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Platelet-activating factor stimulates phospholipase in quiescent Swiss mouse 3T3 fibroblast

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Platelet-activating factor(PAF) induced the rapid hydrolysis of phospholipids in Swiss mouse 3T3 fibroblasts prelabeled with [^{3}H]arachidonic acid or [^{3}H]choline. Up to 23% of the [^{3}H]arachidonic acid incorporated in phospholipids was released into the medium when PAF was added to cells. We also observed increases of diacylglycerol and lysophosphatidylcholine in the medium and cells after the addition of PAF. These results support the possibility that platelet-activating factor stimulates phospholipase A_2 and C activities in 3T3 cells.

Platelet-activating factor *Phospholipase A_2* *Phospholipase C* *Arachidonic acid* *Diacylglycerol*

1. INTRODUCTION

PAF is released from leukocytes after immunologic stimulation in various species [1]; it has recently been identified as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine [2,3]. This bioactive phospholipid causes aggregation and degranulation of platelets, and stimulates arachidonic acid release and subsequent thromboxane synthesis [4]. Authors in [2] demonstrated that alkylacetyl–GPC also possesses marked hypotensive activity when given to 'Goldblatt' 1-kidney,1-clip hypertensive rats. However, little is known as to whether PAF has any effects on PL hydrolysis in cells other than platelets and leukocytes. If other cells are affected directly by PAF, and they are stimulated to release prostaglandins, it may have a physiological importance. To study this possibility, we examined PLase A_2 and C activities in Swiss mouse 3T3 fibroblasts stimulated by PAF.

Abbreviations: PAF, platelet-activating factor; DMEM, Dulbecco's modified Eagle's medium; PLase, phospholipase; PL, phospholipids; Lyso-PC, lysophosphatidylcholine; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; MG, monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol; TLC, thin layer chromatography

2. MATERIALS AND METHODS

[5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid (135 Ci/mmol) and [*methyl*-^{3}H]choline chloride (78 Ci/mmol) were obtained from Amersham. 1-*O*-Hexadecyl-2-acetyl-phosphatidylcholine (C_{16}PAF) was obtained from Sigma. 1-*O*-Octadecyl-2-acetyl-phosphatidylcholine (C_{18}PAF) was purchased from Backen Feinchemikalien. All other materials were of reagent purity. Swiss mouse 3T3 fibroblasts (Flow Laboratory) were maintained in DMEM supplemented with 10% calf serum as previously described [6]. Triplicated cultures of 1.2×10^5 cells plated in 2 ml DMEM containing 0.2% calf serum and 1 μCi of [^{3}H]arachidonic acid or 8 μCi of [^{3}H]choline chloride in 35 mm Falcon dishes were cultured for 24 h. The incorporation of [^{3}H]arachidonic acid and [^{3}H]choline into the cells was about 20 and 0.2%, respectively. The distribution of incorporated arachidonic acid into the 2-position of phosphoglycerides was determined by treating the cells with naja naja venom PLase A_2 which is specific for fatty acid in the

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2-position; 98.3% of the [^{3}H]arachidonic acid was released from cells. The cells were washed on dishes and then incubated at 37°C with the indicated concentration of PAF in 0.6 ml of serum-free DMEM. The lipids released into the medium from cells were extracted with 2 ml of ethyl acetate and analyzed [7,8]. When cellular lipids were obtained for TLC, the medium was removed, the cells were rapidly washed and treated with 1 ml of 70% methanol and then scraped from dishes. PL were extracted and analyzed by the methods previously described [8,9]. When DG and Lyso-PC were determined, the radioactivities in the medium and cells were combined. Neutral lipids were separated by TLC with a previously described solvent system [9]. The rate of release of [^{3}H]arachidonic acid from 3T3 cells in the presence of C_{16} or C_{18}PAF was determined.

3. RESULTS

3T3 cells responded to treatment with both PAFs at 5×10^{-5} M by releasing, in 1 h, up to 23% of the incorporated arachidonic acid into the medium (fig.1A). A detectable release began within 5 min after treatment of 10^{-6} M of PAF and continued for at least 90 min (fig.2). The composition of cellular PL changed rapidly. PC, PI, and PE decreased from 43 to 35%, from 14 to 7%, and from 30 to 23%, respectively, when PAF was added to cells prelabeled with [^{3}H]arachidonic

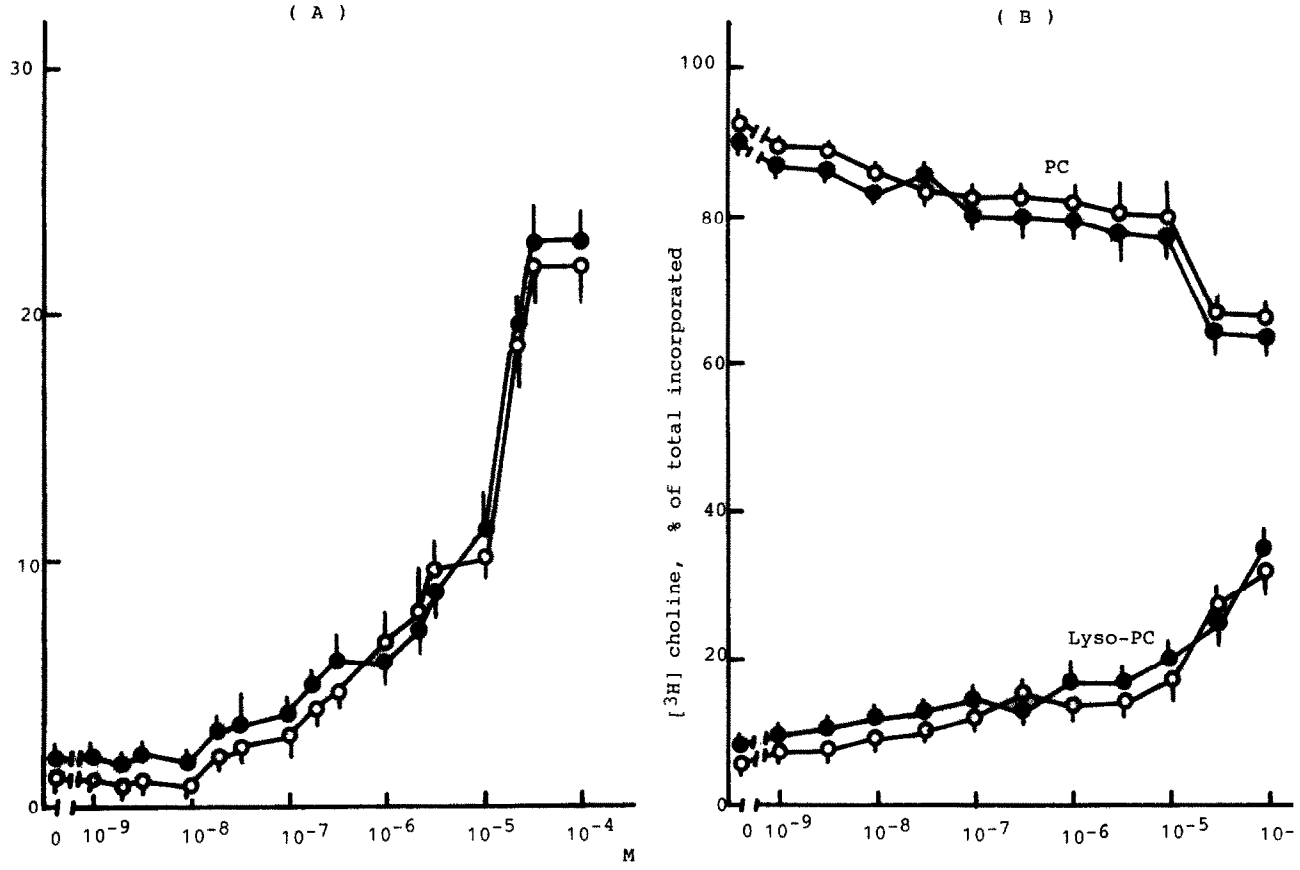

Fig.1. Effect of the concentration of PAF on (A) the release of arachidonic acid and (B) the change of phosphatidylcholine in 3T3 cells. Triplicate cultures of 1.2×10^5 cells were incubated with the indicated concentration of PAF for 60 min at 37°C, and the radioactivities in the medium and cells were extracted and analyzed by TLC as described in section 2. (○) C_{16}PAF; (●) C_{18}PAF.

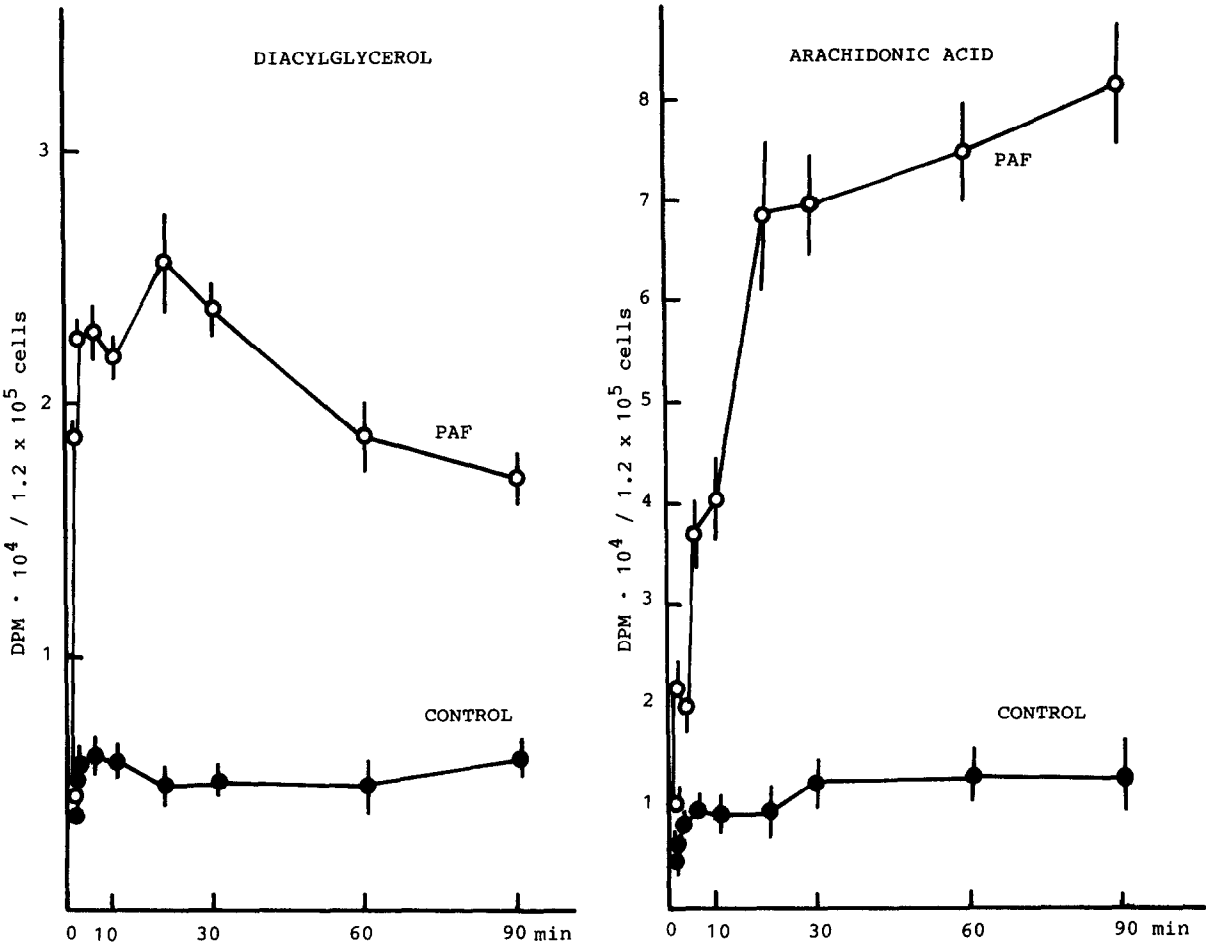

Fig.2. Time course of effect of PAF on the release of arachidonic acid into the medium and the release of diacylglycerol into the medium and the cells. 3T3 cells were incubated with 1 μM of PAF for the indicated period at 37°C as described in section 2. (○) C_{16}PAF; (●) control.

acid. As shown in fig.1B, the detectable decrease of PC began at 10^{-8} M of PAF. On the other hand, the radioactivity in Lyso-PC increased with the concentration of PAF. To study PLase C activity stimulated by PAF, the accumulation of DG was measured. The increase in DG was detectable within 1 min and reached a peak at 20 min (fig.2). 6.7% of radioactivity was released as DG in the medium and the cells. MG accumulation was also observed. However, the accumulation of DG + MG was relatively small compared to the free arachidonic acid released. On the other hand, TG was not changed at any concentration of PAF. It seemed, therefore, that the addition of PAF to 3T3 cells promoted the release of arachidonic acid from PL by the stimulation of PLase A_2 and C activities.

4. DISCUSSION

Previous investigations have shown that PAF stimulates PGs syntheses, PLase A_2 and C activities in platelets and leukocytes. Therefore, we investigated whether PAF stimulates PLase A_2 and C in 3T3 cells. We demonstrated that the degradation of PL was accompanied by the accumulation of DG and Lyso-PC. The decline of DG after 30 min (fig.2) might have been caused by the reincorporation into PL [9].

We showed that PAF stimulated not only PLase A_2 but also PLase C in Swiss mouse 3T3 fibroblasts. These effects may be physiologically important, because other cells as well as platelets and leukocytes may be directly affected by PAF, possibly releasing prostaglandins when stimulated by PAF.

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