Kappa B alpha (inhibitory kappa B alpha) identified as labile repressor of MnSOD (manganese superoxide dismutase) expression

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Abstract:

Cytokines, phorbol esters, radiation and chemotherapeutic drugs up-regulate the expression of MnSOD (manganese superoxide dismutase). Using the VA-13 cell line, we studied the regulation of SOD2 upon treatment with PMA. Pre-treatment with CHX (cycloheximide) followed by PMA led to significantly higher levels of MnSOD mRNA compared with those with either agent alone, suggesting de novo synthesis of an inhibitory protein. PMA treatment modulates redox-sensitive transcription factors, therefore we evaluated the effects of this combination treatment upon AP-1 (activator protein 1) and NF-kappaB (nuclear factor kappaB), two transacting factors suggested to play a role in SOD2 regulation. Coadministration of CHX and PMA led to a time-dependent increase in the binding activity of NF-kappaB. Therefore we evaluated IkappaBalpha (inhibitory kappaBalpha) and found that co-administration decreased its steady-state level compared with either agent alone, suggesting that enhanced NF-kappaB activation is due to inhibition of IkappaBalpha synthesis. PMA activates PKC (protein kinase C) enzymes which phosphorylate IkappaBalpha, leading to its degradation, therefore we used GF109203X to inhibit PKC activity. Stable transfection utilizing a PMA-responsive element in the human SOD2 gene, showed a concentration-dependent decrease in luciferase and NF-kappaB-binding activity with GF109203X. Western blot analysis indicated the presence of several PKC isoforms in the VA-13 cell line; however, PMA pre-treatment specifically down-regulated alpha and betaI, suggesting a role for one or more of these proteins in SOD2 induction. Taken together, these results indicate that the PKC pathway leading to SOD2 induction proceeds at least in part through NF-kappaB and that inhibition of IkappaBalpha synthesis might serve as a potential pharmacological approach to up-regulate MnSOD.

Keywords: cycloheximide; gene regulation; manganese superoxide dismutase (MnSOD); nuclear-factor-kappa B-binding protein; (NF-kappa B-binding protein); phorbol ester; protein kinase C (PKC)

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