Augmentation of Prostaglandin E₂ Production by Mammalian Phospholipase A₂ Added Exogenously

Shuntaro Hara, Ichiro Kudo, and Keizo Inoue
Faculty of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113

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Rat group II phospholipase A₂ added exogenously to A23187-activated HL-60 granulocytes augmented their production of prostaglandin E₂. Human group II phospholipase A₂, and porcine group I phospholipase A₂ augmented the prostaglandin E₂ production in a similar manner. No significant increase in prostaglandin E₂ production was observed when cells were treated with purified phospholipase A₁ in the absence of A23187. Extracellular phospholipase A₂ at inflamed sites may contribute to the generation of pro-inflammatory lipid mediators by hydrolyzing the cellular phospholipids of activated inflammatory cells.

Soluble phospholipase A₂ is released from inflammatory cells, such as platelets (1-3), vascular smooth muscle cells (4), and renal mesangial cells (5), in response to various stimuli, and has been implicated in the pathogenesis of inflammatory diseases. Human rheumatoid arthritis (6), Gram-negative septic shock (7), and psoriasis (8) are associated with high levels of extracellular phospholipase A₂ activity, which may promote inflammation by generating pro-inflammatory lipid mediators. It has been reported that snake venom phospholipase A₂ induces the generation of leukotrienes and other lipoxigenase products in porcine, human, and rat leukocytes (9-11), as well as edema, necrosis, and death when injected into experimental animals (12-16). However, the pro-inflammatory activity of phospholipase A₂ detected at inflamed sites has only been implicated indirectly.

Mammalian extracellular phospholipases A₂ have a molecular weight of about 14,000 and have been classified into two groups based on their primary structures (17). Pancreatic phospholipase A₁ belongs to the group I enzyme family (18, 19), while phospholipase A₂ at inflamed sites belongs to the group II enzyme family (20). In the present study, we investigated whether or not mammalian group II phospholipase A₂ can produce pro-inflammatory lipid mediators under certain conditions.

An HPLC chromatogram of the 9-anthryldiazomethane (ADAM) derivatives of prostaglandins produced by retinoic acid-differentiated HL-60 granulocytes is shown in Fig. 1. Neither A23187 nor rat group II phospholipase A₂ alone affected the production of prostaglandins by the HL-60 granulocytes, but a significant increase in prostaglandin E₂ (PGE₂) production was detected when the cells were treated with both A23187 and the purified enzyme. The augmentation of PGE₂ production by rat group II phospholipase A₂ was dose-dependent (from 0.7 μg/ml to 3 μg/ml), as indicated in Table I.

The other mammalian extracellular phospholipases A₂ also affected the PGE₂ production of HL-60 granulocytes (Table I). Human group II phospholipase A₂ purified from rheumatoid synovial fluid and porcine group I phospholipase A₂ (pancreatic enzyme) both augmented the PGE₂ production.

Several investigators have previously suggested that extracellular phospholipase A₂ exhibits pro-inflammatory effects in vivo (12-16), but the mechanism involved has remained unclear. The present findings suggest that the extracellular enzyme might contribute to the progression of inflammation by hydrolyzing cellular phospholipids to generate pro-inflammatory lipid mediators. Although mammalian pancreatic phospholipase A₂ also augmented the PGE₂ production, it seems unlikely that the group I enzyme contributes to the progression of general inflammation, since phospholipase A₂ detected at inflamed sites belongs to the group II enzyme family (20).

It is noteworthy that rat group II phospholipase A₂ was unable to influence the PGE₂ production without the coexistence of the Ca²⁺ ionophore, A23187. The activity of some phospholipases A₂ on the cell membrane is known to be influenced by the lipid packing in the outer leaflet of the plasma membrane (21). A23187 might change the molecular packing of lipids, making the lipids susceptible to the enzyme. All mammalian group II phospholipases A₂, hydrolyze phosphatidyethanolamine (PE) and phosphatidylserine (PS) more efficiently than phosphatidylcholine (20). Alternatively, A23187 might increase the distribution of both PE and PS in the outer leaflet of the plasma membrane, as found in platelets by Bevers et al. (22). PE and PS, which are both substrates for the group II enzymes and present in the inner leaflet of resting cells (23), may become accessible to the extracellular enzyme under these conditions. We found that injection of rat group II phospholipase A₂ into the hind paws of rats with adjuvant-induced arthritis exacerbated the edema (24). However, no effect was observed in normal rats. It can be concluded that expression of pharmacological activity of the exogenous enzyme requires a certain stage of inflammation induced by some other factors. The change in the transmembrane

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2To whom correspondence should be addressed.

Abbreviations: ADAM, 9-anthryldiazomethane; PGE₂, prostaglandin E₂; PE, phosphatidylethanolamine; PS, phosphatidylserine; HBSS, Hank's balanced salt solution.
then suspended in the same buffer (1 x 10⁷ cells/ml). After preincubation with concentrations of free calcium ions. We assume that, at the time of 34 min with this HPLC system.

The ADAM (acetonitrile/water/phosphoric acid (60 : 40 : 1)) at the flow rate of 0.7 ml/min. Authentic prostaglandin E₂ (PGE₂) was purchased from Boehringer Mannheim (Mannheim, Germany).

distribution of phospholipids may be one of the features of such an "activated" stage.

Although the source of the extracellular phospholipase A₂ detected at inflamed sites has not been identified, it may be secreted by leukocytes in response to inflammatory stimuli. The present study indicated that inflammatory stimuli may increase the release of phospholipase A₂ into the extracellular space, resulting in enhanced production of arachidonic acid, which is taken up by the activated leukocytes and then metabolized to PGE₂.

Recently, evidence has accumulated indicating the existence, in various mammalian cells including granulocytes, of an intracellular phospholipase A₂ with a relatively high molecular weight (25-34). This high molecular weight enzyme might be mainly involved in the stimulus-coupled liberation of arachidonic acid, since it exhibits a preference for arachidonate esterified at the sn-2 position of a phospholipid substrate and is activated by submicromolar concentrations of free calcium ions. We assume that, at inflamed sites, both extracellular group II phospholipase A₂ and high molecular weight phospholipase A₂ may synergistically stimulate PGE₂ production.

REFERENCES


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Prostaglandin Production by Exogenous Phospholipase A₂