CALCIUM INDEPENDENT, PHOSPHOLIPID DEPENDENT RESINIFERATOXIN-KINASE ACTIVATION OF NADPH-OXIDASE IN A CELL FREE SYSTEM

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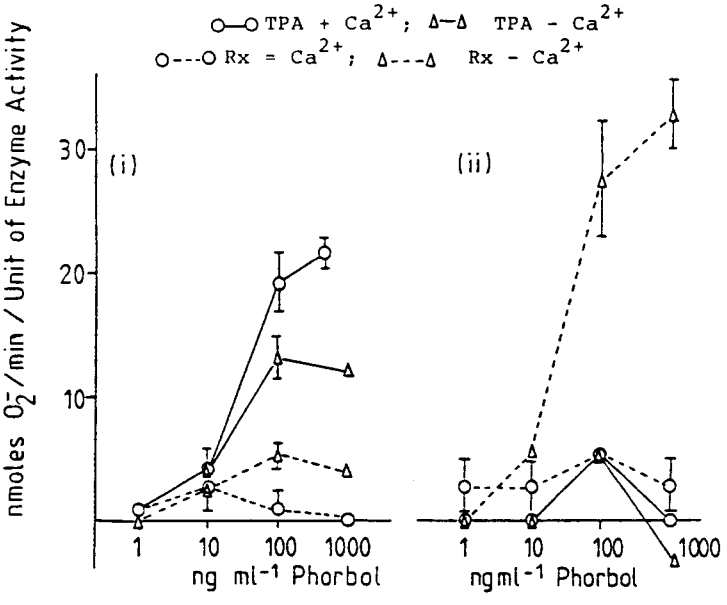
Following activation of the macrophage respiratory burst by various stimuli (e.g. phorbol ester) (Babior, 1984), superoxide anion (O_2^-) is produced by the membrane bound NADPH-oxidase complex (Rossi et al, 1986). Activation by phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), has been linked to the phosphorylation of a component of NADPH-oxidase by protein kinase C (PKC) (Cox et al, 1985). Resiniferatoxin (Rx) is the most potent inflammatory agent of the phorbol related family of diterpenes (Schmidt and Evans, 1979) and is known to activate a novel calcium-independent phospholipid dependent kinase, termed Rx-kinase (Ryves et al, 1989). Activation of NADPH-oxidase was therefore studied in a cell free system of mouse macrophages using both TPA and Rx together with PKC and Rx-kinase.

Membrane containing the bound oxidase, from mouse peritoneal macrophage homogenate was purified on discontinuous sucrose gradient (10%/40% w/v). 10% sucrose layer contained the cytosol fraction and 40% sucrose layer contained the membrane fractions. Membrane containing aliquots and cytosol were activated with phorbol esters and/or PKC and Rx-kinase, and O_2^- production was determined by a continuous kinetic assay of SOD-inhibitable reduction of cytochrome C spectrophotometrically at 550 nm (Bellavite et al, 1985).

Activation of NADPH-oxidase by diterpene esters in the presence of (i) PKC and (ii) Rx-kinase.



Activation of NADPH-oxidase by TPA was partially calcium dependent in the presence of PKC. However, Rx was a weak activator in this system. The activation of the enzyme complex by TPA with or without calcium in the presence of Rx-kinase, and Rx with calcium also in the presence of Rx-kinase were all weakly active or inactive. Rx with calcium absent in the presence of Rx-kinase induced a maximal superoxide release per unit of kinase activity. TPA and Rx alone produced no activation of NADPH-oxidase in this system.

Since TPA activation of PKC is partially Ca^{2+}-dependent and Rx is weakly active on PKC, it is reasonable to assume the observed activation of NADPH-oxidase is due to increased kinase activity. It is also suggested that since Rx activation of Rx-kinase is Ca^{2+}-independent, the apparent activation of the oxidase by Rx is due to phorphorylation of some component of NADPH-oxidase complex by Rx-kinase, independent of PKC.

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