Distinct PKC isoforms mediate the activation of cPLA2 and adenylyl cyclase by phorbol ester in RAW 264.7 macrophages

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摘要

Abstract

1.The modulatory effects of protein kinase C (PKC) on the activation of cytosolic phospholipase A2 (cPLA2) and adenylyl cyclase (AC) have recently been described. Since the signalling cascades associated with these events play critical roles in various functions of macrophages, we set out to investigate the crosstalk between PKC and the cPLA2 and AC pathways in mouse RAW 264.7 macrophages and to determine the involvement of individual PKC isoforms. The cPLA2 and AC pathways were studied by measuring the potentiation by the phorbol ester PMA of ionomycin-induced arachidonic acid (AA) release and prostagladin E1 (PGE1)-stimulated cyclic AMP production, respectively.

2.PMA at 1μM caused a significant increase in AA release both in the presence (371%) and absence (67%) of ionomycin induction, while exposure of RAW 264.7 cells to PMA increased PGE1 stimulation of cyclic AMP levels by 208%.

3.Treatment of cells with staurosporine and Ro 31-8220 inhibited the PMA-induced potentiation of both AA release and cyclic AMP accumulation, while Go 6976 (an inhibitor of classical PKC isoforms) and LY 379196 (a specific inhibitor of PKCβ) inhibited the AA response but failed to affect the enhancement of the cyclic AMP response by PMA.

4.Long term pretreatment of cells with PMA abolished the subsequent effect of PMA in potentiating AA release, but only inhibited the cyclic AMP response by 42%.

5.Neither PD 98059, an inhibitor of MEK, nor genistein, an inhibitor of tyrosine kinases, had any effect on the ability of PMA to potentiate AA or cyclic AMP production.

6.The potentiation of AA release, but not of cyclic AMP formation, by PMA was

sensitive to inhibition by wortmannin. This effect was unrelated to the inhibition of PKC activation as deduced from the translocation of PKC activity to the cell membrane.

7.Western blot analysis revealed the presence of eight PKC isoforms (α, βI, βII, δ, ε, μ λ and ξ) in RAW 264.7 cells and PMA was shown to induce the translocation of the α, βI, βII, δ, ε and μ isoforms from the cytosol to the cell membrane within 2min.

8.Pretreatment of cells with PMA for 2–24h resulted in a time-dependent down-regulation of PKCα, βI, βII, and δ expression, while the levels of the other four PKC isozymes were unchanged after PMA treatment for 24h. A decrease in the potentiation of AA release by PMA was observed, concomitant with the time-dependent down-regulation of PKC.

9.These results indicate that PKCβ has a crucial role in the mediation of cPLA2 activation by the phorbol ester PMA, whereas PMA utilizes PKC ε and/or μ to up-regulate AC activity.