The effect of prostaglandin E2 (PGE2), forskolin, and dibutyryl cAMP on arachidonic acid release, inositol phospholipid metabolism, and Ca2+ mobilization was investigated. The chemotactic tripeptide (formylmethionyl-leucyl-phenylalanine [fMLP])-induced arachidonic acid release in neutrophils was significantly inhibited by PGE2, forskolin, and dibutyryl cAMP. Among them, PGE2 was found to be the most potent inhibitor. However, when neutrophils were stimulated by Ca2+ ionophore A23187, such inhibitory effect by these agents was less marked. PGE2 also suppressed the enhanced incorporation of [32P]Pi into phosphatidic acid (PA) and phosphatidylinositol in a dose-dependent manner in fMLP-stimulated neutrophils. Also in this case, Ca2+ ionophore-induced alterations were hardly inhibited by PGE2. As well, PGE2 inhibited the fMLP-induced decrease of [3H]arachidonic acid in phosphatidylcholine and phosphatidylinositol and the increase in PA very significantly. But the inhibitory effect by PGE2 was found to be weak in Ca2+ ionophore-stimulated neutrophils. Concerning polyphosphoinositide breakdown, PGE2 did not affect the fMLP-induced decrease of [32P]phosphatidylinositol 4,5-bisphosphate which occurred within 10 s but inhibited the subsequent loss of [32P]phosphatidylinositol 4-phosphate and [32P]phosphatidylinositol, suggesting that the compensatory resynthesis of phosphatidylinositol 4,5-bisphosphate was inhibited. On the other hand, fMLP-induced diacylglycerol formation was suppressed for the early period until 1 min, but with further incubation, diacylglycerol formation was rather accelerated by PGE2. Moreover, the inhibition of PA formation by PGE2 became evident after a 30-s time lag, suggesting that the conversion of diacylglycerol to PA is inhibited by PGE2. The formation of water-soluble products of inositol phospholipid degradation by phospholipase C, such as inositol phosphate, inositol 1,4-bisphosphate, and inositol 1,4,5-trisphosphate, was also suppressed by PGE2 treatment. However, the inhibition was not so marked as that of arachidonic acid release and PA formation. Thus, PGE2 appeared to inhibit not only initial events such as polyphosphoinositide breakdown but also turnover of inositol phospholipids. PGE2, forskolin, and dibutyryl cAMP did not block the rapid elevation of intracellular Ca2+ which was observed within 10 s in fMLP-stimulated neutrophils. However, subsequent increase in intracellular Ca2+ which was caused from 10 s to 3 min after stimulation was inhibited by PGE2, forskolin, and dibutyryl cAMP. These data may demonstrate that PGE2 inhibits Ca2+ influx rather than Ca2+ mobilization from intracellular stores. Considering that arachidonic acid release is completely dependent on extracellular Ca2+, PGE2 may inhibit Ca2+ influx through the suppression of inositol phospholipid metabolism and then inhibit arachidonic acid release.

Neutrophils have been considered as playing a major role in host defenses against microorganisms and have been implicated in contributing to inflammatory events. The release of lysosomal enzymes and the generation of superoxide anion in activated neutrophils have been suggested as possible mechanisms by which some of these events are mediated (1–3).

We (4) and other investigators (5–9) have demonstrated that phosphatidylinositol response and arachidonic acid release are closely associated with neutrophil activation. Recently, the degradation of phosphatidylinositol 4,5-bisphosphate (PI-4,5-P2) by phospholipase C rather than phosphatidylinositol (PI) has been proposed to be a primary event in stimulus-response coupling in a variety of tissues (for review see Refs. 10 and 11). Also in chemotactic peptide-stimulated neutrophils, PI-4,5-P2 breakdown to diacylglycerol and inositol 1,4,5-trisphosphate (IP3) has been found to occur very rapidly compared to that of other inositol lipids such as phosphatidylinositol 4-phosphate (PI-4-P) and PI (8, 9, 12). Thus, two biologically important factors, DG and IP3, are produced in response to receptor activation. DG, one of the products of PI-4,5-P2, is known to act as an activator for protein kinase C (for review see Refs. 13 and 14). Therefore, the signal-induced DG accumulation leads to the activation of protein kinase C. Concerning another product, since Streb et al. (15) suggested that IP3 had roles in the mobilization of intracellular free Ca2+, much evidence has been presented that IP3 is able to release Ca2+ from intracellular store in various

1 The abbreviations used are: PI-4,5-P2, phosphatidylinositol 4,5-bisphosphate; fMLP, formylmethionyl-leucyl-phenylalanine; PI, phosphatidylinositol; PI-4-P, phosphatidylinositol 4-phosphate; PA, phosphatidic acid; PC, phosphatidylcholine; PG, prostaglandin; DG, diacylglycerol; IP, inositol phosphate; IP3, inositol 1,4,5-trisphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; EMEM, Eagle’s minimal essential medium; NDGA, nordihydroguaiaretic acid; BPB, bromphenacyl bromide.
tissues and cells (for review see Refs. 16 and 17). Thus, it now appears to be the most promising intermediate of hormone-stimulated inositol phospholipid metabolism capable of acting as a messenger for the mobilization of Ca²⁺ from intracellular storage.

It is well known that in activated neutrophils arachidonic acid is liberated from phospholipids and converted to prostaglandins and leukotrienes that have very strong biological activities. In this case, the liberation of arachidonic acid is thought to be the rate-limiting step. However, it is not clear how arachidonic acid release is regulated. Recently, numerous reports have shown that an increase of CaM²⁺ in leukocytes generally leads to subsequent inhibition of physiological functions (18–20). Substances that are able to elevate Ca²⁺ have therefore been considered as factors that contribute to the modulation of leukocyte activities. Particularly, prostaglandins E and leukotrienes have attracted attention, since they seem to regulate leukocyte under physiological conditions (20). Among the E series of prostaglandins, prostaglandin E₂ (PGE₂) is thought to be one of the most important regulators because PGE₂ is one of the major prostaglandins produced from arachidonic acid in activated neutrophils (21, 22). However, so far it is not known whether PGE₂ is able to affect inositol phospholipid metabolism, arachidonic acid release, and Ca²⁺ mobilization, although PGE₂ can inhibit the subsequent responses such as lysosomal enzyme secretion and superoxide anion production (18–20) in neutrophils.

In this report, the effect of PGE₂ on the metabolism of inositol phospholipids, arachidonic acid release, and Ca²⁺ mobilization is studied.

**MATERIALS AND METHODS**

[5,6,8,9,11,12,14,15-³H]Arachidonic acid (95.4 Ci/mmol), myo-2-³Hinositol (12.5 Ci/mmol), and [³²P]orthophosphoric acid (carrier-free) were obtained from New England Nuclear. Ca²⁺ ionophore A23187 was from Calbiochem-Behring. Nordihydroguaiaretic acid, bromophenacyl bromide, forskolin, and dibutyryl cyclic AMP were from Sigma. Prostaglandin E₂ was from Funakoshi Chemical Co., Osaka. Eagle’s minimum essential medium (EMEM) and Hanks’ balanced salt solution were from Nissui Co., Tokyo. N,N,N',N'-tetraacetic acid, tetraacetoxymethyl ester (Quin 2AM), and Hanks’ balanced salt solution containing calcium (1.6 mM) or 2 mM EGTA (1 × 10⁵ cells/ml) were obtained from Nissui Co., Tokyo. 2-[Amino-5-methyleneoxy]-methyl]-6-methoxy-8-aminquinoline-N,N,N’,N’-tetraacetic acid, tetracetoxyethyl ester (Quin 2AM), was from Dojin Chemical Laboratories, Kumamoto. BW755C was a gift from Mitsubishi Pharmaceutical Co., Tokyo. nordihydroguaiaretic acid (NDGA) and BW755C were found to be strong inhibitors of the metabolism of inositol phospholipids, arachidonic acid release, and Ca²⁺ mobilization.

### RESULTS

**Effect of Inhibitors for Arachidonic Acid Cascade on Superoxide Anion Production and Lysosomal Enzyme Secretion—** The chemotactic tripeptide, fMLP, has been shown to stimulate superoxide anion production and lysosomal enzyme secretion very significantly (3, 26–30). This enhanced production of superoxide anion was further stimulated by the addition of indomethacin, a cyclooxygenase inhibitor (Fig. 1A). On the contrary, a lipoxigenase inhibitor such as nordihydroguaiaretic acid (NDGA) reduced the production of superoxide anion.

**FIG. 1.** Effect of inhibitors for arachidonic acid cascade on superoxide anion (A) and lysosomal enzyme secretion (B). A, neutrophils (2 × 10⁶ cells/ml) were stimulated by 1 μM fMLP in the presence of various concentrations of drugs with 80 μM cytochrome c for 10 min at 37°C, and then cytochrome c reduction was measured at 550 nm with a reference of 540 nm. Before the treatment by fMLP, neutrophils were preincubated with various drugs or vehicle for 8 min. B, after neutrophils were treated with 5 μg/ml colchicin B for 10 min at 37°C, reaction was started by the addition of 1 μM fMLP in the presence of various concentrations of drugs and continued for 5 min. Aliquots of the supernatant were used to measure secreted N-acetyl-β-glucosaminidase. Concentrations of agents are indicated in nanomolar for PGE₂ or micromolar for indomethacin (Ind). NDGA, and BFP.
inhibitors for superoxide anion production. Bromophenacetyl bromide (BPB), a phospholipase A2 inhibitor, also suppressed the production. PGE₂ suppressed the production of superoxide anion. For lysosomal enzyme secretion, similar results were obtained, namely, NDGA, BW755C, BPB, and PGE₂ inhibited the secretion of N-acetyl-β-D-glucosaminidase and indomethacin activated the secretion (Fig. 1B). These results suggest that cyclooxygenase products inhibit the neutrophil functions such as superoxide anion production and lysosomal enzyme secretion, but lipoxygenase products activate those functions. PGE₂ is one of the major products of arachidonic acid metabolites when neutrophils were stimulated by chemotactic peptide (21, 22). Therefore, there is a possibility that PGE₂ causes a negative feedback effect on neutrophils.

**Inhibitory Effect of PGE₂, Forskolin, and Dibutyryl cAMP on Arachidonic Acid Release**—It has become clear that arachidonic acid metabolites make important roles on neutrophil activation (6, 31, 33). Therefore, the effect of PGE₂, forskolin, and dibutyryl cAMP on arachidonic acid release was examined. As shown in Fig. 2, not only PGE₂ but also forskolin and dibutyryl cAMP inhibited arachidonic acid release in a dose-dependent manner which is induced by fMLP or Ca²⁺ ionophore stimulation. Among them, PGE₂ was found to be the most potent inhibitor and 1 μM PGE₂ almost completely inhibited arachidonic acid release in chemotactic peptide-stimulated neutrophils. The inhibitory effect was also obtained by the treatment of forskolin which is known as a potent cAMP-increasing agent, although dibutyryl cAMP caused a slight inhibition. Therefore, some parts of the inhibitory effect of PGE₂, if not all, may be explained by cAMP elevation. This inhibition was more significant in fMLP-stimulated neutrophils than that in Ca²⁺ ionophore-activated neutrophils. Since Ca²⁺ ionophore can activate phospholipase A₂ through intracellular Ca²⁺ elevation, these data may suggest that PGE₂ mainly inhibits a certain step in pathways from receptor activation to arachidonic acid release rather than inhibit directly the arachidonic acid-cleaving enzyme phospholipase A₂.

**Effect of PGE₂ on [³²P]Phospholipid Metabolism**—When neutrophils were stimulated by fMLP, a marked increase of [³²P] incorporation into phosphaticid acid (PA) and PI and the formation of lysophosphatidylcholine were observed (Fig. 3). These increases were significantly antagonized by the addition of PGE₂ in a dose-dependent manner. Metabolisms of other phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine, and phosphatidylserine were not affected by fMLP. As observed on the effect of arachidonic acid release, this inhibitory effect was more significant in chemotactic peptide-activated neutrophils than that in Ca²⁺ ionophore-activated neutrophils.

**Effect of PGE₂ on the Distribution of [³H]Arachidonic Acid**—After neutrophils were prelabeled with [³H]arachidonic acid, the effect of PGE₂ on the redistribution of [³H]arachidonic acid was examined (Fig. 4). When neutrophils were stimulated with fMLP, the decrease of [³H]arachidonic acid in PC and PI and the increase in PA and free arachidonic acid were significant. The decrease in PC and PI was blocked by PGE₂.
by the addition of PGE₂ in a dose-dependent manner. Moreover, the increase in PA and free arachidonic acid was almost completely inhibited by the treatment of more than 100 nM PGE₂. Ca²⁺ ionophore also enhanced the loss of [³²P]arachidonic acid in PC, phosphatidylethanolamine, and PI. However, this decrease was not inhibited so strongly by PGE₂ as that observed in fMLP-stimulated neutrophils. As well, the increase of [¹⁴C]arachidonic acid in PA and free fatty acid which was caused by Ca²⁺ ionophore was also weakly inhibited.

Effect of PGE₂ on [³²P]-Labeled PI-4,5-P₂, PI-4-P, PI, and PA—It has been reported that polyphosphoinositide breakdown is closely related to Ca²⁺ mobilization and arachidonic acid release (for review see Refs. 17 and 34). As shown in Fig. 5, chemotactic peptide caused very rapid decreases in [³²P]PI-4,5-P₂ and [³²P]PI-4-P, followed by the compensatory resynthesis of PI-4,5-P₂ and PI-4-P. On the other hand, PA labeling by [³⁵S]P increased with time without transient decrease by chemotactic peptide. PGE₂ did not inhibit the decrease in [³²P]PI-4,5-P₂ significantly but suppressed the subsequent decrease in [³²P]PI-4-P. This experiment was repeated four times. PGE₂ always inhibited the decrease of PI-4-P more markedly than that of PI-4,5-P₂, although the inhibitory effect was varied in each experiment. Concerning [³²P]PI, both the initial decrease which occurred within 1 min and the subsequent resynthesis were inhibited by PGE₂. These results may show that recovery synthesis of PI-4,5-P₂ from PI is inhibited by PGE₂. Under these conditions, [³²P] incorporation into PA was markedly inhibited. However, this inhibition became evident in 30 s after stimulation, suggesting that the conversion of DG to PA is blocked by PGE₂. Ca²⁺ ionophore did not cause the significant decrease of PI-4-P and PI-4,5-P₂ (data not shown).

Effect of PGE₂ on Inositol Phosphates Formation—It seemed likely that PGE₂ did not inhibit the initial reactions such as PI-4,5-P₂ breakdown in the [³²P] experiment, although PI-4-P decrease was inhibited. Thus, the effect of PGE₂ on the formation of inositol phosphate which should be formed by the result of inositol phospholipids breakdown was studied. As shown in Fig. 6, fMLP enhanced the formation of various inositol phosphates such as IP₅, IP₃, and IP₁. In this case, formation of IP₅ occurred rapidly and reached a maximum at 10 s after stimulation. The formation preceded that of IP₁ or IP₃. PGE₂ could inhibit not only IP and IP₃ formation but also IP₅ formation. But the inhibition seemed to be slight compared to that of arachidonic acid release and PA formation. These results suggest that there are some steps to be inhibited by PGE₂ except PI-4,5-P₂ breakdown to DG and IP₅. Under these conditions, arachidonic acid release and PA formation were strongly blocked.
**Fig. 6.** Effect of PGE₂ on fMLP-induced formation of inositol phosphates. Neutrophils labeled with myo[2-³H]inositol were incubated with 10 mM LiCl for 10 min and then treated with 1 μM PGE₂ or vehicle for 3 min. The cells were then stimulated with 1 μM fMLP. The treatment by PGE₂ without fMLP did not show the significant effect on the formation of inositol phosphates. Each point represents the mean ± S.E. of triplicate determinations. *p < 0.01. C, control; ○, fMLP; Δ, PGE₂; ▲, PGE₂ + fMLP.

**Fig. 7.** Time course of fMLP- or A23187-induced accumulation of [³H]arachidonic acid and [³H]arachidonoyl-DG. [³H]Arachidonic acid-labeled neutrophils were preincubated with 1 μM PGE₂ or vehicle for 3 min at 37 °C. The cells were stimulated by 1 μM fMLP or 1 μM A23187 for the indicated time at 37 °C. PGE₂ alone did not affect the basal [³H]arachidonic acid or [³H]arachidonoyl-DG. Each point represents the mean ± S.E. of triplicate determinations. *p < 0.01. C, control; ○, fMLP; Δ, A23187; ▲, fMLP + PGE₂; ▲, A23187 + PGE₂.

**Time Course of the Formation of [³H]Arachidonoyl-DG and Free [³H]Arachidonic Acid—DG formation was increased by fMLP during the incubation time up to 3 min (Fig. 7). This increase seemed to be inhibited for the early period until 1 min, but with further incubation the increase was, on the contrary, potentiated a little with PGE₂. On the other hand, increased formation of DG by Ca²⁺ ionophore was not affected significantly by PGE₂. fMLP-induced liberation of arachidonic acid was inhibited very strongly. However, Ca²⁺ ionophore-induced liberation was partially blocked by the addition of PGE₂. Considering the results that DG accumulation after 1 min was rather stimulated by PGE₂, the conversion of DG to PA may be inhibited. This may be the reason why PA formation was markedly inhibited.

**Effect of PGE₂ and Forskolin on fMLP- or Ca²⁺ Ionophore A23187-induced cAMP Elevation—**As shown in Table I, PGE₂ alone caused approximately a 2-fold elevation of cAMP levels relative to control values, but stimulated a 5–6-fold rise in the presence of 1 μM fMLP or Ca²⁺ ionophore A23187. On the other hand, forskolin alone also elicited a 1.4-fold rise in cAMP levels. These results strongly suggest that the inhibitory effect of PGE₂ on inositol phospholipids metabolism is produced mainly through cAMP elevation.

**Effect of PGE₂ on Intracellular Ca²⁺ Level—**It was already demonstrated that arachidonic acid release was completely dependent on extracellular Ca²⁺ (4). Therefore, the effect of PGE₂ on free intracellular Ca²⁺ levels was monitored by Quin...
2 in the presence or absence of extracellular Ca++. When neutrophils were activated by fMLP in Ca++ containing medium, the intracellular Ca++ level increased from 170 to 370 nM very rapidly within 10 s and then gradually increased up to 900 nM until 3 min (Fig. 8A). In this case, PGE2 did not inhibit the rapid increase in Ca++ which occurred within 10 s, but it suppressed the following increase in Ca++ which occurred between 10 s and 3 min in a dose-dependent manner. Forskolin and dibutyryl cAMP also gave the same effect on Ca++ mobilization, namely, they did not inhibit the initial increase in Ca++ but inhibited the following increase (data not shown). Similar experiments were carried out in the presence of 2 mM EGTA (Fig. 8B). Even under this condition, the initial increase by fMLP stimulation still occurred, although the amount of increased Ca++ was less significant; but the secondary increase in Ca++ was not observed. In this condition, PGE2 did not block Ca++ increase which was induced by fMLP.

These data suggest that PGE2 inhibits the secondary Ca++ increase which may be due to Ca++ influx; but PGE2 does not inhibit the initial burst increase, which may be due to Ca++ release from the intracellular store.

**DISCUSSION**

PGE2 inhibited the functions of leukocytes such as lysosomal enzyme secretion, superoxide anion production, and aggregation. This effect was thought to be caused through intracellular cAMP elevation (18, 19). But it is not certain how cAMP inhibited those events. On the other hand, when neutrophils are activated with fMLP, rapid alterations of inositol phospholipid metabolism are caused. Such enhanced inositol phospholipid metabolism is believed to have played an important role in neutrophil functions through Ca++ mobilization and protein kinase C activation. In this case, breakdown of PI-4,5-P2 to DG and IP3 rather than that of PI to DG and IP has been proposed as an initial event (12). If this is the case, inhibition of these steps must lead to suppression of neutrophil functions. However, this initial breakdown of PI-4,5-P2 appeared not to be inhibited markedly by the addition of PGE2, although fMLP-induced decrease of PI and PI-4-P was blocked. If the decrease of PI and PI-4-P mainly caused by the conversion to PI-4,5-P2 through the pathway, PI to PI-4-P, and then to PI-4,5-P2 as suggested by Berridge (35), inhibition of loss of 32P-labeled PI and PI-4-P may indicate the suppressive conversion from PI to PI-4,5-P2. In this experiment, although fMLP-induced decrease of PI-4,5-P2 was not significantly altered by PGE2, IP3 formation was found to be inhibited. This discrepancy may be explained by the reason why inhibition of IP3 formation by PGE2 breakdown toDG and IP3 is apparently masked, because basal labeling of [32P]PI-4,5-P2 is slightly lower in PGE2-treated neutrophils. A second possibility is that PGE2 stimulates the phosphomonoesterase that hydrolyzes PI-4,5-P2 to PI-4-P (36) in addition to the inhibition of PI-4,5-P2 breakdown to DG and IP3. A third possibility is that the inhibition of decrease in [32P]PI-4,5-P2 is hardly detected since the decrease is rapidly recovered with the resynthesis. On the contrary, formed IP3 may be detected cumulatively, although IP3 gradually degraded to IP2 and then to IP with the further incubation. Therefore, inhibition of IP3 formation may be easily measured. On the other hand, synthesis of PA and IP was strongly inhibited to PGE2. These results suggest that PGE2 inhibits not only an initial event such as PI-4,5-P2 breakdown weakly but also turnover of inositol phospholipids strongly. Forskolin and dibutryl cAMP seemed to mimic PGE2 effect on arachidonic acid release and phospholipid metabolism. If the inhib-

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**TABLE I**

Effect of PGE2 and forskolin on fMLP- or Ca++ ionophore A23187-induced cAMP elevation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>fMLP</th>
<th>Ca++ ionophore</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/10^6 cells</td>
<td>pmol/10^6 cells</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>7.06 ± 0.20</td>
<td>7.55 ± 1.28</td>
<td>8.11 ± 1.05</td>
</tr>
<tr>
<td>PGE2</td>
<td>14.71 ± 1.34</td>
<td>38.24 ± 1.00</td>
<td>36.28 ± 1.97</td>
</tr>
<tr>
<td>Forskolin</td>
<td>10.39 ± 0.39</td>
<td>15.00 ± 1.27</td>
<td>15.69 ± 2.23</td>
</tr>
</tbody>
</table>

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**FIG. 8.** Time course of changes in fluorescence of Quin 2-loaded neutrophils. A, effect of PGE2 on fluorescence response of Quin 2-loaded, fMLP-activated neutrophils in Ca++-containing medium; B, effect of PGE2 on fluorescence response of Quin 2-loaded, fMLP-activated neutrophils in 2 mM EGTA-containing medium. The concentration of PGE2 used in this experiment was 1 μM. Other details were the same as described under "Materials and Methods."
itory effect is derived from the increase in cAMP, the potency must be dependent on the ability for raising cAMP. Indeed, the inhibitory effect was correlated with cAMP elevation. Interestingly, the combined treatment of PGE$_2$ and fMLP caused the dramatic increase of cAMP (Table 1). This result also supports the idea that the potent effect of PGE$_2$ may be derived mainly from cAMP elevation. When neutrophils were activated with Ca$^{2+}$ ionophore, the inhibitory effect of PGE$_2$ on arachidonic acid release and phospholipid metabolism was not so effective as that in fMLP-activated neutrophils. This fact may indicate that events leading to intracellular Ca$^{2+}$ increase such as enhanced metabolism of inositol phospholipids are mainly suppressed by PGE$_2$. But, it is still unclear whether the inhibitory effect of PGE$_2$ on lysosomal enzyme secretion and superoxide anion production is caused by the inhibition of enhanced inositol phospholipid turnover.

It is well known that Ca$^{2+}$ plays an important role in lysosomal enzyme secretion and superoxide anion production, although extracellular Ca$^{2+}$ is not an absolute requirement for the activation of these functions (37, 38). In this experiment, we showed that PGE$_2$ could inhibit Ca$^{2+}$ influx but not Ca$^{2+}$ mobilization from intracellular stores. Recently, much evidence has been presented that newly formed IP$_3$, which is a resultant product of PI-4,5-P$_2$ breakdown by phospholipase C, can mobilize Ca$^{2+}$ from the intracellular store (for review see Refs. 15 and 34) and another product of PI-4,5-P$_2$ breakdown, DG, activates protein kinase C (for review see Refs. 13 and 14). However, in this experiment, IP$_3$ and DG formation induced by fMLP could not be inhibited effectively by PGE$_2$. If IP$_3$ could release Ca$^{2+}$ from intracellular store, this may be the reason why the initial Ca$^{2+}$ increase within 10 s was hardly blocked by PGE$_2$. Recently, Togni et al. (39) have also reported that the inhibitory effect of PGE$_2$ on neutrophil responses is not due to the suppression of the initial burst elevation of Ca$^{2+}$. Since arachidonic acid release is completely dependent on extracellular Ca$^{2+}$ (4), inhibition of Ca$^{2+}$ influx perhaps causes the suppression of arachidonic acid release in fMLP-stimulated neutrophils. However, PGE$_2$ also inhibited the release of arachidonic acid and inositol phospholipid metabolism that was induced by Ca$^{2+}$ ionophore, although its effect was not so marked. These results may indicate that PGE$_2$ also inhibits a step in the pathways from Ca$^{2+}$ influx to arachidonic acid release, such as phospholipid metabolism, phospholipase A$_2$ activation, and DG lipase-catalyzed arachidonic acid liberation (40). Considering the fact that PA can act as Ca$^{2+}$ ionophore in membranes (41) and PA synthesis is tightly coupled to lysosomal enzyme secretion (42), enhanced metabolism of inositol phospholipids may play an important role in not only Ca$^{2+}$ mobilization from intracellular store but also Ca$^{2+}$ influx. In this case, PGE$_2$ seemed to affect Ca$^{2+}$ influx through inhibiting the enhanced metabolism of inositol phospholipids rather than inhibiting directly phospholipase C activity which catalyzes the degradation of PI-4,5-P$_2$ to IP$_3$ and DG. In fact, phospholipase C, which was isolated from neutrophils treated with PGE$_2$ or dibutyryl cAMP, was found to have a similar activity to that of nontreated neutrophils.

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T. Takenawa, J. Ishitoya, and Y. Nagai, unpublished data.