Arachidonic Acid Metabolism in Polymorphonuclear Leukocytes from Patients with Chronic Granulomatous Disease

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The effect of the calcium ionophore A23187 on the release and metabolism of [3H]arachidonic acid was examined in normal polymorphonuclear leukocytes and those obtained from patients with chronic granulomatous disease. The ionophore A23187 which stimulates oxidative metabolism in normal polymorphonuclear leukocytes was ineffective in increasing oxidative metabolism (chemiluminescence) in polymorphonuclear leukocytes from patients with chronic granulomatous disease. However, the ionophore A23187 stimulated the release of [3H]arachidonic acid from chronic granulomatous disease neutrophil phospholipids and stimulated its metabolism into hydroxyeicosatetraenoic acids and leukotrienes.

Chronic granulomatous disease (CGD) is characterized by an increased susceptibility to bacterial infections especially in infants or young children (20, 25). Morphological events associated with CGD have shown that polymorphonuclear leukocytes (PMNs, neutrophils) effectively phagocytize bacteria and fungi but fail to kill these microorganisms (14, 20, 23, 26). Biochemical evidence indicates that this defect in the microbicidal activity of PMNs from patients with CGD has been linked to the absence of the "respiratory burst" associated with phagocytosis of the microorganisms by PMNs (19).

Normal human PMNs undergo marked alterations in oxidative metabolism, collectively referred to as the respiratory burst (3, 15), in response to a number of stimuli, including phagocytosis (4), concanavalin A (30), detergents (18), the tumor-promoting agent phorbol myristate acetate (17), and the calcium ionophore A23187 (29, 31). Evidence by Allen (1) indicates that PMN-generated chemiluminescence correlates well with the respiratory burst activity. The calcium ionophore A23187 has previously been shown to be a potent stimulator of PMN chemiluminescence (16). The calcium ionophore A23187 has been shown also to mobilize calcium ions (11, 27) and to stimulate the release of radiolabeled arachidonic acid from human neutrophil phospholipids (33, 35, 36).

Phosphatidylinositol and phosphatidylcholine were shown to be the primary sources of [3H]arachidonate released upon stimulation of PMNs with opsonized zymosan (34) and the calcium ionophores A23187 and Ionomycin (35, 36). However, the ionophores but not zymosan led to the production of [3H]hydroxyeicosatetraenoic acid (HETE) and [3H]diHETE. There was no significant production of [3H]-labeled prostaglandins or [3H]-labeled thromboxanes detected with either stimulant. In contrast to zymosan and calcium ionophores, phorbol myristate acetate, another potent stimulator of neutrophil oxidative metabolism (17) and degranulation, did not release [3H]arachidonate.

PMNs were isolated from peripheral venous blood from normal healthy volunteers and from two patients with CGD as has been previously described (32). These cells were suspended in Dulbecco phosphate-buffered saline (PBS) containing both calcium and magnesium ions. For the chemiluminescence experiments, the PBS contained glucose (100 mg/dl) since the cells appeared to be maintained for longer periods of time under these conditions. In these experiments, the cells were maintained in plastic tubes on ice and were diluted to $5 \times 10^7$/ml with PBS containing glucose at room temperature immediately before use. PMN chemiluminescence in a luminol-enhanced system was done as previously described (16). For the lipid assays, the cells were suspended at $3.5 \times 10^7$/ml in PBS without glucose. Neutrophils ($3.5 \times 10^7$ cells per ml in PBS) were incubated with [3H]arachidonic acid (0.5 µCi, 0.1 nmol) and [14C]stearic acid (0.1

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μCi, 1.77 nmol) for 2 h at 37°C. The cells were washed once in PBS and resuspended in 0.5 ml of PBS. The reactions were initiated with either 0.5 ml of PBS (resting) or 0.5 ml of the ionophore A23187 in PBS (final concentration, 2 μM A23187) for 5 min at 37°C. Trypan blue exclusion revealed that greater than 95% of the cells remained viable even after a 15-min incubation period with 2 μM ionophore A23187. The incubations were terminated by the addition of 3 ml of chloroform-methanol (1:2, vol/vol). The lipids were extracted by the method of Bligh and Dyer (10) as modified by Bills et al. (9) and separated by thin-layer chromatography (TLC). The neutral lipids were separated in a chloroform-methanol-acetic acid-water (75:50:10:6, vol/vol) system (system I). 11-, 12-, and 15-HETE comigrate with diglyceride in one-dimensional TLC with solvent system I, whereas 5-HETE migrates separately between diglyceride and monoglyceride. Leukotrienes comigrate with MG in one-dimensional TLC with system I. Further identification of [3H]arachidonate lipoxygenase products has been previously made (13, 36). Briefly, the combined HETE and leukotriene fractions were separated from diglyceride and monoglyceride by a two-dimensional TLC system, and the individual HETE and leukotrienes were identified by both normal and reverse-phase high-pressure liquid chromatography. Leukotrienes were further identified by their characteristic spectral absorbance pattern. The major [3H]arachidonate lipoxygenase products formed upon stimulation with the ionophore A23187 were shown to be 5-HETE and leukotriene B_4. Phospholipids were separated by the hexane-ether-formic acid (90:60:6, vol/vol) system (system II). The compounds were visualized by iodine vapors, scraped into scintillation mini-vials, and counted in Budget-Solve scintillation cocktail.

In agreement with reports by Allen et al. (2) and Castranova et al. (12), we found that the ionophore A23187 was unable to stimulate chemiluminescence in PMNs from patients with CGD. The calcium ionophore A23187 stimulated the release of [3H]arachidonate from cellular phospholipids (primarily phosphatidylinositol and secondarily phosphatidylcholine) from both normal and CGD cells (Table 1). It is interesting to note, however, that only approximately half as much [3H]arachidonate was released from phosphatidylinositol in the CGD cells as in the normal cells. In both normal and CGD cells, the [3H]arachidonate released was further metabolized to produce [3H]HETE (primarily 5-HETE) and [3H]-labeled leukotrienes to an equal extent.

There are at least two possible reasons for the difference in the amount of [3H]arachidonate released between CGD and normal cells. First, there may be differences in the precursor pool size as shown by the differences in the amount of [3H]arachidonate incorporated into the phosphatidylerine-phosphatidylinositol fractions of the CGD and normal cells. Second, there may be some deficiency in the phospholipase A_2 activity in neutrophils from patients with CGD.

There have been several proposed mechanisms for the release of arachidonic acid at least from platelet cellular phospholipids: a direct release by phospholipase A_2 (8) or an indirect release by a phosphatidylinositol-specific phos-

### TABLE 1. Distribution of [3H]arachidonate-labeled neutrophil lipids

<table>
<thead>
<tr>
<th>System</th>
<th>Normal</th>
<th>% Distribution</th>
<th>Δ%</th>
<th>CGD</th>
<th>% Distribution</th>
<th>Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBS</td>
<td>A23187</td>
<td></td>
<td></td>
<td>PBS</td>
<td>A23187</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA</td>
<td>5.8 ± 1.2</td>
<td>8.3 ± 1.6</td>
<td>2.5</td>
<td>7.4 ± 3.8</td>
<td>8.9 ± 2.1</td>
<td>1.5</td>
</tr>
<tr>
<td>HETE</td>
<td>8.4 ± 2.1</td>
<td>15.2 ± 2.3</td>
<td>6.8</td>
<td>8.8 ± 5.5</td>
<td>13.4 ± 4.4</td>
<td>4.6</td>
</tr>
<tr>
<td>Leukotrienes</td>
<td>0.9 ± 0.2</td>
<td>5.0 ± 1.3</td>
<td>4.1</td>
<td>1.0 ± 0.5</td>
<td>5.4 ± 1.2</td>
<td>4.4</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td>8.2 ± 1.0</td>
<td>7.8 ± 1.8</td>
<td>-0.4</td>
<td>7.0 ± 0.5</td>
<td>6.9 ± 0.7</td>
<td>-0.1</td>
</tr>
<tr>
<td>PS-PI</td>
<td>23.7 ± 1.6</td>
<td>11.0 ± 0.8</td>
<td>-12.7</td>
<td>17.4 ± 4.1</td>
<td>12.0 ± 2.8</td>
<td>-5.4</td>
</tr>
<tr>
<td>PC</td>
<td>11.1 ± 1.2</td>
<td>8.0 ± 1.0</td>
<td>-3.1</td>
<td>11.8 ± 0.9</td>
<td>8.9 ± 1.4</td>
<td>-2.9</td>
</tr>
</tbody>
</table>

*Neutral lipids were separated by TLC in solvent system I, and phospholipids were separated in solvent system II. Total incorporation of [3H]arachidonate into PMN cellular lipids from both normal and CGD cells ranged from 3 × 10^6 to 5 × 10^6 dpm. There was no significant difference between the two groups in the amount of radiolabel incorporated into cellular lipids. Values for the percent distribution of the [3H]radiolabel represents the mean ± the standard deviation of two to three separate experiments, each done in duplicate. Values for the change in percentages (Δ%) represent the difference between the ionophore (stimulated) and the PBS (resting). Abbreviations: CGD, chronic granulomatous disease; PBS, phosphate-buffered saline; FFA, free fatty acids; HETE, hydroxyeicosatetraenoic acids; PE, phosphatidylethanolamine; PS-PI, phosphatidylserine-phosphatidylinositol; PC, phosphatidylcholine.*
pholipase C which produces an arachidonate-rich diglyceride fraction, which is further broken down, or both. This diglyceride then either may be degraded by a diglyceride lipase that releases free arachidonate and monoacylglycerol (5, 28) or, as has recently been shown by Prescott and Majerus (24), may be degraded by a diglyceride lipase which produces a 2-arachidonoylglycerol that is hydrolyzed by a monoglyceride lipase releasing arachidonate and glycerol. Lapetina et al. (21), and Lapetina and Cuatrecasas (22) have suggested a pathway employing diglyceride kinase, producing phosphatidic acid. Subsequent action by phospholipase A₂ on phosphatidic acid produces free arachidonate and lysophosphatidic acid. To investigate these possibilities in human neutrophils, we employed [¹⁴C]stearic acid (C₁₈:₀) and [³H]arachidonic acid (C₂₀:₄) as precursors for positions 1 and 2 of the phospholipids, respectively. Of the several possible pathways described that could account for the release of free arachidonic acid, our data favor that involving phospholipase A₂, even though we were unable to demonstrate the formation of a lysophospholipid. The reason for this tentative conclusion is that any pathway involving phospholipase C should produce double-labeled diglycerides, which could not be detected at any time of incubation. Recently, Billah and Lapetina (6, 7) have demonstrated, at least in platelets, a metabolic pool of phosphatidylglycerol that is susceptible to degradation by phospholipase A₂ when stimulated with the ionophore A²₃₁₈₇. Our results are highly suggestive of a phospholipase A₂ release mechanism from both phosphatidylglycerol and phosphatidylinositol, although more work may be necessary before the precise mechanism of C₂₀:₄ turnover can be defined.

It had previously been shown that the initiation of the respiratory burst activity in human neutrophils does not necessarily stimulate the release and metabolism of arachidonic acid, since PMA (which is a potent stimulator of the respiratory burst in normal neutrophils) does not stimulate arachidonate release and metabolism (25). The results presented here also indicate a dissociation between the respiratory burst activity and arachidonate release and metabolism, since the ionophore A²₃₁₈₇ does not stimulate the respiratory burst in neutrophils from patients with CGD. The ionophore does, however, stimulate the release and further metabolism of [³H]arachidonate by the lipoxygenase system.

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LITERATURE CITED


