The Effect of Sodium Salicylate and Aspirin on NF-κB

Elizabeth Kopp and Sankar Ghosh find that activation of the transcription factor nuclear factor-κB (NF-κB) is inhibited by aspirin and salicylate, which suggests an explanation for the anti-inflammatory nature of these drugs (1). Because the conclusion has significant implications for the development of novel anti-inflammatory agents, we explored the phenomenon further. We found that at concentrations required for inhibition of NF-κB-dependent transcription, sodium salicylate inhibits activation of a variety of transcription factors. This appears to result from the ability of salicylate to nonspecifically inhibit cellular kinases. Consistent with the previous report (1), we found that salicylate inhibited phorbol 12-myristate 13-acetate (PMA)/ionomycin-dependent induction of NF-κB DNA binding activity (not shown) and similarly induced transcription from an NF-κB-dependent enhancer (Fig. 1A). To ascertain the nature of this event, we examined the specificity of salicylate. Salicylate inhibited transcription from an AP-1–dependent enhancer induced by PMA/ionomycin (Fig. 1B). This effect is not secondary to the inhibition of NF-κB, as in these cells the immunosuppressive drug FK-506 also inhibits induction of NF-κB–dependent promoter activity by PMA/ionomycin, but has no inhibitory effect on induction of AP-1–dependent activity (2). As activation of NF-κB and AP-1 share the same stimuli,
they might also share a common activation pathway that might contain a component uniquely sensitive to salicylate. We therefore examined the effect of salicylate on an independent signal transduction pathway, activation of a cAMP (adenosine 3',5'-monophosphate) Responsive Element (CRE) (3).

Salicylate was as effective at inhibiting transcription from a CRE-dependent enhancer induced by dibutyryl-cAMP as it was from the other promoters tested (Fig. 1C). To our knowledge there is no specific component shared by the PMA/ionomycin- and cAMP-dependent signal transduction pathways. Salicylate affects neither transcription itself nor the translation or activity of the reporter enzyme (1). Thus, our observations suggested some general effect of salicylate on the activity of independent signal transduction pathways. These pathways are activated by numerous kinases that might all be targets of salicylate. To assess whether salicylate nonspecifically affects cellular kinases, we measured bulk transfer of phosphate from [γ-32P]ATP (adenosine triphosphate) to basic material in lysates of nonstimulated, proliferating Jurkat cells. Salicylate inhibited 80% of all kinase activity detectable in these lysates; one half of its effective dose (ED50) in this assay (2.5 to 5 mM) is identical to its ED50 on activation of DNA binding and induction of transcription (Fig. 1D).

From these results we conclude that the report by Kopp and Ghosh vastly underestimates the effects of high concentrations of salicylate on cellular physiology. The data they presented, while unquestionably accurate, do not support the contention that activation of NF-κB plays a unique role in inflammation or is a unique target of high-dose salicylate. Inhibition of NF-κB activation is not a unique effect of this anti-inflammatory agent, since other cellular events are equally sensitive to its action. Rather, salicylate given in high doses appears to exhibit nonspecific pharmacological effects on cellular kinases. Salicylate concentrations of 1 to 2 mM in serum are required to achieve an anti-inflammatory effect, and concentrations of 6.5 mM are extremely toxic (4). Because salicylate concentrations that cause nonspecific inhibition of kinase activity in vitro are close to concentrations reported to be required for both clinical effect and broad toxicity, we believe that the lack of specificity may explain both the clinical effectiveness and the toxicity of salicylate.

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REFERENCES

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Response: Frantz and O'Neill raise a concern that salicylates are not specific for the inhibition of NF-κB and instead suggest that they nonspecifically inhibit all cellular kinases. However, we find that in cells in tissue culture, 2 mM sodium salicylate (which inhibits NF-κB induction on average by 70%) has no effect on cellular viability and growth kinetics (1), indicating that even if salicylates inhibit cellular kinases, they do not affect general cellular metabolism or growth. Since NF-κB is an important transcription factor that is clearly involved in inflammation, it was reasonable to speculate that these drugs work in part by interfering with the pro-inflammatory activity of NF-κB. Our intent was not to suggest that salicylates are uniquely specific for NF-κB (it is well-known that aspirin inhibits prostaglandin production), we were merely demonstrating a possible relationship between a known inflammatory mediator, NF-κB, and a family of known anti-inflammatory drugs, the salicylates. Recently, two other groups have identified NF-κB as one of the cellular targets of another class of widely prescribed anti-inflammatory drugs, the corticosteroids (2). Like the salicylates, these drugs do not specifically target NF-κB, however, the inhibition of NF-κB is at present a persuasive explanation for their therapeutic effects in vivo.

As described in our report, the presence of salicylates blocked the degradation of IκB, which suggests that they were interfering with a component of the signaling pathway, most likely a serine-threonine kinase. Although we did not further examine the mechanism of this interference, we dispute the contention of Frantz and O'Neill that there is no precedence for the cAMP-dependent pathway and the PMA/ionomycin pathway converging with the activation of NF-κB. In Drosophila, the cAMP-dependent protein kinase activates the NF-κB homolog dorsal by phosphorylating it (3). Furthermore, the activation of NF-κB by cAMP analogs and forskolin have also been reported (4). Also, all members of the Iκ protein family share a conserved, canonical IκB site which has not yet been adequately investigated for function.

Finally, we hope that our results and the