Fatty Acid-dependent Benzo(a)pyrene Oxidation in Colonic Mucosal Microsomes: Evidence for a Distinct Metabolic Pathway

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ABSTRACT

The present study examines and compares the ability of reduced nicotinamide adenine dinucleotide phosphate or fatty acids to increase the conversion of \(^{3}\)Hbenzo(a)pyrene (BP) to protein-bound metabolites. The results demonstrate the presence of a pathway for fatty acid-dependent BP oxidation in colonic microsomes, which is distinct from reduced nicotinamide adenine dinucleotide phosphate-dependent cytochrome P-450 activity. The unsaturated fatty acids (arachidonate or linoleate, but not palmitate) increased \(^{3}\)HBP metabolism approximately 2-fold. The product of fatty acid-induced increases in \(^{3}\)HBP metabolism was tightly bound to protein and could not be extracted, indicating the generation of reactive BP metabolites during cooxidation. In contrast to fatty acids, no increase in protein-bound metabolites was observed with reduced nicotinamide adenine dinucleotide phosphate compared to that in the heated microsome control. Similar concentration response relationships were observed between arachidonate-induced increases in immunoreactive prostaglandin E (IPGE) synthesis and conversion of \(^{3}\)HBP to protein-bound metabolites. Half-maximal effects of arachidonate on both parameters were observed at 10 \(\mu\)M with optimal effects expressed at 25 \(\mu\)M arachidonate. The prostaglandin endoperoxide synthetase inhibitor, indomethacin, suppressed basal and arachidonate-stimulated \(^{3}\)HBP metabolism (35 to 65%) and inhibited microsomal IPGE synthesis (90 to 95%). In contrast, 2-diethylaminoethyl-2,2-diphenylvalerate (20 \(\mu\)M) or 7,8-benzoflavone (50 \(\mu\)M), cytochrome P-450 inhibitors, did not influence basal or fatty acid-stimulated \(^{3}\)HBP metabolism. The ability of linoleic acid, which did not increase microsomal IPGE accumulation, to increase \(^{3}\)HBP metabolism and the failure of indomethacin to completely suppress arachidonate-induced increases in \(^{3}\)HBP metabolism suggest a pathway for fatty acid-induced \(^{3}\)HBP metabolism independent of prostaglandin synthesis. A role for fatty acid hydroperoxides in colonic \(^{3}\)HBP metabolism was supported by the ability of 5,8,11,14-eicosatetraynoic acid, an inhibitor of fatty acid oxidation, to suppress both arachidonate- and linoleate-induced increases in \(^{3}\)HBP metabolism. These results support the existence of a distinct fatty acid-responsive pathway for \(^{3}\)HBP metabolism in colonic microsomes. The relative importance of this pathway and the cytochrome P-450 pathway for local colonic metabolism of this and other procarcinogens requires further study.

INTRODUCTION

BP is metabolically activated by several mammalian tissues, including colon, to electrophilic derivatives which covalently bind to protein and DNA and are mutagenic (3, 12, 14, 18). The NADPH-dependent cytochrome P-450 system has been studied extensively in liver and is known to mediate this activation process (13, 16, 39). There is evidence that colonic mucosal microsomes and colonic explants in culture metabolize BP and other polycyclic hydrocarbons via a NADPH-dependent cytochrome P-450 system similar to that found in the liver (1–3, 11, 12). Nevertheless, in the absence of inducers of microsomal cytochrome P-450 activity, the NADPH-dependent pathway for BP hydroxylation is approximately 20-fold lower in colon than in liver microsomes (11). It has recently been shown in several tissues that xenobiotics may also be metabolized via cooxidation during the synthesis of prostaglandins (5, 7, 10, 24–26, 32, 33, 41). These studies have demonstrated the presence of a distinct (NADPH-dependent) arachidonic acid-responsive, cyclooxygenase-dependent pathway for drug and carcinogen metabolism with broad substrate specificity in sheep seminal vesicles (10, 24–26), rat renal inner medulla (40, 41), urinary bladder (5), and lung (32, 33). In a previous report, we described a fatty acid-responsive pathway in colonic mucosal microsomes for the metabolism of drugs and carcinogens (7). This pathway is at least in part linked to cyclooxygenase activity (7). Previous studies have suggested that the products of BP metabolism may differ when microsomal oxidation is stimulated by NADPH versus arachidonic acid (32, 33). In lung, the percentage of metabolites that bound to protein was higher when microsomes were incubated with arachidonic acid than when NADPH was used as a stimulus (32, 33).

Accordingly, in the present study, we examined the effects of fatty acids versus NADPH on the binding of metabolites to protein in rat colonic mucosal microsomes. The prostaglandin synthetase inhibitor indomethacin and the cytochrome P-450 inhibitors 7,8-benzoflavone and SKF-525A were used to differentiate between stimulatory effects of fatty acids on the cyclooxygenase and mixed-function oxidase pathways. The results support the existence of a distinct, NADPH-independent, fatty acid-responsive pathway(s) in colonic mucosal micro-

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somomes, linked in part to prostaglandin synthesis, which oxidizes BP to reactive metabolites that bind to protein.

**MATERIALS AND METHODS**

**Preparation of the Microsomal Fraction.** Female Sprague-Dawley rats (Zivic Miller Laboratories, Pittsburgh, Pa.) were anesthetized with pentobarbital (5 mg/100 g i.p.), and the distal colon was excised and placed in 0.85% NaCl solution at 4°C. The microsomal fraction was prepared from mucosal scrapings as described previously (7). Briefly, mucosal scrapings were washed 3 times by centrifugation at 2000 × g in cold 10 mM Tris-HCl, pH 7.4, containing 0.14 M KCl and 10 mM EDTA. The washed scrapings were then homogenized in the Tris-KCl buffer and centrifuged at 8,500 × g for 6 min. The 8,500 × g supernatant was centrifuged at 105,000 × g for 60 min, washed once in the Tris-KCl buffer, and resuspended in 0.1 M phosphate, pH 7.8.

**Incubation and Extraction of Microsomes for the Determination of [3H]BP Metabolism.** The freshly prepared microsomal fraction was suspended in O2-saturated 0.1 mM phosphate buffer, pH 7.8, at a protein concentration of 0.25 to 0.50 mg/ml. Aliquots of 3 ml were incubated at 37°C in 25-ml flasks with vigorous agitation under an atmosphere of 100% O2. In some experiments, O2 was excluded, and the buffer was equilibrated with 100% N2. The microsomal incubates were preincubated for 5 min during which time test agents were added where indicated in the text. Following the 5-min preincubation, additional test agents plus [3H]BP dissolved in 5 μl dimethyl sulfoxide (25 μCi/flask) were added, and the incubation was continued for an additional 30 min. The final concentration of BP in the flask was 0.5 μM. At the end of the incubation, microsomal incubates were extracted with vigorous shaking in 3 ml of chilled acetone followed by 12 ml of ethyl acetate as described previously by Sivarajah et al. (32). The organic layer was removed, and the water layer was mixed with an equal volume of chilled 10% trichloroacetic acid for 30 min and centrifuged. The precipitated protein was alternately extracted (at least 4 times) with CH3OH:H2O (4:1) and CHCl3:CH3OH (2:1) until no further detectable counts could be extracted. The washed protein was solubilized in 1 N NaOH. Aliquots were counted, and protein content was determined by the method of Lowry et al. (22). A heated microsome blank was included in each experiment, and values shown have been corrected accordingly. Incubation of heated microsomes for 30 min with arachidonate (10 to 100 μM) did not significantly influence [3H]BP binding to protein compared to that observed in heated microsomes incubated in the absence of arachidonate. All experimental values shown are at least twice the heated microsome blank. In 3 additional experiments, vitamin E (1 mM) was added to a portion of the microsomal extracts and to the organic solvents used to wash the precipitated protein. Vitamin E has previously been shown to prevent spontaneous oxidation of phenols to quinones (4:1): and CH2Cl2:CH3OH (2:1) until no further detectable counts could be extracted. The washed protein was solubilized in 1 N NaOH. Aliquots were counted, and protein content was determined by the method of Lowry et al. (22). A heated microsome blank was included in each experiment, and values shown have been corrected accordingly. Incubation of heated microsomes for 30 min with arachidonate (10 to 100 μM) did not significantly influence [3H]BP binding to protein compared to that observed in heated microsomes incubated in the absence of arachidonate. All experimental values shown are at least twice the heated microsome blank. In 3 additional experiments, vitamin E (1 mM) was added to a portion of the microsomal extracts and to the organic solvents used to wash the precipitated protein. Vitamin E has previously been shown to prevent spontaneous oxidation of phenols to quinones during the extraction procedure (27). No effect of addition of vitamin E to the extracts on the conversion of [3H]BP to protein-bound metabolites was observed (without vitamin E, basal, 1.0 ± 0.12; S.E.; plus 100 μM arachidonate, 7.4 ± 0.9; 500 μM plus NADPH and 5 mM MgCl2, 0.97 ± 0.16; with vitamin E, basal, 1.1 ± 0.14; plus arachidonate, 8.6 ± 1.2; plus NADPH and MgCl2, 0.93 ± 0.11.)

**Determination of Microsomal Prostaglandin E Synthesis.** Incubations for determination of IPGE production were conducted as described above for [3H]BP metabolism, except that [3H]BP was omitted from the incubation mixture. At the end of the incubation, microsomal incubates were acidified to pH 3 to 3.5, and [3H]IPGE (1500 cpm/ml incubate) was added to assess recovery. Samples were then extracted twice in 3 volumes of CHCl3, and the prostaglandin fraction was isolated by silica acid column chromatography as described previously (6). Samples were assayed for IPGE content by radioimmunoassay as described previously (6).

[3H]BP (17.4 Ci/mmol) and [3H]IPGE2 were obtained from American/Searle Corp., Arlington Heights, Ill., and New England Nuclear, Boston, Mass., respectively. Arachidonic and linoleic acids were obtained from Nu-Chek Prep, Elysian, Minn. Antiserum to prostaglandin E was purchased from Regis Chemical Co., Morton Grove, Ill. TYA and SKF-525A were gifts of Hoffman-LaRoche Inc., Nutley, N. J., and Smith, Kline & French Laboratories, Philadelphia, Pa., respectively.

**RESULTS**

Table 1 compares the effects of several fatty acids to those of NADPH plus MgCl2 on conversion of [3H]BP to protein-bound metabolites. Arachidonate (100 μM) increased [3H]BP binding to colonic mucosal microsomal protein by approximately 12-fold. Indomethacin (300 μM) suppressed basal and arachidonate-induced increases in protein binding approximately 36 and 57%, respectively. Linoleate also increased [3H]BP metabolism to protein-bound derivatives (Table 1). However, in contrast to results with arachidonate, stimulatory effects of linoleate were not significantly suppressed by indomethacin. In contrast to the effects of the unsaturated fatty acids, palmitate had no effect on [3H]BP binding to protein (Table 1). Moreover, addition of NADPH plus MgCl2 to colonic microsomal incubates did not influence conversion of [3H]BP to protein-bound metabolites.

Chart 1 illustrates the concentration response relationship between arachidonate, linoleate, and [3H]BP binding to protein. Half-maximal stimulation of [3H]BP binding to protein was observed at 10 μM arachidonate or linoleate. Optimal effects of these fatty acids were expressed at 25 μM. No further increase was observed when the concentration of fatty acids was increased to 100 μM. Indomethacin suppressed [3H]BP binding to protein at all concentrations of arachidonate tested but was without influence on linoleate-induced increases in [3H]BP binding to protein under the conditions tested in Chart 1. As is also shown in Chart 1, arachidonate increased IPGE production approximately 9-fold. Analogous to the effects of arachidonate on [3H]BP binding to protein, half-maximal stimulation of IPGE was observed at 10 μM arachidonate with optimal effects expressed at the 25 μM concentration of this fatty acid. In the presence of indomethacin, microsomal IPGE synthesis was not significantly suppressed by indomethacin. In contrast to the effects of the unsaturated fatty acids, palmitate had no effect on [3H]BP binding to protein (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Conversion of [3H]BP to protein-bound metabolites (pmol/mg protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Microsomes</td>
</tr>
<tr>
<td></td>
<td>+ Indomethacin</td>
</tr>
<tr>
<td>+ Arachidonate</td>
<td>+ Arachidonate + indomethacin</td>
</tr>
<tr>
<td>+ Linoleate</td>
<td>+ Linoleate + indomethacin</td>
</tr>
<tr>
<td>+ Palmitate</td>
<td>+ Palmitate + MgCl2</td>
</tr>
<tr>
<td>+ NADPH + MgCl2</td>
<td>+ NADPH + MgCl2 + Indomethacin</td>
</tr>
<tr>
<td>+ Indomethacin + NADPH + MgCl2</td>
<td>+ Indomethacin + NADPH + MgCl2</td>
</tr>
<tr>
<td>Mean ± S.E. of 6 determinations from 4 separate experiments (d.f. = 6, comparing any 2 groups by Student’s independent t test).</td>
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<tr>
<td>Mean ± S.E. of 6 determinations from 4 separate experiments (d.f. = 6, comparing any 2 groups by Student’s independent t test).</td>
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</table>

* Significant increase; p < 0.05 compared to corresponding value in the absence of indomethacin.

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chidonate-induced, and linoleate-induced increases in [3H]BP binding to protein. As shown, BHA, BHT, and NDGA (100 to 500 μM) suppressed basal [3H]BP binding to protein 30 to 45%. At 100 μM concentrations of these antioxidants, arachidonate- and linoleate-induced increases in [3H]BP binding to protein were reduced 67 to 79%, while at 500 μM the antioxidants completely abolished the stimulatory effects of the fatty acids on [3H]BP binding to protein. Under anaerobic conditions, [3H]BP binding to protein was not detectable in the presence or absence of fatty acids (Table 3).

To the extent that antioxidants suppress the expression of the mutagenic and carcinogenic activity of BP, the inhibitory effects of O2 exclusion and antioxidants on fatty acid-induced increases in [3H]BP binding to protein are consistent with a role for this pathway in the conversion of BP to mutagenic and carcinogenic derivatives. Nevertheless, the effect of O2 exclusion and antioxidants on the inhibition of [3H]BP binding to protein could be expressed at the level of fatty acid oxidation or at that of [3H]BP oxidation mediated by either cytochrome P-450 or the hydroperoxidase associated with prostaglandin synthetase activity. Inhibitors of cytochrome P-450 activity have previously been used to assess the interrelationships between cytochrome P-450 and prostaglandin synthetase-hy-

suppressed 70 to 95%. In contrast to arachidonate, linoleate had no effect on microsomal IPGE synthesis at any concentration tested in Chart 1.

Our own previous observations in colonie microsomes had suggested that oxidation of unsaturated fatty acids to either prostaglandin endoperoxides or fatty acid hydroperoxides may be involved in the expression of fatty acid-induced increases in drug and carcinogen metabolism. TYA is a nonmetabolizable analogue of arachidonic acid which has been shown to antagonize the metabolism of arachidonate via cyclooxygenase and lipoxygenase pathways (9). As shown in Table 2, preincubation of colonie microsomes with TYA suppressed by 65% the increases in [3H]BP incorporation into protein induced by arachidonate or linoleate. These studies suggested that a metabolite of arachidonate or linoleate, rather than the fatty acids per se, was mediating fatty acid-induced increases in [3H]BP binding to protein.

The antioxidants BHA, BHT, and NDGA have previously been shown to inhibit cytochrome P-450-catalyzed BP hydroxylation and conversion to mutagens in rat liver microsomes (29). BHA and BHT have also been shown to inhibit BP-induced neoplasia in mouse forestomach (35, 36). Table 3 illustrates the effects of BHA, BHT, and NDGA on basal, arachidonate- and linoleate-induced increases in [3H]BP binding to protein. As shown, BHA, BHT, and NDGA (100 to 500 μM) suppressed basal [3H]BP binding to protein 30 to 45%. At 100 μM concentrations of these antioxidants, arachidonate- and linoleate-induced increases in [3H]BP binding to protein were reduced 67 to 79%, while at 500 μM the antioxidants completely abolished the stimulatory effects of the fatty acids on [3H]BP binding to protein. Under anaerobic conditions, [3H]BP binding to protein was not detectable in the presence or absence of fatty acids (Table 3).

To the extent that antioxidants suppress the expression of the mutagenic and carcinogenic activity of BP, the inhibitory effects of O2 exclusion and antioxidants on fatty acid-induced increases in [3H]BP binding to protein are consistent with a role for this pathway in the conversion of BP to mutagenic and carcinogenic derivatives. Nevertheless, the effect of O2 exclusion and antioxidants on the inhibition of [3H]BP binding to protein could be expressed at the level of fatty acid oxidation or at that of [3H]BP oxidation mediated by either cytochrome P-450 or the hydroperoxidase associated with prostaglandin synthetase activity. Inhibitors of cytochrome P-450 activity have previously been used to assess the interrelationships between cytochrome P-450 and prostaglandin synthetase-hy-

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Table 2

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Conversion of [3H]BP to protein-bound metabolites (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ O2</td>
<td>0.93 ± 0.13μM TYA 0.52 ± 0.07μM + TYA</td>
</tr>
<tr>
<td>+ Arachidonate</td>
<td>8.6 ± 1.3μM + Arachidonate 2.8 ± 0.4μM + BHT</td>
</tr>
<tr>
<td>+ Linoleate</td>
<td>7.3 ± 1.2μM + Linoleate 2.6 ± 0.4μM + NDGA</td>
</tr>
</tbody>
</table>

- Mean ± S.E. of 9 determinations pooled from 3 separate experiments (d.f. = 4).
- Significant decrease; p < 0.01 compared to corresponding value in the absence of TYA.
- Significant increase; p < 0.01 compared to corresponding value in the absence of arachidonate or linoleate.

Table 3

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Conversion of [3H]BP to protein-bound metabolites (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.91 ± 0.12μM + O2 0.57 ± 0.07μM + BHA 0.52 ± 0.08μM + BHT 0.63 ± 0.12μM + NDGA</td>
</tr>
<tr>
<td>Arachidonate</td>
<td>8.3 ± 1.2μM + O2 2.7 ± 0.3μM + BHA 0.63 ± 0.09μM + BHT 2.38 ± 0.34μM + NDGA 0.51 ± 0.09μM + NDGA</td>
</tr>
<tr>
<td>Linoleate</td>
<td>9.7 ± 1.3μM + O2 3.1 ± 0.3μM + BHA 0.51 ± 0.07μM + BHT 2.73 ± 0.33μM + NDGA 0.62 ± 0.09μM + NDGA</td>
</tr>
</tbody>
</table>

- Mean ± S.E. of 9 determinations from 3 separate experiments (d.f. = 4).
- Significant increase; p < 0.01 compared to corresponding value in the absence of arachidonate or linoleate.
- Significant decrease; p < 0.01 compared to corresponding value in the absence of arachidonate or linoleate.
- ND, not detectable.

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droperoxidase-mediated increases in drug metabolism (10). As shown in Table 4, 7,8-benzoflavone (10 to 50 μM) and SKF-525A (500 to 2 mM) were without effect on basal or arachidonate-induced increases in this parameter. These concentrations of benzoflavone and SKF-525A have previously been shown to inhibit colonic mucosal microsomal mixed-function oxidase activity (11, 12).

DISCUSSION

Several proposed pathways exist for the activation of procarcinogens targeted for the colon. Procarcinogens may be activated in the liver and transported to the colon as glucuronidic acid conjugates (30, 37). Colonic bacteria may then deconjugate these products and allow absorption to occur (37). Alternatively, microorganisms present in the colon could generate active carcinogenic metabolites from procarcinogens (4, 15, 21). Recent studies in cultured colonic segments and microsomal fractions also support a role for colonic mucosa per se in the absence of bacteria in the activation of procarcinogens. Thus, cultured human colon can metabolize several classes of procarcinogens, including the aromatic hydrocarbons BP and 7,12-dimethylbenzanthracene, to products which bind covalently to DNA and protein (1–3). Moreover, BP metabolites formed DNA adducts in cultured human colon which were identified as BP-diol-epoxide I (72 to 100%) and BP-diol-epoxide II. The major BP metabolites extracted from the culture medium were tetrols and triols (3). While BP has not been shown to produce colonic tumors, it did induce cancer in mouse forestomach (35, 36). Moreover, 3-methylcholanthrene, an aromatic hydrocarbon structurally related to BP, is carcinogenic in rodents (17). In cultures of human colon, the finding of diol-epoxides and tetrols, which are solvation products of the unstable diol-epoxides, implies that this tissue is capable of metabolizing BP to its proximate carcinogenic form (18, 20, 31, 38). The involvement of the cytochrome P-450 system in the metabolism of BP by cultured human colon is suggested, but not proved, by the ability of 7,8-benzoflavone to suppress BP metabolism (1). Studies in colonic mucosal microsomes have extended these observations and demonstrated the existence of a NADPH-dependent cytochrome P-450 system capable of catalyzing the oxidation of a number of aromatic hydrocarbons including BP (11, 12). In the absence of inducers of cytochrome P-450, colonic microsomal activity is about 20-fold lower than that of liver (11). This observation led to the present studies which were aimed at determining whether additional pathways exist for the metabolism of BP, and potentially other procarcinogens by the colon.

Recent studies in seminal vesicle microsomes, renal inner medulla, urinary bladder, lung, and colon have demonstrated an alternate pathway for drug and carcinogen oxidation which is linked at least in part to prostaglandin synthesis (5, 7, 10, 24–26, 33, 40, 41). Studies of prostaglandin synthetase purified from seminal vesicle microsomes have shown that the latter enzyme catalyzes 2 distinct reactions: bis-dioxygenation of arachidonate to PGG2; and hydroperoxide reduction of PGG2 to PGH2 (10). The hydroperoxidase activity catalyzes the peroxidative cooxidation of drugs with a broad substrate specificity similar to that of cytochrome P-450. Nevertheless, the hydroperoxidase purified from seminal vesicle microsomes appears distinct from cytochrome P-450 and is not inhibited by a number of cytochrome P-450 inhibitors including metyrapone, 7,8-benzoflavone, and SKF-525A (10). The arachidonate-dependent oxidation of the urinary bladder carcinogen N-[4-(5-nitro-2-furyl)-2-thiazoyl]formamide by rabbit bladder microsomes was also not inhibited by metyrapone or 7,8-benzoflavone (5) and thus appears to be distinct from cytochrome P-450 activity.

The present study clearly demonstrates in colonic microsomes the presence of a pathway for fatty acid-dependent BP oxidation which is distinct from the NADPH-dependent cytochrome P-450 pathway. This is supported by the observations that: (a) arachidonate and linoleate increased the conversion of [3H]BP to protein-bound metabolites, whereas NADPH plus MgCl2 was ineffective; (b) indomethacin, an inhibitor of prostaglandin endoperoxide synthetase, and TYA, an analogue of arachidonate which inhibits fatty acid oxidation, inhibited arachidonate-induced increases in [3H]BP metabolism; and (c) 7,8-benzoflavone and SKF-525A, inhibitors of colonic mucosal cytochrome P-450 activity (11, 12), were without influence on arachidonate-induced increases in [3H]BP metabolism.

Basal and arachidonate-induced increases in [3H]BP metabolism appeared to be linked at least in part to prostaglandin synthesis. Thus, similar concentration response relationships were observed between arachidonate, [3H]BP metabolism, and microsomal prostaglandin E synthesis. Moreover, indomethacin suppressed basal and arachidonate-induced increases in [3H]BP metabolism (35 to 65%) and inhibited prostaglandin E production by 90 to 95%. Nevertheless, the failure of indomethacin to completely abolish arachidonate stimulation of [3H]BP metabolism and the ability of linoleate, which does not increase prostaglandin E synthesis in microsomes, to stimulate [3H]BP metabolism, suggested a pathway for fatty acid-induced [3H]BP metabolism that is independent of prostaglandin synthesis. The cooxidation of organic substrates by seminal vesicle microsomes is known to be stimulated by 15-hydroperoxy-arachidonic acid as well as by PGG2 (24). Therefore, fatty acid hydroperoxides generated by lipooxygenase activity could also serve as peroxidative substrates for the cooxidation of [3H]BP by colonic microsomes. In the present study, a role for fatty acid hydroperoxides in colonic [3H]BP metabolism was supported by the ability of TYA, an inhibitor of fatty acid oxidation to prostaglandin endoperoxides or fatty acid hydroperoxides (9), to suppress increases in [3H]BP metabolism induced by...
either arachidonate or linoleate. However, the presence of lipoxigenase activity in colonic mucosa remains to be demonstrated. We cannot completely rule out the possibility that the stimulatory action of linoleic acid on [3H]BP metabolism is linked to the oxidation of linoleic acid to 9-hydroperoxyoctadecadienoic acid by prostaglandin synthetase followed by cooxidation of [3H]BP via a peroxidase-mediated reaction. However, the observation that indomethacin did not significantly suppress linoleic acid-induced increases in conversion of [3H]BP to protein-bound derivatives argues against this mechanism.

Recent evidence suggests that BP-diol-epoxides I and II may be the proximate carcinogenic forms of BP (18, 20, 31, 38). The diol-epoxides are mutagenic (18, 31), highly reactive toward cellular DNA (20), and carcinogenic (31). Previous studies in pulmonary tissue using BP-7,8-diol (32) as substrate have provided evidence for a role for the arachidonate-responsive cyclooxygenase-dependent pathway in the metabolism of BP-7,8-diol to the proximate carcinogenic form. Thus, incubation of BP-7,8-diol with arachidonate and lung microsomes resulted in the formation of 4-tetrols known to be hydrolysis products of BP-diol-epoxide I and an increase in the covalent binding of metabolites to protein (33). By contrast, NADPH-dependent BP-7,8-diol oxidation resulted in the formation of 4-tetrols, hydrolysis products of BP-diol-epoxides I and II, and no binding of metabolites to protein (33). Incubation of BP with guinea pig lung microsomes resulted in approximately 2 to 2.5 times more total BP metabolism with NADPH than with arachidonate (32). However, a larger percentage of the metabolites produced by arachidonate stimulation were protein bound (12 to 15%), compared to that observed with NADPH (4 to 5%). In contrast to results with the BP-7,8-diol, the major metabolites formed by microsomes from BP in the presence of arachidonate were identified by high-pressure liquid chromatography as quinones (32).

In the present study, the products of fatty acid-stimulated [3H]BP were not identified. However, the ability of arachidonate and linoleate to increase protein-bound metabolites implies the generation of highly reactive metabolites of BP and supports a role for a fatty acid-responsive pathway in colonic carcinogen and drug metabolism. Only free unesterified fatty acids are oxygenated via the cyclooxygenase or lipoxigenase pathways (19). The concentration of free fatty acids in cells is quite low (19). Thus, activation of this pathway of carcinogen metabolism would presumably be dependent upon and triggered by release of unsaturated fatty acids from tissue lipid stores and the cellular content of cyclooxygenase or lipoxigenase. A number of stimuli of fatty acid release from membrane stores, including hormones (8, 19), are recognized and provide a potential pathway through which these stimuli might modify carcinogen metabolism.

The system for metabolizing carcinogens in colon is clearly complex and may involve several different pathways. The present results in no way exclude or minimize the contribution of either the mucosal cytochrome P-450 system or the intestinal bacteria to overall drug-metabolizing activity in the colon. The relative importance of an arachidonate-activated cyclooxygenase- or lipoxigenase-dependent pathway for carcinogen metabolism in the colon is not known. In this regard, indomethacin has been reported to inhibit the growth of dimethylhydrazine-induced intestinal tumors (28). However, this appears to be an effect at the level of tumor growth or promotion rather than carcinogen activation (28). Other studies also support the notion that indomethacin suppresses the growth of transplantable tumors (23, 34). By contrast, one recent study has suggested that aspirin may suppress the development of precarcinogenic lesions in urinary bladder and that this effect may be related to an inhibition of carcinogen activation (5).

The fatty acid-responsive pathway may represent an important alternate route for drug and carcinogen metabolism in tissues (e.g., the colon) which have relatively low cytochrome P-450 activity. This is supported by studies of drug and carcinogen metabolism in the kidney. Thus, in the renal cortex, drug metabolism occurs primarily via the cytochrome P-450 system. By contrast, cytochrome P-450 activity is low in the renal medulla, and the arachidonate-dependent pathway predominates (40, 41). Clearly, further work is required to delineate the role of the fatty acid-stimulated pathway and the involvement of cyclooxygenase or lipoxigenase activity in the expression of the cellular actions of chemical carcinogens in the colon.

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