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Hydrolysis of inositol phospholipids precedes cellular proliferation in asbestos-stimulated tracheobronchial epithelial cells

(cell signaling/diacylglycerol/tumor promotion/proliferation/phosphatidylinositol)

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ABSTRACT **Metabolism of inositol phospholipids and phosphatidylcholine was investigated in tracheobronchial epithelial cells exposed to mitogenic concentrations of crocidolite asbestos. Alterations in levels of diacylglycerol, the endogenous activator of protein kinase C, and inositol polyphosphates, presumed mobilizers of intracellular calcium, were examined. Cultures labeled with [^{3}H]glycerol and exposed to proliferative concentrations of crocidolite asbestos demonstrated significant elevations in [^{3}H]diacylglycerol. In contrast, crocidolite-exposed cells labeled with [^{3}H]myristic acid or [^{3}H]choline did not display elevated production of [^{3}H]diacylglycerol or release of [^{3}H]choline metabolites—i.e., evidence of phosphatidylcholine hydrolysis. The soluble tumor promoter phorbol 12-myristate 13-acetate catalyzed both of these changes. *myo*-[^{3}H]Inositol-labeled cells exposed as briefly as 10 min to mitogenic concentrations of crocidolite demonstrated elevations in [^{3}H]inositol mono-, tris-, and terakisphosphates, phenomena indicating turnover of inositol phospholipids. The detection of diacylglycerol and inositol phosphates in crocidolite asbestos-exposed cells suggests that this fibrous tumor promoter activates phospholipase C as it stimulates cellular proliferation.**

Occupational exposure to asbestos results in development of bronchogenic carcinoma, mesothelioma, and asbestosis (1). The pathogenesis of carcinoma caused by asbestos has been studied experimentally in tracheobronchial epithelial cells and organ cultures. Results suggest that asbestos is a classical tumor promoter in the development of bronchogenic carcinoma. For example, many of the effects reported in mouse skin cells exposed to tumor-promoting phorbol esters (2) occur in tracheobronchial epithelial cells exposed to asbestos (3). Additionally, asbestos acts as a tumor promoter in a "two-stage" model of carcinogenesis. Insertion of chrysotile asbestos into rodent tracheal grafts previously exposed to a chemical initiator results in the development of tumors arising from the tracheal epithelium. In contrast, fibers alone do not induce tumors (4).

Many of the morphological and biochemical changes expressed in cells exposed to soluble tumor promoters such as phorbol 12-myristate 13-acetate (PMA) occur after direct interaction of PMA with calcium- and phospholipid-dependent protein kinase C (PKC) (5), an enzyme implicated in cellular growth control. For example, dramatic morphological changes have been seen in rat fibroblasts that overproduce a transfected isoform of PKC (6). Recent work from this laboratory implicates PKC in crocidolite asbestos-induced proliferation of hamster tracheal epithelial (HTE) cells, a cell type giving rise to bronchogenic tumors. For example, asbestos-stimulated induction of ornithine decarboxylase is reduced by inhibitors of PKC (7). Because it is unlikely that soluble asbestos fibers bind directly to PKC as do the lipophilic phorbol esters, we sought to investigate other plausible mechanisms whereby asbestos fibers could initiate PKC-mediated proliferation. In this report, we present evidence that diacylglycerol (acyl$_2$Gro), the endogenous activator of PKC, is produced upon hydrolysis of inositol phospholipids in crocidolite-stimulated HTE cells.

MATERIALS AND METHODS

Chemicals. Union Internationale Contre le Cancer reference samples of crocidolite [$(Na_2Fe_3^{2+}Fe_2^{3+}(Si_8O_{22})(OH)_2$] asbestos were used in these studies. Riebeckite (a nonfibrous particle) and glass beads (8) were used as negative controls to determine whether fibrous geometry was critical for inducing altered lipid metabolism. Physiochemical characterization and size dimensions of fibers and particles have been published (8). PMA from Cancer Research Chemicals (Brewster, NY) was added to the cultures such that the solvent concentration did not exceed 0.1% (vol/vol). Control groups also received the solvent. Phospholipase C (EC 3.1.4.3) from *Bacillus cereus*, bombesin, and *myo*-inositol were obtained from Sigma. [1,2,3-^{3}H]Glycerol, [9,10(n)-^{3}H]myristic acid, [*methyl*-^{3}H]choline chloride, and *myo*-[2(n)-^{3}H]inositol were purchased from DuPont/NEN. Lipid standards were obtained from Avanti Polar Lipids or from Nu-Chek Prep (Elysian, MN). Radiolabeled inositol phospholipid standards were a gift of S. Rittenhouse (University of Vermont College of Medicine). HTE cells, cloned from the tracheobronchial epithelium of a female neonatal golden Syrian hamster, were maintained as described (9).

Radiolabeling and Quantitation of Cellular Lipid Fractions. Late logarithmic phase cultures of HTE cells were labeled with [^{3}H]glycerol (0.5 μCi/ml, 20–40 Ci/mmol; 1 Ci = 37 GBq) for 24 hr and at confluency were incubated briefly (1 hr) with unlabeled medium. Agents (phospholipase C, PMA, or asbestos) then were added in fresh serum-free medium, and exposures were continued for 10–240 min. Next, cultures were rinsed with ice-cold phosphate-buffered saline, fixed in ice-cold methanol/acetic acid, 98:2 (vol/vol), scraped with a rubber policeman into glass scintillation vials, and lipids were extracted (10). This procedure resulted in the quantitative recovery of radioactivity in the lipid fraction. Finally, samples were concentrated, solubilized in methanol/chloroform 2:1 (vol/vol), and resolved by TLC.

Lipid metabolites were analyzed in one of two ways. Radiolabeled samples applied to silica gel G plates (Analtech) were resolved in hexane/diethylether/glacial acetic acid, 50:50:1 (vol/vol/vol) followed by diethylether alone (developed 3 cm from the origin) to separate neutral lipid species

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Abbreviations: acyl$_2$Gro, diacylglycerol; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; HTE, hamster tracheal epithelial; PtdCho, phosphatidylcholine; ANOVA, one-way analysis of variance.

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while phospholipids remained at the origin. Alternatively, chloroform/methanol/petroleum ether/96% (vol/vol) acetic acid/boric acid, 40:20:30:10:1.8 (vol/vol/vol/vol/wt) (12) was used to separate phospholipid species on boric acid-treated plates. Migration of all species was verified with unlabeled standards. Fractions were visualized with iodine vapor and scraped into scintillation vials for quantitation as described (13). Results were normalized to 100,000 cpm of total lipid radioactivity per dish.

Assay of Choline Release. Medium from logarithmic phase cultures was supplemented with [^{3}H]choline chloride (0.60 μCi/ml, 80 Ci/mmol) for ≈48 hr until cells reached confluency. Cultures then were chased with unlabeled medium for 2 hr and crocidolite or PMA was added in fresh serum-free medium. Incubations for various durations were followed by liquid scintillation counting of the radioactivities in both medium and rinsed monolayers solubilized in 1% Triton X-100. The percentage of [^{3}H]choline metabolites released to the medium was calculated as cpm released to medium/[total cpm (cell-associated plus released)] × 100.

Hydrolysis of Inositol Phospholipids. Growth medium of HTE cells was replaced with inositol-free Ham's F-12 medium (GIBCO) containing 10% dialyzed calf serum, 5 μM unlabeled *myo*-inositol, and [^{3}H]*myo*-inositol (5 μCi/ml, 20 Ci/mmol) for 48 hr before adding stimulatory agents. Pilot experiments indicated that this procedure allowed maximal labeling of cellular inositol pools without loss of cell viability. LiCl (final concentration, 10 mM) was added just before incubations with crocidolite or bombesin to inhibit inositol-1-phosphatase (14). Bombesin was used as a positive control because it induced dosage-dependent increases in DNA synthesis and production of inositol polyphosphates in HTE cells (A.S., unpublished data). After exposures of 10–120 min, medium was removed from cultures, and monolayers were fixed in ice-cold chloroform/methanol/hydrochloric acid, 1:2:0.05 (vol/vol/vol) as described (15). Cells then were scraped into glass vials and extracted by adding chloroform and H_2O. After brief centrifugation at 200 × *g*, inositol and inositol polyphosphates were separated on Dowex (formate form) columns and quantified as described (16). The efficiency of this extraction procedure was >90% when 0.01 μCi of tritiated inositol phospholipid standards were tested under identical conditions.

Statistical Methods. Data were analyzed by a one-way analysis of variance (ANOVA) using independent comparisons and Fisher's *F* test of statistical significance (17). The sample population generally consisted of $n = 3$ or $n = 4$ per group, and experiments were repeated two to three times.

RESULTS

Crocidolite asbestos induces a biphasic response in assays of colony-forming efficiency, a highly sensitive indicator of cellular proliferation. Concentrations of crocidolite at 0.05 or 0.1 μg/cm^2 dish induce proliferation, whereas concentrations exceeding 1 μg/cm^2 dish are cytotoxic (18). In studies here, we examined the influence of growth-stimulatory concentrations of crocidolite on the distribution of radioactivity in neutral lipid fractions of HTE cells (Table 1). Lengthy exposures (10–120 min) were used because many cellular responses to asbestos fibers are protracted compared with those induced by soluble tumor promoters. For instance, PMA-induced activation of ornithine decarboxylase occurs in 60–120 min, whereas maximal induction of ornithine decarboxylase by asbestos requires continuous exposure of cells for 240 min (7). Pilot experiments indicated that addition of phospholipase C at 0.025 unit/ml resulted in the rapid production of acyl$_2$Gro from [^{3}H]glycerol-labeled phospholipid pools (data not shown). Indeed, phospholipase C elicits responses similar to those induced by PMA (19) or peptide hormones (20) in many cell types.

Reductions in phospholipid radioactivity at all concentrations of crocidolite (Table 1) suggested that fibers induced phospholipid metabolism at 120-min exposure. However, statistical analysis of the data could distinguish only 0.1 μg of crocidolite per cm^2 dish as significant when compared independently with the untreated control. Notably, this concentration of crocidolite also caused proliferation of HTE cells in the colony-forming efficiency assay (18). The variability inherent in these measurements was evident by the temporally inconsistent reductions in phospholipid radioactivity induced by phospholipase C.

The radioactivity appearing in acyl$_2$Gro and monoacylglycerol fractions also is presented in Table 1. Phospholipase C catalyzed accumulation of acyl$_2$Gro at all exposure periods and also induced formation of monoacylglycerol metabolites (probably through the concerted action of endogenous acyl$_2$Gro lipase). Treatment of cells with crocidolite at 0.1

Table 1. Alterations in radiolabeled phospholipid metabolites of HTE cells upon treatment with phospholipase C or asbestos

Metabolite	Time, min	Control	Phospholipase C	Crocidolite 0.1 μg/cm^2 dish	Crocidolite 0.5 μg/cm^2 dish	Crocidolite 1.0 μg/cm^2 dish
Phospholipid	0	96,714 ± 132				
	10	92,988 ± 274	87,157 ± 2290*	92,173 ± 1680	91,809 ± 1276	94,331 ± 1092
	30	96,702 ± 178	95,303 ± 296	96,503 ± 66	96,965 ± 280	97,270 ± 267
	60	95,459 ± 312	92,633 ± 772*	94,232 ± 1008	95,133 ± 137	95,137 ± 160
	120	95,542 ± 321	94,100 ± 235	93,991 ± 272†	94,738 ± 206	94,434 ± 385
Monoacylglycerol	0	320 ± 26				
	10	242 ± 41	327 ± 24*	292 ± 38	199 ± 31	224 ± 55
	30	323 ± 115	774 ± 86*	521 ± 43	345 ± 131	290 ± 84
	60	249 ± 22	1,107 ± 245*	362 ± 55	346 ± 84	430 ± 78
	120	303 ± 17	734 ± 47*	621 ± 82†	521 ± 26‡	284 ± 25
Acyl$_2$Gro	0	472 ± 46				
	10	394 ± 14	1,064 ± 25*	437 ± 8	405 ± 50	388 ± 10
	30	465 ± 58	1,247 ± 102*	527 ± 14	521 ± 22	471 ± 19
	60	556 ± 99	2,181 ± 506*	542 ± 983	602 ± 17	576 ± 52
	120	603 ± 36	1,682 ± 120*	1430 ± 127†	672 ± 36	793 ± 139

Phospholipase C was at a concentration of 0.025 unit/ml. Data for each time point are from a representative experiment that was repeated once. Values are reported as mean ± SEM for $n = 3$ plates per group.

*$P < 0.05$; significantly different from all other treatment groups by ANOVA and Fisher's *F* test of significance.

†$P < 0.01$; significantly different from unexposed control by ANOVA and Fisher's *F* test.

‡$P < 0.05$; significantly different from that obtained with crocidolite at 1.0 μg/cm^2 dish by ANOVA and Fisher's *F* test.

$\mu g/cm^2$ dish also elevated both monoacylglycerol and $acyl_2Gro$ metabolites at 120-min exposure as compared with the untreated control. Other treatments (120-min exposure to crocidolite at 0.5 $\mu g/cm^2$ dish) appeared to induce similar increases in these minor lipid fractions.

Both growth-stimulatory (0.1 $\mu g/cm^2$ dish) and cytotoxic (5.0 $\mu g/cm^2$ dish) concentrations of crocidolite were examined further for their capacity to stimulate phospholipid metabolism (Fig. 1). Exposures lasted 120 min because this duration maximally changed neutral lipid metabolism in previous experiments. Crocidolite at 0.1 $\mu g/cm^2$ dish significantly decreased radiolabeled phospholipids and concomitantly increased radiolabeled $acyl_2Gro$. The magnitude of these changes resembled those obtained with phospholipase C. Other concentrations of asbestos did not increase $acyl_2Gro$, although crocidolite at 5 $\mu g/cm^2$ dish, a cytotoxic concentration of asbestos, elevated triacylglycerol; the physiological significance of this effect is unclear. The lowest concentration of crocidolite increased triacylglycerol radioactivity but did not affect levels of radioactive monoacylglycerol. Separation of the phospholipids by TLC using a second solvent system failed to reveal the source of the $acyl_2Gro$ induced by crocidolite at 0.1 $\mu g/cm^2$ dish. In that analysis, statistically significant changes were evident only in the neutral lipid fraction, whereas the various phospholipid fractions appeared unchanged (data not shown).

To determine whether the observed stimulation of phospholipid metabolism was caused by nonspecific perturbation of cell membranes by nonfibrous particles, HTE cells were exposed to riebeckite (the chemically identical nonfibrous analog of crocidolite) and glass beads (8). Exposure of cells for 120 min to either particulate at concentrations from 0.1 to 5 $\mu g/cm^2$ dish caused no detectable changes in phospholipid metabolites (data not shown). These results are consistent with the biological behavior of these particulates in biological assays. Glass beads are not growth-stimulatory, and riebeckite is much less potent than crocidolite in eliciting cellular proliferation (18).

Effects of Phorbol Esters. To assess the influence of soluble tumor promoters on phospholipid metabolism, neutral lipid fractions of cultures exposed for 30 min to PMA at 10 and 100 ng/ml were examined (Fig. 2). These concentrations of PMA alter HTE cells morphologically and induce ornithine decarboxylase activity (7) but did not cause accumulation of $acyl_2Gro$ (Fig. 2). Decreased radioactivity in the phospholipid fraction and increased radioactivity in the triacylglycerol fraction were noted in cells exposed to PMA at 100 ng/ml. These phenomena have been reported (21, 22) and may be related to the PtdCho turnover induced by PMA. Because activation of PKC by PMA circumvents the inositol phospholipid pathway of second messenger generation, it is consistent that $acyl_2Gro$ was not increased in response to PMA.

Turnover of PtdCho in HTE Cells. To determine whether elevated $acyl_2Gro$ seen in [^{3}H]glycerol-labeled cells exposed to crocidolite had arisen from PtdCho turnover, HTE cells were labeled with [^{3}H]myristate (11) such that 78% of radiolabel occurred in PtdCho pools and then were exposed to asbestos or PMA. No elevations in radiolabeled $acyl_2Gro$ or reductions in radiolabeled phospholipids were seen in asbestos-stimulated cells at any exposure (data not shown). In contrast, cells exposed to PMA had reduced phospholipid radioactivity at all times with significant elevations in radiolabeled $acyl_2Gro$ seen as early as 30 min after addition. Enhanced radioactivity in the triacylglycerol fraction occurred after 60- and 120-min incubations with PMA, suggesting that elevated triacylglycerol noted earlier in [^{3}H]glycerol-labeled cells (Fig. 2) may have resulted from PtdCho hydrolysis.

To confirm that asbestos did not induce turnover of PtdCho, we measured the release of [^{3}H]choline-containing metabolites from prelabeled cells using a described assay (27) for PMA-induced phospholipid metabolism. Fig. 3 shows that cells exposed to PMA at 100 ng/ml released metabolites of [^{3}H]choline into the growth medium at 30 min of exposure and continuously thereafter, whereas crocidolite-exposed cultures did not differ from untreated controls. Thus, results of experiments using both [^{3}H]myristic acid and [*methyl*-^{3}H]choline chloride suggest that crocidolite asbestos, unlike PMA, does not induce PtdCho turnover.

Inositol Phosphate Production in Asbestos-Exposed Cells. To investigate whether growth-stimulatory concentrations of crocidolite elicited hydrolysis of inositol phospholipids, HTE cells were labeled with [^{3}H]*myo*-inositol, and the production of water-soluble metabolites of inositol was monitored (Table 2). While bombesin appeared to induce production of inositol polyphosphates at all time periods, statistically significant increases in inositol tetrakisphosphates were seen only at 10-min exposure.

Significant increases were detected in the inositol tetrakisphosphate fractions of cells exposed to crocidolite at 0.1 $\mu g/cm^2$ dish at 10-, 30-, and 60-min exposure. Additionally, elevations were seen in the inositol trisphosphate fraction of cells exposed briefly (10 min) to this concentration of fibers. At later times (30-min exposure), increased inositol bisphosphate was seen (data not shown), as was accumulation of inositol monophosphates (Table 2).

Consistent increases in inositol metabolites also were seen in response to crocidolite at 0.05 $\mu g/cm^2$ dish, a proliferative concentration of asbestos in the colony-forming efficiency

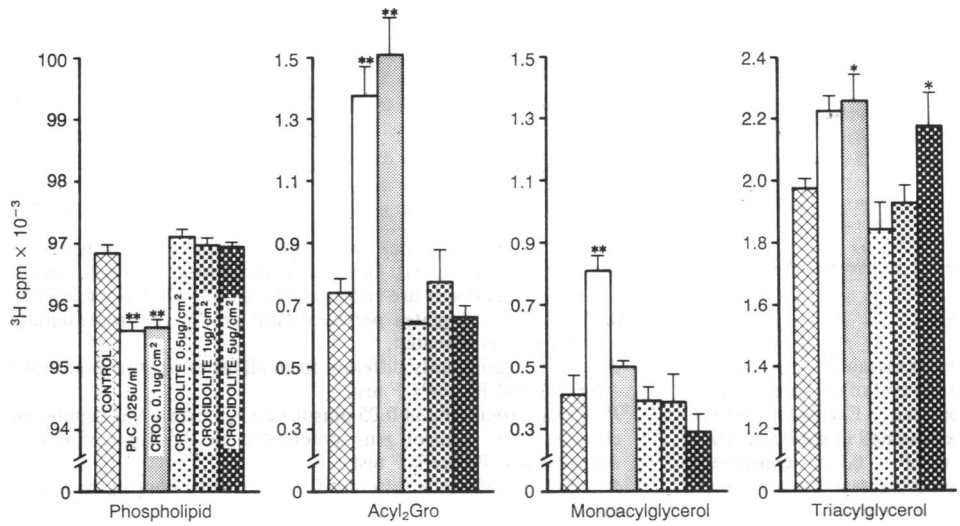

FIG. 1. Asbestos- and phospholipase C (PLC)-induced alterations in neutral lipid radioactivity of HTE cells; exposures were for 120 min in serum-free medium. Bars represent the mean ± SEM of $n = 3$ dishes per treatment group. *, $P < 0.05$; **, $P < 0.01$; significantly different in independent comparisons by ANOVA and Fisher's F test. Croc., crocidolite.

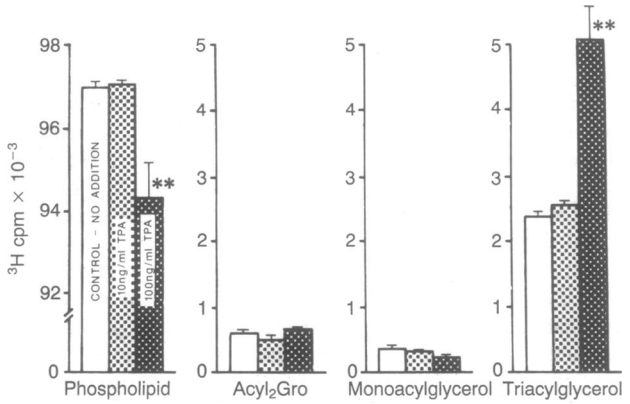

FIG. 2. Cellular lipid metabolism of [^{3}H]glycerol-labeled HTE cells exposed to PMA (TPA). The histogram shows radioactivity appearing in lipid fractions of [^{3}H]glycerol-labeled HTE cells exposed to 0, 10, or 100 ng of PMA per ml for 30 min. Bars represent mean ± SEM of $n = 3$ dishes per group. **, $P < 0.01$; significantly different from control by ANOVA and Fisher's F test.

assay (18). Crocidolite at 0.5 μg/cm^2 dish, a concentration of asbestos that appears neither stimulatory nor cytotoxic, elicited levels of inositol phosphates approximating those of untreated controls. The nonfibrous mineral, riebeckite (at 0.05, 0.1, 0.5, or 1 μg/cm^2 dish), did not induce turnover of inositol phospholipids when tested in similar exposures (data not shown).

DISCUSSION

Occupational exposure to asbestos has been associated with increased risks of malignancies and fibrotic lung disease (1). However, the mechanism(s) of altered cellular proliferation and differentiation by asbestos are unclear. Results here indicate that crocidolite asbestos elicits changes in phospholipid metabolism distinct from those induced by phorbol diesters. Detection of products of inositol phospholipid hydrolysis—i.e., acyl$_2$Gro and inositol phosphate(s)—in asbestos-exposed cells strongly suggests that activation of phospholipase C occurs in response to fibers.

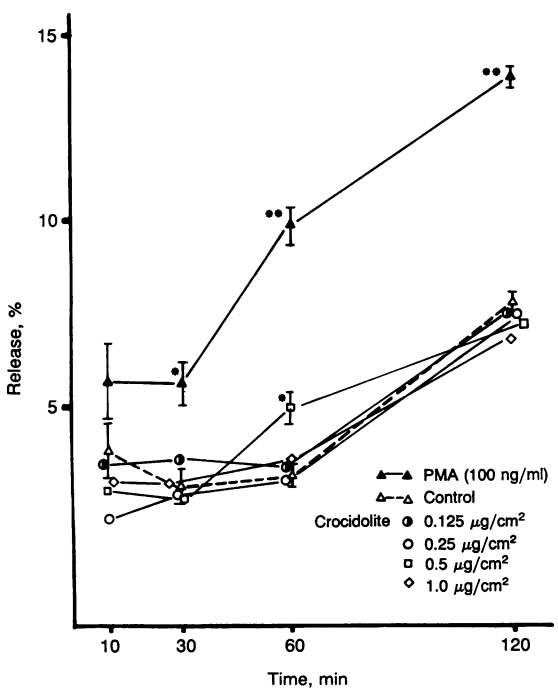

FIG. 3. Release of [^{3}H]choline-containing metabolites from HTE cells treated with PMA or crocidolite. At the end of the incubations, media and cells were sampled for radioactivity. Each point represents the mean ± SEM of a release index obtained in duplicate from $n = 4$ dishes per group. *, $P < 0.05$; **, $P < 0.01$; significantly different from control by ANOVA and Fisher's F test. Data are from a representative experiment performed three times.

The observation that growth-stimulatory, but not cytotoxic, concentrations of crocidolite elicit metabolism of inositol-containing phospholipids is exciting and has broad implications for research on fiber carcinogenesis. Work here indicates that crocidolite fibers, which appear to stimulate cells in a nonreceptor-mediated manner, may activate PKC in target cells of the respiratory tract by eliciting production of acyl$_2$Gro, the physiological activator of PKC. In contrast to the immediate stimulation of PKC by PMA, the time interval that occurs between application of asbestos fibers and measurable cellular responses indicates that other physiological processes (such as hydrolysis of phospholipids) may be required before PKC activation. Indeed, studies from this laboratory have shown that 60- to 180-min exposures of HTE cells to crocidolite fibers result in PKC activation (28). Data also indicate that crocidolite asbestos does not compete with [^{3}H]phorbol dibutyrate for binding to the enzyme. Thus, the fibers do not appear to activate PKC directly.

Table 2. Production of inositol polyphosphates after stimulation by 10 μM bombesin or asbestos

Treatment	Inositol trisphosphate	Inositol tetrakisphosphate	Inositol monophosphate
10 min			
Control	1328 ± 91	2726 ± 78	32,776 ± 1525
Bombesin	1650 ± 63	3333 ± 107*	34,814 ± 5642
Crocidolite			
0.05 μg/cm^2	1941 ± 155†	3372 ± 175†	39,951 ± 4337†
0.1 μg/cm^2	1843 ± 116‡	3129 ± 103‡	43,265 ± 2769
0.5 μg/cm^2	1385 ± 50	2904 ± 150	27,383 ± 1802
30 min			
Control	1218 ± 86	2698 ± 109	5,466 ± 845
Bombesin	1561 ± 156	3204 ± 181	4,807 ± 1027
Crocidolite			
0.05 μg/cm^2	1482 ± 55	3031 ± 46	16,586 ± 4272†
0.1 μg/cm^2	1491 ± 110	3354 ± 151‡	8,910 ± 2914
0.5 μg/cm^2	1322 ± 28	2844 ± 119	6,548 ± 1876
60 min			
Control	1446 ± 62	2634 ± 111	13,652 ± 1420
Bombesin	1509 ± 63	3106 ± 151	15,608 ± 1309
Crocidolite			
0.05 μg/cm^2	1584 ± 89	2893 ± 101	10,075 ± 1363†
0.1 μg/cm^2	1584 ± 58	3114 ± 147‡	18,286 ± 1698‡
0.5 μg/cm^2	1516 ± 119	2678 ± 186	16,688 ± 1605
120 min			
Control	1768 ± 129	3866 ± 94	8,070 ± 2459
Bombesin	1873 ± 129	4089 ± 293	14,916 ± 2291
Crocidolite			
0.05 μg/cm^2	1768 ± 102	4173 ± 76	9,739 ± 2121†
0.1 μg/cm^2	1775 ± 100	4371 ± 245	9,722 ± 2178
0.5 μg/cm^2	1579 ± 61	3912 ± 148	18,880 ± 1532

Inositol polyphosphates were eluted from anion-exchange columns as described in text. Only those fractions critical to the inositol phospholipid pathway are shown. Data are reported as mean dpm ± SEM from $n = 4$ plates per treatment group; this representative experiment was repeated.

*$P < 0.05$; significantly different from all other treatment groups by ANOVA and Fisher's F test.

†$P < 0.01$; crocidolite at 0.05 plus 0.1 μg/cm^2 dish was significantly elevated over control plus crocidolite at 0.5 μg/cm^2 dish by ANOVA and Fisher's F test.

‡$P < 0.05$; significantly different from untreated control by ANOVA and Fisher's F test.

The generation of $acyl_2Gro$ and inositol phosphate(s) by a fibrous mineral agrees with previous observations from this laboratory (7, 18) and others (29) demonstrating the importance of fibrous geometry in eliciting cellular responses. In studies here, nonfibrous particulates such as riebeckite and glass beads did not stimulate production of either $acyl_2Gro$ or inositol phosphate(s).

The delay in appearance of $acyl_2Gro$ after exposure of HTE cells to crocidolite was suggestive of a recently described pathway for generation of $acyl_2Gro$ from PtdCho (11, 24, 30, 31). However, experiments here demonstrate that asbestos does not induce PtdCho turnover.

Radiolabeling of inositol phospholipid pools with [^{3}H]*myo*-inositol enabled detection of inositol phosphates within 10 min after asbestos addition. The rapidity of this response is considerably slower than the metabolism of inositol phospholipids measured in hormone-stimulated cells (23). However, a 10-min delay preceding a biological response to asbestos is not unreasonable because crocidolite fibers (*i*) must contact the target cells physically (i.e., settle out of suspension) before stimulating biochemical responses, and (*ii*) activate cellular responses nonspecifically (in a nonreceptor-mediated manner).

Longer exposures (120 min) were required for asbestos-induced generation of $acyl_2Gro$ in [^{3}H]glycerol-labeled cells. This delay is similar to a second phase of inositol phospholipid hydrolysis described in other experimental systems (25). Other investigators have shown that rapid increases in $acyl_2Gro$ result from hydrolysis of inositol phospholipids, a minor fraction of cellular phospholipids, whereas prolonged elevations in $acyl_2Gro$ may arise from hydrolysis of phosphatidylinositol (26). The $acyl_2Gro$ detected at 120-min exposure of HTE cells to asbestos may result from the hydrolysis of [^{3}H]glycerol-labeled phosphatidylinositol because this phospholipid species (*i*) constitutes a much larger fraction of cellular phospholipids radiolabeled with [^{3}H]glycerol than does phosphatidylinositol bisphosphate and (*ii*) has been shown to be hydrolyzed during the prolonged phase of the biphasic $acyl_2Gro$ response (26). In contrast, inositol polyphosphates detected in [^{3}H]*myo*-inositol-labeled cells exposed to crocidolite presumably arise from hydrolysis of phosphatidylinositol bisphosphate because all inositol polyphospholipids are readily labeled by [^{3}H]*myo*-inositol. Thus, our demonstration of relatively rapid increases in inositol phosphate(s) and delayed elevations in $acyl_2Gro$ suggest that crocidolite may elicit a biphasic hydrolysis of inositol-containing lipids.

Asbestos is multifactorial in its effects on target cells (3). Therefore, other explanations for the protracted accumulation of $acyl_2Gro$ cannot be overlooked. *De novo* synthesis of membrane components necessary for endocytosis could result in significant delays before appearance of $acyl_2Gro$. However, the $acyl_2Gro$ detected in our studies probably did not arise *de novo* because HTE cells were labeled to metabolic equilibrium and not to high specific activity at glycolytic pools.

Our studies provide insight into the mechanisms of cellular stimulation by crocidolite asbestos, a putative tumor promoter in cells of the respiratory tract. Although both fibrous asbestos and the soluble tumor promoter PMA cause proliferative changes in HTE cells, the metabolism of phospholipids induced by the two agents is dissimilar. PMA activates hydrolysis of PtdCho either before or after its direct and exogenous activation of PKC. In contrast, crocidolite does not induce metabolism of PtdCho but stimulates turnover of inositol phospholipids to initiate an activational cascade of events culminating in cellular proliferation. Thus, these seemingly unrelated tumor promoters may exert some of their promotional effects through activation of distinct phospholipid pathways initiated at the plasma membrane.

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