes the active portion of the skeletal muscle protein inhibitor (the N-terminal 31 amino acids). Transfection of this recombinant inhibitor into cells specifically decreased the cAMP-induced expression of a cotransfected reporter gene.

In previous reports we have demonstrated that interleukin-1 (IL-1), a potent mediator of immune and inflammatory responses, stimulates the production of cAMP in a variety of responsive cells (4, 20). We also found that several IL-1-mediated responses such as interleukin-2 (IL-2) receptor α expression, induction of κ immunoglobulin light-chain synthesis, and stimulation of thymocyte proliferation are mimicked by cAMP analogs and cAMP-elevating drugs (4, 20). The IL-1 receptor is coupled to adenylate cyclase via a pertussis toxin-sensitive, adenylate cyclase-stimulatory G protein (4). In addition, IL-1 (15, 18, 19) and cAMP analogs (18, 19) can induce the activation of NF-κB, a DNA-binding protein that exhibits specificity for DNA sequences present in the regulatory regions of several genes, including the IL-2 receptor α-subunit gene, κ immunoglobulin light-chain gene, and the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (2, 5, 8, 12, 13, 18). Furthermore, we have also found that PKA, as well as PKC, can induce the in vitro activation of NF-κB as well as its translocation into isolated nuclei (19). Although these in vitro observations are consistent with a role for PKA in IL-1 signal transduction in vivo, it was essential to demonstrate this point with intact cells and a highly specific method for inhibiting the activity of PKA.

Construction and expression of PKA inhibitor oligonucleotide. An approach similar to the strategy of Grove et al. (6) was used to investigate the involvement of PKA in the signal transduction pathway for IL-1. A cDNA fragment encoding the N-terminal 31 amino acids of the PKA inhibitor described by Scott et al. (16) was prepared by first synthesizing two sets of oligonucleotides, 74 (A) and 72 (B) base pairs in length, that contained a *Bam*HI (A) or *Bgl*II (B) restriction site. The individual oligonucleotides of each set were purified and annealed. The oligonucleotide pairs were digested with *Sfi*I (to create the appropriate cohesive ends for ligation of the A and B oligonucleotides) and ligated. The ligated oligonucleotide was then digested with *Bam*HI and *Bgl*II and cloned into the *Bgl*II site of pSV2neo (pSV2neo-PKI). Following transformation of *Escherichia coli* MM294, pSV2neo-PKI was purified and digested with *Nar*I, and the insert ends were filled in by using the Klenow fragment of DNA polymerase I. *Bgl*II linkers were blunt end ligated to the insert followed by digestion with *Bgl*II. A 197-base-pair insert was purified and cloned into the *Bcl*I site of the expression vector pSBL (3) (kindly provided by Brian Grinnel, Lilly Research Laboratories). Plasmids containing the PKA inhibitor in the sense (pSBL-PKIs) and antisense (pSBL-PKIas) orientations were used in transfection studies

Involvement of PKA in the signal transduction pathway for IL-1. Our initial experiments focused on the role played by PKA in the activation of the κ immunoglobulin gene enhancer in the pre-B-cell line 70Z/3. Cells were transfected by the DEAE-dextran method with EκCAT (a plasmid that contains the Vκ21E gene enhancer and promoter sequences linked to the reporter gene choramphenicol acetyltransferase [CAT]) (2) and cultured for 48 h in the presence of different stimuli. In confirmation of our earlier work (18), we found that IL-1 and forskolin, a potent activator of adenylate cyclase, stimulated an increase in CAT activity (Fig. 1A). In addition, phorbol myristate acetate (PMA), a PKC activator, also promoted an increase in CAT activity. No CAT activity was detected when 70Z/3 cells were transfected with an enhancer-lacking construct (E-CAT) and stimulated with

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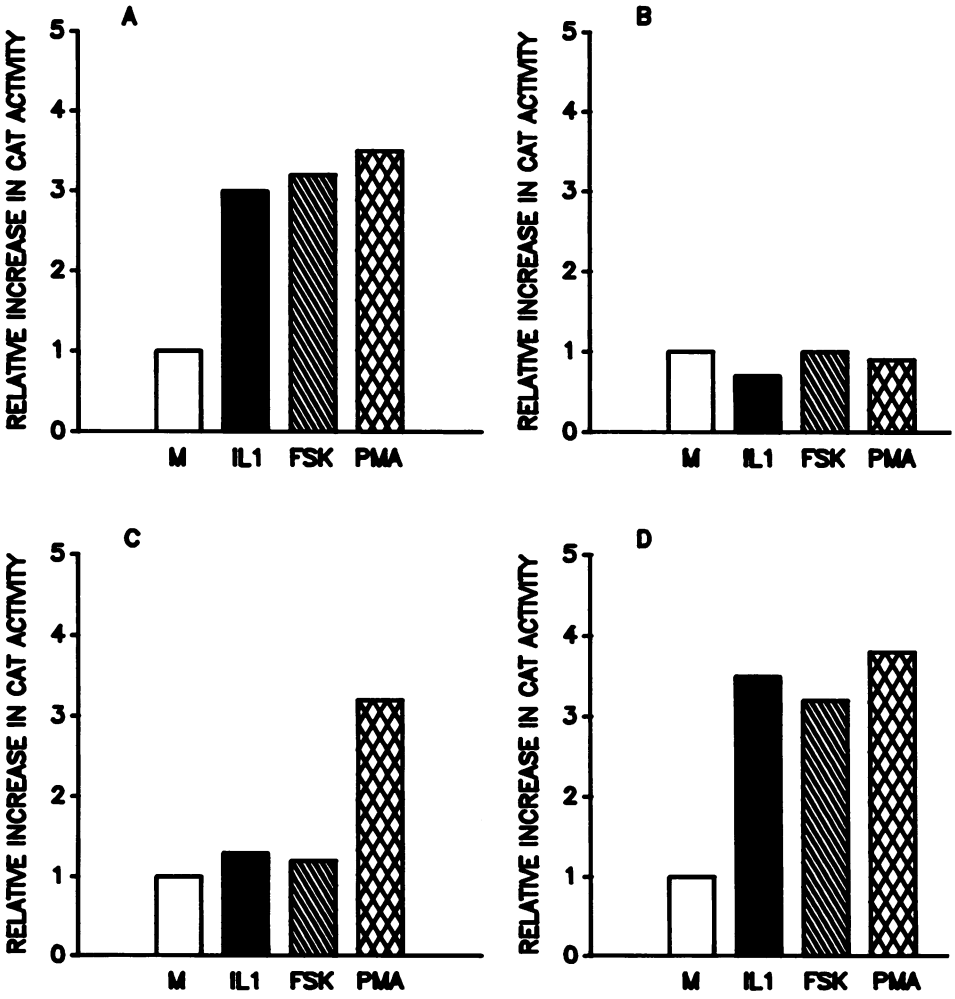

FIG. 1. Effect of PKA inhibitor protein expression on IL-1-induced gene transcription in 70Z/3 cells. (A and B) 70Z/3 cells (4×10^7) were transfected with 10 μg of the EκCAT plasmid (A) or E-CAT (B) and an equal amount of the carrier plasmid pBR322 by the DEAE-dextran method as described previously (19). After transfection, cells were cultured for 48 h with medium only, 2×10^{-11} M human IL-1α, 3 μM forskolin, or 50 ng of PMA per ml. (C and D) 70Z/3 cells (4×10^7) were cotransfected with equal amounts (10 μg of each plasmid DNA) of EκCAT and pSBL-PKIs (C) or EκCAT and pSBL-PKIas (D) by the DEAE-dextran method. After transfection, cells were cultured with medium only, 2×10^{-11} M human IL-1α, 3 μM forskolin, or 50 ng of PMA per ml for 48 h. CAT activity was assayed by thin-layer chromatography. Acetylated forms of chloramphenicol were detected by scanning the plates with a radioanalytic imaging system (Ambis Systems, San Diego, Calif.). The relative increase in CAT activity in stimulated cultures was calculated relative to the CAT activity in cultures incubated in medium only. The results presented here are from a single representative experiment. M, Medium; IL-1, interleukin-1; FSK, forskolin; PMA, phorbol myristate acetate. The results presented here are representative of the results obtained in several identical experiments.

IL-1, forskolin, or PMA (Fig. 1B). In order to investigate the participation of PKA in the activation of the κ enhancer, 70Z/3 cells were cotransfected with EκCAT and pSBL-PKIs plasmids and cultured for 48 h in the presence of IL-1 or the cAMP-inducing agent forskolin. As shown in Fig. 1C, the ability of IL-1 to induce gene transcription was abolished in cells transfected with pSBL-PKIs. However, when 70Z/3 cells cotransfected with EκCAT and pSBL-PKIs were stimulated with PMA, CAT activity was observed. These results indicated that the PKI was not acting in a nonspecific manner, since PKC-mediated responses were unaffected (compare Fig. 1A and C). When 70Z/3 cells were cotransfected with EκCAT and pSBL containing the PKA inhibitor insert in the antisense orientation (pSBL-PKIas), no significant inhibitory activity was observed (Fig. 1D). Cotransfection of 70Z/3 cells with pSBL-PKIs or pSBL-PKIas and pRSV-CAT did not result in a level of CAT activity that was different from that obtained with cells transfected with only pRSV-CAT (data not shown). Taken together these results indicate that cAMP-dependent protein kinase(s) is involved in the in vivo activation of the κ enhancer in response to IL-1.

We extended these studies to two other IL-1-responsive cell lines, the human natural killer cell line YT that is induced by IL-1 to express the IL-2 receptor α subunit (20) and the murine thymoma cell line EL 4 6.1 C10 that produces IL-2 in response to IL-1 (14). In YT cells, transcriptional activation of HIV-1 has been shown to be mediated by NF-κB (14). When YT (Fig. 2A) and EL 4 (Fig. 3A) cells were transfected with HIV-1–CAT, IL-1 and PMA independently induced CAT expression. It should be noted, however, that PMA and IL-1 did not act synergistically when

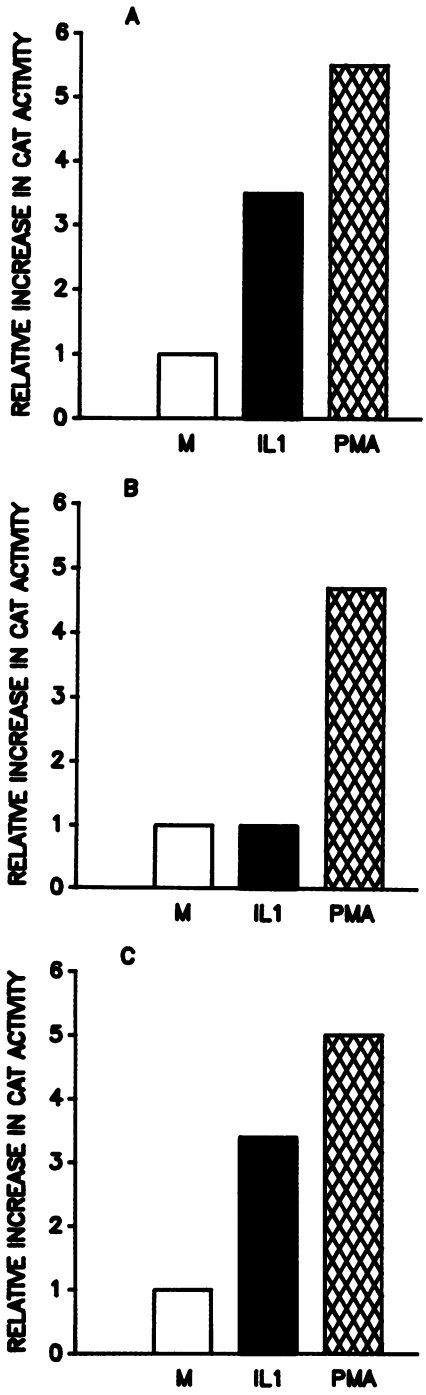

FIG. 2. Effect of PKA inhibitor protein expression on IL-1-induced gene transcription in YT cells. (A) YT cells (1.4×10^6) were transfected with 1.4 μg of plasmid HIV-1–CAT and an equal amount of the carrier plasmid pBR322 and cultured for 48 h in the presence of medium, 2×10^{-11} M human IL-1α, or 10 ng of PMA per ml. (B) A second sample of YT cells (1.4×10^6) was cotransfected with equal amounts of HIV-1–CAT and pSBL-PKIs plasmids (1.4 μg of DNA from each plasmid) and cultured for 48h in the presence of medium, human IL-1-α, or PMA (same concentrations as given above). (C) cells (1.4×10^6) cells were also cotransfected with equal amounts of HIV-1–CAT and pSBL-PKIas (1.4 μg of DNA from each plasmid) and cultured as described above. The results presented here are representative of the results obtained in several identical experiments. M, Medium.

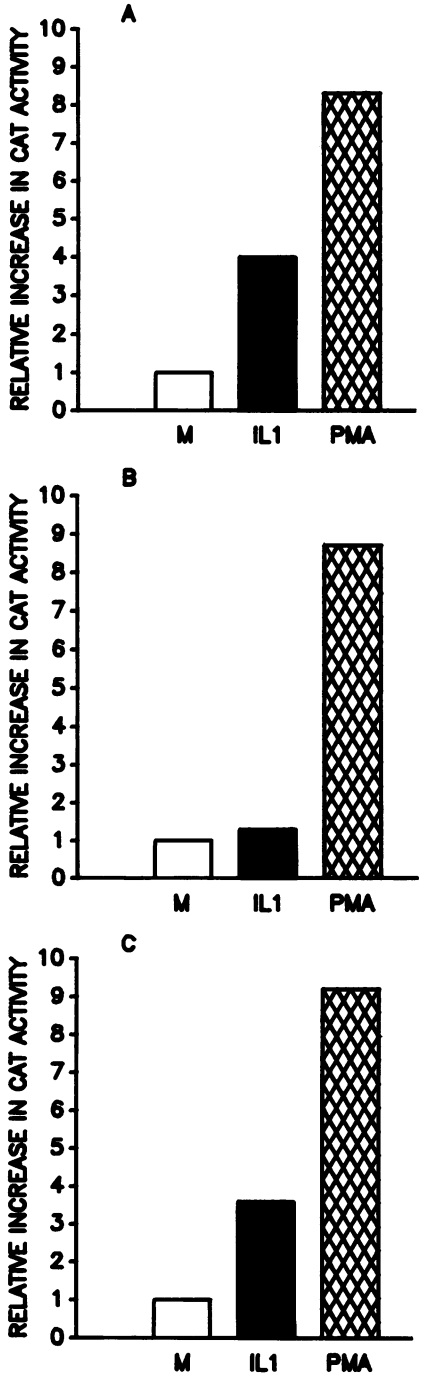

FIG. 3. Effect of PKA inhibitor protein expression on IL-1-induced gene transcription in EL 4 6.1 C10 cells. (A) EL 4 6.1 C10 cells (1×10^7) were transfected with 10 μg of plasmid HIV-1–CAT and an equal amount of the carrier plasmid pBR322 and cultured for 48 h in the presence of medium, 2×10^{-11} M human IL-1α, or 10 ng of PMA per ml. (B) A second sample of EL 4 6.1 C10 cells (10^7) was cotransfected with equal amounts of HIV-1–CAT and pSBL-PKIs (10 μg of DNA from each plasmid) and cultured for 48 h in the presence of medium, human IL-1α, or PMA (same concentrations as given above). (C) EL 4 6.1 C10 cells (10^7) were also cotransfected with equal amounts of HIV-1–CAT and pSBL-PKIas (10 μg of DNA from each plasmid) and cultured as described above. The results presented here are representative of the results obtained in several identical experiments. M, Medium.

they were added together to cultures of HIV-1–CAT-transfected cells (data not shown). When the cell lines were cotransfected with HIV-1–CAT and pSBL-PKIs and stimulated with IL-1, CAT activity was markedly reduced (Fig. 2B and 3B), suggesting that PKA is involved in the activation of the HIV-1 long terminal repeat by IL-1. In contrast, when YT and EL 4 cells cotransfected with HIV-1–CAT and pSBL-PKIs were stimulated with PMA, CAT activity did not significantly differ from that seen with cells transfected with HIV-1–CAT only (Fig. 2B and 3B). As would be expected, pSBL-PKIas had no effect on IL-1 activity (Fig. 2C and 3C). The presence of pSBL-PKIs or pSBL-PKIas in the cells did not affect CAT expression from the pRSV-CAT plasmid (data not shown). These results suggest that in the pre-B-cell line 70Z/3, the natural killer cell line YT, and the T-cell line EL 4 6.1 C.10, PKA is involved in a signal transduction pathway for IL-1 that is linked to the activation of gene transcription. On the basis of our previous findings (19), it is quite likely that PKA is directly involved in the activation of specific transcriptional factors such as NF-κB.

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LITERATURE CITED

1. **Akerblom, I. E., E. P. Slater, M. Beato, J. D. Baxter, and P. L. Mellon.** 1988. Negative regulation by glucocorticoids through interference with a cAMP responsive enhancer. Science **241:** 350–353.
2. **Atchinson, M. L., and R. P. Perry.** 1987. The role of the kappa enhancer and its binding factor NF-κB in the developmental regulation of kappa gene transcription. Cell **48:**121–128.
3. **Berg, D. T., P. Q. Mooney, M. Baez, and B. W. Grinnell.** 1988. Tandem promoter/enhancer units create a versatile regulatory element for the expression of genes in mammalian cells. Nucleic Acids Res. **16:**1635.
4. **Chedid, M., F. Shirakawa, P. Naylor, and S. B. Mizel.** 1989. Signal transduction pathway for IL-1. Involvement of a pertussis toxin-sensitive GTP-binding protein in the activation of adenylate cyclase. J. Immunol. **142:**4301–4306.
5. **Cross, S. L., N. F. Holden, M. J. Lenardo, and W. J. Leonard.** 1989. Functionally distinct NF-κB binding sites in the immunoglobulin and IL-2 receptor α chain genes. Science **244:**466–469.
6. **Grove, J. R., D. J. Price, H. M. Goodman, and J. Avruch.** 1987. Recombinant fragment of the protein kinase inhibitor blocks cyclic AMP-dependent gene transcription. Science **238:**530–533.
7. **Hansen, C., F. Javaux, G. Juvenal, G. Vassart, and D. Christophe.** 1989. cAMP-dependent binding of a trans-acting factor to the thyroglobulin promoter. Biochem. Biophys. Res. Commun. **160:**722–731.
8. **Lenardo, M. J., and D. Baltimore.** 1989. NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell **58:**227–229.
9. **Mellon, P. L., C. H. Clegg, L. A. Correl, and G. S. McKnight.** 1989. Regulation of trancription by cyclic AMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA **86:**4887–4891.
10. **Montminy, M. R., and L. M. Bilezikjian.** 1987. Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature (London) **328:**175–178.
11. **Montminy, M. R., K. A. Sevarino, J. A. Wagner, G. Mandel, and R. H. Goodman.** 1986. Identification of a cyclic-AMP responsive element within the rat somatostatin gene. Proc. Natl. Acad. Sci. USA **83:**6682–6686.
12. **Nabel, G. J., and D. Baltimore.** 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (London) **326:**711–713.
13. **Nilson, J. H., J. A. Bokar, B. Andersen, R. Bohinski, G. Kennedy, R. A. Keri, T. A. Farmerie, and R. A. Fenstermaker.** 1989. CRE-binding proteins interact cooperatively to enhance placental-specific expression of the glycoprotein hormone alpha-subunit gene. Ann. N.Y. Acad. Sci. **564:**77–85.
14. **Osborn, L., S. Kunkel, and G. J. Nabel.** 1989. Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. Proc. Natl. Acad. Sci. USA **86:**2336–2340.
15. **Roesler, W. J., G. R. Vandenbark, and R. W. Hanson.** 1988, Cyclic AMP and the induction of eukaryotic gene transcription. J. Biol. Chem. **263:**9063–9066.
16. **Scott, J. D., E. H. Fischer, K. Takio, J. G. Damaille, and E. G. Krebs.** 1989. Amino acid sequence of the heat-stable inhibitor of the cAMP-dependent protein kinase from rabbit skeletal muscle. Proc. Natl. Acad. Sci. USA **82:**5732–5736.
17. **Sen, R., and D. Baltimore.** 1986. Inducibility of kappa immunoglobulin enhancer-binding protein NF-kappa B by its posttranslational mechanism. Cell **47:**921–928.
18. **Shirakawa, F., M. Chedid, J. Suttles, B. A. Pollok, and S. B. Mizel.** 1989. Interleukin-1 and cyclic AMP induce κ immunoglobulin light-chain expression via activation of an NF-κB-like DNA binding protein. Mol. Cell. Biol. **9:**959–964.
19. **Shirakawa, F., and S. B. Mizel.** 1989. In vitro activation and nuclear translocation of NF-κB catalyzed by cyclic AMP dependent protein kinase and protein kinase C. Mol. Cell. Biol. **9:**2424–2430.
20. **Shirakawa, F. U. Yamashita, M. Chedid, and S. B. Mizel.** 1988. Cyclic AMP—an intracellular second messenger for interleukin 1. Proc. Natl. Acad. Sci. USA **85:**8201–8205.
21. **Short, J. M., A. Wynshaw-Boris, H. P. Short, and R. W. Hanson.** 1986. Characterization of the phosphoenolpyruvate carboxykinase (GTP) promoter-regulatory region. J. Biol. Chem. **261:**9721–9726.
22. **Silver, B. J., J. A. Bokar, J. B. Virgin, E. A. Vallen, A. Milstead, and J. H. Nilson.** 1987. Cyclic AMP regulation of the human glycoprotein hormone α-subunit gene is mediated by an 18 base-pair element. Proc. Natl. Acad. Sci. USA **84:**2198–2202.
23. **Whitehouse, S., and D. A. Walsh.** 1983. Inhibitor protein of the cAMP-dependent protein kinase. Characteristics and purification. Methods Enzymol. **99:**80–93.