Fatty Acid-stimulated Oxidation of Methylazoxymethanol by Rat Colonic Mucosa

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ABSTRACT

The present study examined fatty acid-initiated metabolism of methylazoxymethanol (MAM) to formaldehyde (HCHO) by the 10,000 x g soluble fraction of rat colonic mucosa, and the role of prostaglandin synthase and lipoxygenase activities in mediating this process. Incubation of MAM with soluble fractions of rat colonic mucosa, in the absence of arachidonate, resulted in the expression of prostaglandin synthase and lipoxygenase activities in mediator NAD+ dehydrogenase pathway and the fatty acid-stimulated pathway. NADPH did not stimulate HCHO formation by 10,000 x g soluble fractions of rat colonic mucosa, and the role of fatty acid-initiated cooxidation in the expression of MAM metabolism and the potential role of such pathways in activation or detoxification of MAM are not known.

In this regard, the cooxidative metabolism of drugs and carcinogens by the PG synthase system has been demonstrated in several tissues including seminal vesicle microsomes, renal medulla, and colonic mucosa (1, 2, 6, 8, 19–21, 28, 29, 33). The PG synthase complex catalyzes the oxygenation of arachidonate to PGG2, and its subsequent reduction to PGH2 (8). Oxidizing equivalents generated during reduction of PGH2 can mediate the oxidation of several classes of xenobiotics (8). Fatty-acid hydroperoxides may also serve as substrates for the peroxidatic activity of PG synthase (19). Our previous studies have supported a role for both colonic cyclooxygenase and lipoxygenase activities in the fatty acid-stimulated cooxidative metabolism of benzo(a)pyrene to metabolites which bind to colonic microsomal protein (6). A potential role of fatty acid-initiated cooxidation in the metabolic activation or detoxification of MAM by colon has not been assessed previously. In the present study, fatty acid-initiated MAM decomposition to formaldehyde (HCHO) was examined in 10,000 x g soluble fractions of colonic mucosa in the presence and absence of inhibitors of cyclooxygenase and lipoxygenase activities. Since previous studies had demonstrated enhanced PG production in superficial compared to proliferative colonic epithelium (4), fatty acid-stimulated MAM metabolism was also assessed in these 2 isolated cell populations.

INTRODUCTION

Expression of the carcinogenic activity of 1,2-DMH, a specifically reactive colon carcinogen (31), is thought to require acti-

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3 The abbreviations used are: 1,2-DMH, 1,2-dimethylhydrazine; MAM, methylazoxymethanol; SKF-525A, 2-diethyleniminomethyl-2,2-dihydropyridine; PG, progastaglind; ETYA, 5,8,11,14-eicosatetraynoic acid; dThd, thymidine.

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MATERIALS AND METHODS

Tissue Preparation. Female Sprague-Dawley rats (Zivic-Miller Laboratories, Pittsburgh, PA) were anesthetized with pentobarbital (5 mg/100 g i.p.), the distal colon resected from the colorectal flexure to 1 cm above the anal orifice and placed in 0.85% NaCl solution (saline) at 4°C which contained 30 mg of penicillin and 5 mg of gentamicin/100 ml. The colons were cleaned and opened longitudinally, and mucosal scrapings were prepared with a metal spatula. Alternatively, superficial and proliferative colonic epithelial cells were prepared as described previously (3, 23). Briefly, the colons were everted, filled with saline, and tied at both ends.
with surgical suture. Each everted colonic loop, from which mesenteric fat had been removed, was incubated in a 50-ml flask in 10 ml of Medium 199 bicarbonate buffer containing 2 mM ethyleneglycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, penicillin (30 mg/100 ml), gentamicin (5 mg/100 ml), and 10% (v/v) heat-treated (56° for 60 min) fetal bovine serum. After a preliminary incubation for 15 min at 37° that resulted in release of a number of cells which did not exclude trypan blue, the colonic epithelial cells were isolated by timed sequential incubations of the colonic loops in the same buffer with the following additions and for the following times: incubation I, 2 mM dithiothreitol, 40 min; incubation II, 27 mM citrate, 15 min; incubation III, 2 mM dithiothreitol, 15 min; incubation IV, 2 mM dithiothreitol, 30 min; and incubation V, 2 mM dithiothreitol, 30 min. Cells from incubations I and II were pooled and are referred to as superficial cells, while cells from incubations IV and V were pooled and are termed proliferative cells. Cells were washed 3 times in Krebs-Ringer-bicarbonate-glucose buffer which had been equilibrated with 95% O2, 5% CO2. Approximately 90 to 95% of the isolated cells were epithelial cells as determined by light microscopy. A section of residual colon was also routinely examined histologically to ensure complete removal of epithelium. Greater than 95% of the isolated cells were viable as determined by trypan blue exclusion. Thorough washing of cells was necessary to remove dithiothreitol, which was found to suppress colonic PG synthesis.

As reported previously (3), the in vivo rate of [3H]dThd incorporation into DNA of cells from normal adult rat colon isolated in Fraction V, is 5- to 10-fold higher than that in Fraction I. The rate of [3H]dThd incorporation into DNA of cells from rat colon in pooled Fractions IV and V is 4- to 7-fold higher than that in pooled Fractions I and II. The in vivo rate of [3H]dThd incorporation into DNA was determined in epithelial cells which had been isolated from the colons of rats sacrificed 2 hr after injection of 50 μCi of [3H]dThd as described in detail previously (3). Pooled Fractions IV and V contained approximately 65 to 68% of the total protein and DNA recovered in Fractions I to V, whereas pooled Fractions I and II contained 17 to 25% of the total recovered protein and DNA (3).

Mucosal scrapings (100 mg tissue/1 ml buffer) or epithelial cells (100 mg wet weight of cells/1 ml buffer) were homogenized in 50 mM Tris-2 mM EDTA, pH 7.4. Homogenates were centrifuged at 10,000 × g. The freshly prepared 10,000 × g supernatant, which contains microsomes, was incubated for various periods of time in a final volume of 0.5 ml with test agents present at the final concentration indicated in the text. Between 0.3 and 0.8 mg of protein from the 10,000 × g soluble fraction of mucosal scraping and epithelial cell homogenates was routinely used for measurements of MAM metabolism to HCHO. In each experiment, a buffer blank which contained MAM was routinely incubated with the experimental samples at 37°. All results were corrected for the spontaneous generation of HCHO in the buffer blank.

Determination of HCHO. For determination of HCHO, incubations were stopped by addition of 0.25 ml of cold 20% trichloroacetic acid. Following centrifugation, 0.5 ml of the clear supernatant was assayed for HCHO by the procedure of Nash (23). Standard curves were linear between 0.007 and 0.14 μmol of HCHO. Under the conditions described, HCHO generation was linear for at least 30 min with 0.3 to 0.8 mg of protein from the mucosal 10,000 × g soluble fraction.

Preparation of MAM. MAM was prepared from MAM acetate by reaction with horse serum cholinesterase as described previously (16). Approximately 90% conversion of MAM acetate to MAM was observed as judged by reaction with m-nitrophenol (27). MAM was used in all the studies described in the present communication.

Preparation of Arachidonate Hydroperoxide. 15-Hydroperoxy-5,8,11,13-eicosatetraenoic acid was prepared by incubation of arachidonate with soybean lipoxygenase in 50 mM borate buffer at pH 9.0 for 5 min at 0° as described previously (14).

Results were repeated 3 or 4 times and the average value of duplicate determinations from each experiment entered as a single number (d.f. = 4 or 6, respectively, comparing any 2 parameters by the independent t test).

Materials. MAM acetate and azoxymethane were obtained from the IIT Research Institute, Chicago, IL. ETYA and SKF-525A were gifts of Hoffmann-LaRoche Inc., Nutley, NJ, and Smith Kline & French Laboratories, Philadelphia, PA, respectively. Lipoxygenase (type I), pyrazole, and 1,2-DMH were obtained from Sigma Chemical Co., St. Louis, MO. Arachidonate and linoleate were purchased from NuChek Prep, Inc., Elysian, MN. The sources of all other reagents have been described previously (3, 4, 6).

RESULTS

MAM is known to spontaneously decompose to the methylating agent, methyldiazonium and HCHO with a half-life of about 12 hr at 37° (16, 22). Chart 1 illustrates the time course of decomposition of MAM to HCHO in buffer alone compared to that observed when MAM was incubated with the colonic mucosal 10,000 × g soluble fraction. All incubations contain 100 μM arachidonate. As illustrated, incubation of the 10,000 × g soluble fraction of colonic mucosal homogenates with MAM in the presence of 100 μM arachidonate resulted in enhanced generation of...
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HCHO compared to that observed when MAM was incubated in buffer alone. The initial rate of HCHO production from MAM during the first 5 min of incubation was increased 2- and 10-fold in the presence of 0.38 and 1.9 mg of protein, respectively. All subsequent results were corrected for HCHO production in buffer alone incubated at 37° under the same conditions as the experimental samples.

As shown in Chart 2, significant HCHO production occurred with 1 mM MAM, and optimal HCHO generation was observed with 25 to 50 mM MAM. Results shown for MAM metabolism were corrected for spontaneous decomposition of MAM in buffer alone under the same conditions. The concentration of MAM used for the indicated concentration of MAM (C––C), azoxymethane (C––C) or 1,2-DMH (C––C). At the end of 30 min, the HCHO content of the incubation mixture was extracted with trichloroacetic acid and assayed as described in "Materials and Methods." Results shown are means of duplicate determinations from a representative experiment verified in 2 additional separate studies.

The results of Charts 1 and 2 were obtained in the presence of 100 μM arachidonate. As illustrated in Table 1, significant HCHO production from MAM was also observed in the absence of arachidonate. Inclusion of arachidonate in the incubation medium resulted in a 50% stimulation of HCHO production from MAM. Linoleate and arachidonate hydroperoxide also stimulated HCHO production at each concentration of MAM used. By contrast, peritoneal macrophages and reaction with arachidonate (100 mM) suppressed HCHO formation from MAM in superfi
cial colon epithelial cells are more active in generating PGs than are proliferative cell homogenates approximately 50 to 60% in the presence or absence of arachidonate. ETYA (100 mM) inhibited HCHO formation by approximately 90% in the presence or absence of arachidonate.

Previous studies have supported a role for MAM metabolism via alcohol dehydrogenase in expression of the toxic and carcinogenic action of MAM in the colon (16, 24). Metabolism of MAM to methyazaohydroxymethanal via alcohol dehydrogenase is measured by reduction of NAD+ in the presence of MAM (16). Accordingly, we compared the ability of the colon mucosal 10,000 x g soluble fraction to metabolize MAM to HCHO via the fatty acid hydroperoxide-induced increases in HCHO. ETYA (500 μM) suppressed HCHO production by MAM by 75 to 80% in the absence of fatty acids and abolished the stimulatory actions of arachidonate and linoleate on this parameter. Analogous to results obtained with indomethacin, arachidonate hydroperoxide overcame the inhibitory effects of ETYA on HCHO production. Spontaneous HCHO production in incubates of MAM in buffer alone at 37° was not influenced by any of the test agents shown in Table 1.

Table 2 illustrates the effect of varying concentrations of indomethacin or ETYA on metabolism of MAM to HCHO. As illustrated, indomethacin at 25 μM reduced HCHO formation by 30 to 40% in the presence or absence of arachidonate. No significant effect was detectable with 10 μM indomethacin (not shown). Raising the concentration of indomethacin to 100 μM further reduced HCHO formation by 50 to 60% compared to the values obtained in the absence of indomethacin. Further increases in indomethacin to 300 μM concentration did not result in further reductions in HCHO formation compared to that observed at 100 μM. Moreover, indomethacin did not completely abolish arachidonate stimulation of HCHO formation at any of the concentrations tested in Table 2. As is also shown, 25 μM ETYA reduced HCHO formation by 60 to 65% in the presence or absence of arachidonate. No significant effect was detectable with 10 μM ETYA (not shown). Increasing the ETYA concentration to 100 μM further reduced HCHO formation by 75 to 80% compared to the values obtained in the absence of ETYA, but did not completely abolish arachidonate-induced increases in HCHO. As is also shown in Table 2, 500 μM ETYA did not further suppress basal HCHO formation compared to that observed with 100 μM ETYA. However, 500 μM ETYA completely blocked the arachidonate-induced increase in HCHO formation.

As illustrated in Table 3, inclusion of NADPH plus MgCl2 in the incubation mixture did not influence HCHO production from MAM. Moreover, 2 inhibitors of cytochrome P-450 activity in colonic mucosa, 7,8-benzoflavone, and SKF-525A (12, 13), and an inhibitor of NADPH-dependent amine oxidase, methimazole (26), were without effect on HCHO generation from MAM in the presence or absence of NADPH.

Previous studies from this laboratory have demonstrated that isolated superficial colonic epithelial cells are more active in generating PGs than are proliferative cells (4). Accordingly, we compared oxidation of MAM to HCHO by 10,000 x g soluble fractions of superficial or proliferative epithelial cell homogenates. As shown in Table 4, the soluble fractions of superficial cells were 3 to 4 times more active in the metabolism of MAM to HCHO than were proliferative epithelial cell fractions in both the presence and absence of exogenous arachidonate. Indomethacin (100 μM) suppressed HCHO formation from MAM in superficial or proliferative cell homogenates approximately 50 to 60% in the presence or absence of arachidonate. ETYA (100 μM) inhibited HCHO formation by approximately 90% in the presence or absence of arachidonate.

Previous studies have supported a role for MAM metabolism via alcohol dehydrogenase in expression of the toxic and carcinogenic action of MAM in the colon (16, 24). Metabolism of MAM to methyazaohydroxymethanal via alcohol dehydrogenase is measured by reduction of NAD+ in the presence of MAM (16). Accordingly, we compared the ability of the colon mucosal 10,000 x g soluble fraction to metabolize MAM to HCHO via the fatty acid hydroperoxide-induced increases in HCHO.
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Table 1
Effects of fatty acids, arachidonate hydroperoxide, and inhibitors of cytochrome P-450 on the metabolism of MAM in colonic mucosal homogenates

The 10,000 x g soluble fraction of colonic mucosal scraping homogenates (0.52 mg protein) was preincubated for 10 min at 37°C with no addition, indomethacin (300 μM), or ETYA (500 μM), as indicated under initial conditions. MAM (5 mM) was then added to all the incubates. Where shown under final additions, arachidonate (100 μM), linoleate (100 μM), palmitate (100 μM) or arachidonate hydroperoxide (10 μM) were added and the incubation continued for an additional 30 min.

<table>
<thead>
<tr>
<th>Initial additions</th>
<th>None</th>
<th>Indomethacin</th>
<th>ETYA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCHO production (nmol/min/mg protein)</td>
<td>6.2 ± 0.9</td>
<td>6.4 ± 0.8</td>
<td>6.2 ± 1.0</td>
</tr>
</tbody>
</table>

Table 2
Concentration-response relationship between indomethacin or ETYA and HCHO production from MAM

The 10,000 x g soluble fraction of colonic mucosal scraping homogenates was preincubated for 10 min at 37°C with no addition, indomethacin, or ETYA at the final concentration shown. MAM (5 mM) and, where indicated, arachidonate (100 μM) were added and the incubation continued for 30 min.

<table>
<thead>
<tr>
<th>Initial additions</th>
<th>None</th>
<th>Arachidonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCHO production (nmol/min/mg protein)</td>
<td>8.1 ± 1.0</td>
<td>8.1 ± 1.0</td>
</tr>
</tbody>
</table>

Table 3
Effects of NADPH and inhibitors of mixed function oxidase activity on HCHO production from MAM in colonic mucosal homogenates

The 10,000 x g soluble fraction of colonic mucosal homogenates was preincubated for 10 min at 37°C with no addition or the test agents shown under initial additions at a final concentration of 0.5 mM. MAM (5 mM) was then added to all the incubates. NADPH (0.5 mM) plus MgCl₂ (5 mM) was then added where indicated under final additions, and the incubation continued for an additional 30 min.

<table>
<thead>
<tr>
<th>Initial addition</th>
<th>Final addition</th>
<th>HCHO (nmol/min/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>NADPH</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>7,8-Benzoflavone</td>
<td>NADPH</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>SKF-525A</td>
<td>NADPH</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>Methimazole</td>
<td>NADPH</td>
<td>4.5 ± 0.6</td>
</tr>
</tbody>
</table>

Table 4
Arachidonate-stimulated HCHO production from MAM in superficial versus proliferative colonic epithelial cell homogenates

The 10,000 x g soluble fraction of homogenates of superficial (0.31 mg of protein) or proliferative (0.82 mg of protein) epithelial cells was preincubated for 10 min at 37°C with or without indomethacin (100 μM) or ETYA (100 μM) as indicated. MAM (5 mM) was then added to all the incubates. Where shown, arachidonate was added at a 100 μM final concentration, and the incubation continued for an additional 30 min.

<table>
<thead>
<tr>
<th>Arachidonate absent</th>
<th>Arachidonate present</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCHO (nmol/min/mg protein)</td>
<td>Superficial cells</td>
</tr>
<tr>
<td>None</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>+ Indomethacin</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>+ ETYA</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>+ arachidonate</td>
<td>5.9 ± 0.7</td>
</tr>
<tr>
<td>+ arachidonate + ETYA</td>
<td>0.21 ± 0.03</td>
</tr>
</tbody>
</table>

DISCUSSION
Expression of the carcinogenic activity of 1,2-DMH is thought to require metabolic activation in the liver to the more proximate

MAM. As shown in Table 5, addition of exogenous arachidonate increased the metabolism of MAM to HCHO about 50%. Addition of NAD⁺ to the incubation had no effect on HCHO production in the presence or absence of arachidonate. Moreover, pyrazole, an inhibitor of colonic alcohol dehydrogenase activity (15), had no effect on HCHO formation in the presence or absence of arachidonate or NAD. By contrast, indomethacin and ETYA suppressed HCHO production from MAM by 50 to 80%, respectively. However, as is also shown in Table 5, incubation of NAD⁺ with the 10,000 x g soluble fraction of colonic mucosal homogenates in the presence of MAM resulted in significant reduction of NAD⁺ as measured by an increase in absorption at 340 nm. The capacity of colonic mucosal 10,000 x g soluble fractions to reduce NAD⁺ in the presence of 5 mM NAD⁺ was similar to its capacity to metabolize MAM to HCHO. When determined under these conditions, 5 mM MAM did not result in optimal NAD⁺ reduction, as reported previously (16). Raising the concentration of MAM to 9 or 25 mM resulted in increased metabolism of MAM by the NAD⁺ dependent dehydrogenase activity (data not shown), an observation also in agreement with earlier reports (16). However, as shown in Chart 2, increasing the concentration of MAM also increased its metabolism via the fatty acid-initiated pathway. Addition of arachidonate, indomethacin, or ETYA to the incubation did not influence NAD⁺ reduction (Table 5). By contrast, pyrazole, an inhibitor of alcohol dehydrogenase activity, abolished NAD⁺ reduction (Table 5).

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Table 5

<table>
<thead>
<tr>
<th>Initial additions</th>
<th>Final additions</th>
<th>HCHO (nmol/min/mg protein)</th>
<th>NADH (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>4.1 ± 0.5a</td>
<td>NDb</td>
</tr>
<tr>
<td></td>
<td>Arachidonate</td>
<td>6.8 ± 0.9c</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>NAD+</td>
<td>3.9 ± 0.5</td>
<td>6.2 ± 0.9c</td>
</tr>
<tr>
<td></td>
<td>NAD+ + arachidonate</td>
<td>6.7 ± 0.9c</td>
<td>6.1 ± 1.0c</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>NAD+</td>
<td>2.2 ± 0.4d</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>ETYA</td>
<td>NAD+</td>
<td>1.1 ± 0.1d</td>
<td>6.2 ± 1.0</td>
</tr>
<tr>
<td>Pyrazole</td>
<td>None</td>
<td>3.9 ± 0.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>NAD+</td>
<td>4.1 ± 0.7</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Arachidonate</td>
<td>6.4 ± 1.0</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Mean ± S.E. of 6 determinations pooled from 3 separate experiments.

b ND, not determined.
c p < 0.05 compared to corresponding value in the absence of a final addition.
d p < 0.05 compared to corresponding value in the absence of an initial addition.

carcinogen MAM (9, 32). Azomethane and azoxymethane are intermediates in this proposed pathway (9, 32). Studies in intact animals and perfused liver have identified MAM, azomethane, and azoxymethane in the hepatic perfusates or urine of rats exposed to [14C]1,2-DMH (9, 32). Moreover, hepatic microsomes, but not colonic microsomes, convert azomethane to MAM in vitro (9). The spontaneous decomposition of MAM results in the formation of HCHO plus a methylating agent, methylidiazonium. Methylation of critical cellular macromolecules may mediate the carcinogenic effects of 1,2-DMH and its metabolites. However, several lines of evidence support a role for the enzymatic decomposition of MAM in the expression of its carcinogenic activity (11, 16, 24, 32). Studies in perfused rat liver have demonstrated more rapid decomposition of MAM than expected based on spontaneous decomposition alone (32). Recent work by Grab and Zedeck (16) has clearly demonstrated metabolism of MAM by an NAD+-dependent dehydrogenase activity in soluble fractions of colonic mucosa. The ability of pyrazole, an inhibitor of alcohol dehydrogenase activity, to suppress MAM-induced reduction of NAD+, and of MAM to serve as a substrate for purified alcohol dehydrogenase activity, led to the suggestion that an alcohol dehydrogenase-like activity was responsible for MAM metabolism in colon mucosa (16). Administration of pyrazole to rats abolished the carcinogenic effects of MAM acetate in the rat colon (24), also suggesting that metabolism of MAM by alcohol dehydrogenase activity may be essential for expression of its carcinogenic activity in colon mucosa. Paradoxically, treatment of rats with pyrazole, while suppressing MAM-induced colonic tumor formation, led to an increase in the development of tumors in skin and kidney (24). This raised the possibility that there may be other pyrazole-insensitive enzymatic pathways for MAM activation.

Results of the present study clearly demonstrate that MAM decomposition to HCHO can be accelerated as much as 10-fold above its spontaneous rate of decomposition by the addition of the colonic mucosal soluble fraction plus arachidonate. Several observations suggested a link between arachidonate-stimulated MAM decomposition and colonic mucosal PG synthesis. Indomethacin suppressed MAM decomposition in the presence or absence of arachidonate. In the present study, the concentrations of indomethacin which suppressed basal and arachidonate-induced increases in generation of HCHO from MAM were in the range previously reported to suppress cooxidative metabolism of N-alkyl compounds by the PG endoperoxide synthetase system of ram seminal vesicles (30). Our own previous studies have demonstrated that superficial colonic epithelial cells are more active at producing PGs than are proliferative colonic epithelial cells (4). In the present study, superficial cell-soluble fractions were more active than proliferative cell-soluble fractions in converting MAM to HCHO.

Evidence was also obtained for a role for lipid peroxidation, possibly mediated by lipoxygenase activity in the decomposition of MAM to HCHO. Linoleic acid, which is not a substrate for cyclooxygenase, increased MAM decomposition. The effects of linoleic acid were not suppressed by indomethacin but were suppressed by ETYA, an inhibitor of both cyclooxygenase and lipoxygenase activities in other tissues (7). Moreover, the inhibitory effects of ETYA on basal and arachidonate-stimulated MAM decomposition were clearly greater than those of indomethacin. In the presence of ETYA or indomethacin, 15-hydroperoxy-ETYA increased MAM decomposition. This also implied a peroxidative mechanism for MAM decomposition. The present results are consistent with the possibility that both the cyclooxygenase and lipoxygenase pathways may be involved in the mediation of basal and arachidonate-stimulated MAM decomposition. A similar conclusion was also reached in our previous studies of the fatty acid-dependent metabolism of benzo(a)pyrene to protein-bound metabolites (6). In this regard, it is notable that recent observations, demonstrating the production of 12-hydroxyeicosatetraenoic acid by colonic mucosa, support the presence of lipoxygenase activity of this tissue (5).

Under certain conditions of incubation in the present study, the increase in HCHO formed from MAM upon addition of arachidonate or arachidonate hydroperoxide to the incubation mixtures is actually greater than the amount of arachidonate (Charts 1 and 2) or arachidonate hydroperoxide (Table 1) added. Previous studies of cooxidative metabolism by the PG synthase system of ram seminal vesicles have also demonstrated that the stoichiometry of diphenylisobenzofuran oxidized to arachidonate added greatly exceeds 1:1 (19). In the case of diphenylisobenzofuran, this is likely explained by a mechanism of reaction involving radical intermediates (8, 19). A free radical mechanism resulting in the sequential one electron reduction of oxidized heme to ferric heme has also been proposed for PG synthase-mediated cooxidation of phenylbutazone and trans-7,8-dihydro-7,8-dihydrobenzo(a)pyrene (8). By contrast, other substrates have been shown to be oxidized by the PG synthase system in 1:1 stoichiometry with 15-hydroperoxy PGE2 (8). The mechanism of fatty acid-dependent oxidation of MAM by colonic mucosal 10,000 × g supernatants is not known. Moreover, it is not possible to exclude the possibility that other fatty acid in dependent enzymatic mechanisms, in addition to PG synthase and lipoxygenase-mediated oxidation, are contributing to the high rate of HCHO formation observed relative to arachidonate added. Notably, significant HCHO production was observed in
the 10,000 × g soluble fraction in the absence of exogenous arachidonate. The latter might be explained by the release of endogenous fatty acids during tissue processing. However, indomethacin or ETYA did not completely abolish this activity, suggesting the involvement of other mechanisms for MAM metabolism. Clearly, further work aimed at identifying the intermediates in MAM oxidation is needed to elucidate the pathways involved.

The fatty acid-stimulated pathway for MAM decomposition was clearly distinct from the NADPH-dependent cytochrome P-450 and amine oxidase mechanism. Thus, consistent with previous observations in hepatic microsomes (16), NADPH did not stimulate the decomposition of MAM to HCHO in the presence or absence of arachidonate. Moreover, SKF-525A and 7,8-benzoflavone, inhibitors of colonic cytochrome P-450 activity (12, 13), and methimazole, an inhibitor of amine oxidase activity (26) did not influence MAM decomposition to HCHO in the presence or absence of arachidonate. The fatty acid-stimulated pathway for metabolism of MAM to HCHO was also clearly distinct from the NAD+-dependent dehydrogenase activity previously described in colonic mucosa (11, 16). The product of the action of the NAD+-dependent dehydrogenase on MAM is presumed to be methylazoxymethanol (19). HCHO is not a proposed product in the decomposition of MAM via the NAD+-dependent dehydrogenase pathway. In the present study, addition of NAD+ to the mucosal soluble fractions of homogenates did not increase the production of HCHO from MAM. However, significant reduction of NAD+ to NADH was observed when NAD+ was incubated with the colonic mucosal soluble fraction in the presence of MAM. The MAM-dependent reduction of NAD+ by the colonic mucosal 10,000 × g soluble fraction was not influenced by arachidonate, indomethacin, or ETYA, but was abolished by pyrazole. Conversely, the fatty acid-stimulated decomposition of MAM to HCHO was not influenced by NAD+ or pyrazole but was suppressed by indomethacin or ETYA. Thus, our results support the existence of at least 2 distinct enzymatic pathways for the metabolism of MAM in colonic mucosa: (a) an NAD+-dependent dehydrogenase pathway; and (b) a fatty acid-stimulated cooxidation pathway. On a molar basis, the relative rates of decomposition of MAM by these 2 pathways were similar.

Pyrazole has previously been shown to abolish colon tumor formation in response to MAM (24). Since fatty acid-stimulated MAM metabolism is not pyrazole sensitive, this might suggest that the fatty acid-dependent pathway for MAM oxidation does not play a key role in expression of the carcinogenic action of MAM. However, the suppressive effects of pyrazole may not be specific for alcohol dehydrogenase activity and may be expressed at more than one step in the carcinogenic process. Thus, in addition to MAM oxidation, pyrazole inhibits the conversion of azoxymethane to MAM which is not thought to be catalyzed by alcohol dehydrogenase (11). Moreover, the in vivo and in vitro rate of MAM metabolism in mice devoid of alcohol dehydrogenase activity was the same as that in control mice, and pyrazole suppressed MAM metabolism in both strains of mice (10). The ability of pyrazole to completely block colonic tumor formation in response to MAM (24), despite the fact that MAM can decompose spontaneously to yield a methylating agent (16, 22), raises the possibility that pyrazole may inhibit MAM carcinogenesis at steps other than MAM metabolism.

Numerous previous studies have shown that indomethacin suppresses colonic tumor growth in response to several carcinogens including MAM (17) and 1,2-DMH (25). However, in these studies indomethacin was given after carcinogen treatment was completed and microscopic lesions had already appeared in the colon (17, 25). Thus, in these studies, an effect of indomethacin on tumor promotion was demonstrated. This action of indomethacin may occur by mechanisms quite independent of those of the drug on either MAM metabolism or local colonic generation of PGs.

Clearly, further work is needed to define the significance of fatty acid-stimulated cooxidation in the expression of the carcinogenic action of MAM. Specifically, the influence on tumor development of administration of inhibitors of cyclooxygenase and/or lipoxygenase activity when given during or prior to the stage of carcinogen activation must be examined. Since fatty acid-initiated metabolism of MAM occurs preferentially in the superficial epithelial cells of colon, which are destined to slough, it is possible that this pathway represents a protective mechanism against the potential carcinogenic effects of MAM on the dividing cells found in the deeper levels of the crypts.

REFERENCES

OXIDATION OF METHYLAZOXYMETHANOL BY COLONIC MUCOSA


Fat ty Acid-stimulated Oxidation of Methylazoxymethanol by Rat Colonic Mucosa

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