Control by Gastrointestinal Hormones of the Hydroxylation of the Carcinogen Benzo(a)pyrene and Other Xenobiotics in Rat Colon

Wan Fen Fang and Henry W. Strobel

Department of Biochemistry and Molecular Biology, The University of Texas Medical School at Houston, Houston, Texas 77025

ABSTRACT

The cytochrome P-450-dependent drug metabolism system activities of rat colon microsomes are increased by gastrointestinal hormones. Pentagastrin, secretin, and cholecystokinin octapeptide increase the specific content of cytochrome P-450 and stimulate O-dealkylation and polycyclic aromatic hydrocarbon hydroxylation to different degrees. Increase in hydroxylation activity by pentagastrin is dose dependent with a stimulation of 250% of control occurring at a dose level of 250 μg/kg body weight. The increase in hydroxylation activity is prevented by cycloheximide treatment showing dependence on protein synthesis. Pentagastrin and cholecystokinin increase the specific content of colon microsomal P-450 77%, while secretin increases cytochrome P-450 content 138% of the control value. Pentagastrin induces a 4-fold increase in ethylmorphine demethylation, while the major effect by secretin is on p-nitrophenetole dealkylation activity (2-fold increase), and the major effects of cholecystokinin are on benzphetamine and benzo(a)pyrene hydroxylation (2-fold induction). These hormones induce the hydroxylation of other substrates also, but the pattern of effects varies with the hormone used. Liver microsomal hydroxylation activities are also increased significantly by pretreatment with these hormones, although to a lesser degree than colon hydroxylation activities. Several tissue substances induce the hydroxylation of specific substrates, although no pattern of inductive effects is evident. For example, reserpine pretreatment doubles the rate of benzo(a)pyrene hydroxylation in liver and colon microsomes but decreases or has no effect on other hydroxylation activities in liver microsomes and induces only ethylmorphine dealkylation in colon microsomes. On the other hand, 16,16-dimethylprostaglandin E₂ induces benzphetamine hydroxylation in the colon but only benzo(a)pyrene hydroxylation in the liver. The gastrointestinal hormones induce drug metabolism with an apparent pattern of specificities and an increase in cytochrome P-450 specific content not seen in animals pretreated with the tissue substances tested.

INTRODUCTION

The metabolism of xenobiotics such as benzo(a)pyrene to products of altered pharmacological potency occurs in many organs and tissues including the liver (20, 21), kidney (6), small intestine (2, 27, 32, 35), and lung (1, 29). Lu et al. (20) have shown that the substrate specificity for the drug metabolism system rests with the cytochrome component using benzo(a)pyrene as a test substrate, and several laboratories (12–14, 30) have established that the cytochrome component has multiple forms with distinct but overlapping substrate preferences. In addition, Kawalak and Lu (19) have shown that analogous cytochromes purified from different species (i.e., cytochrome P-448 isolated from rat and liver) can also have quite different catalytic activities. For instance, these authors (19) reported that rat liver cytochrome P-448 hydroxylated benzo(a)pyrene at a rate 10 times higher than that of rabbit liver cytochrome P-448. Using various forms of the cytochrome from the same animal and organ, Wood et al. (38) and Wislocki et al. (37) have shown that various forms of the cytochrome hydroxylate benzo(a)pyrene and that this activation of benzo(a)pyrene is associated with a marked increase in carcinogenicity and mutagenicity.

Furthermore, various tissues within the same species hydroxylate substrates such as benzo(a)pyrene with differing efficiencies and turnover numbers (2, 19, 27, 32, 35). Fang and Strobel have shown that benzo(a)pyrene is hydroxylated by the rat colon (9) and, more specifically, that benzo(a)pyrene is activated to mutagenic products by the colon (8). Moreover, the hydroxylation and activation of benzo(a)pyrene by the colon, like those actions by the liver, are markedly responsive to exogenous inducers (e.g., β-naphthoflavone). The present study examines the regulation of both benzo(a)pyrene hydroxylation and the metabolism of other drugs by endogenous hormones and tissue substances. Specifically, this report presents the first evidence that the peptide gastrointestinal hormones modulate the rate of drug metabolism in the colon.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 150 to 200 g were obtained from Flow Laboratories, Dublin, Va., for this study. The rats were housed in wire-bottomed cages and maintained on laboratory chow and water ad libitum throughout the course of the study. The gastrointestinal hormones and tissue substances were administered to experimental animals once or twice daily for each of 3 consecutive days, and animals were sacrificed between 8 and 9 a.m. on the fourth day unless otherwise stated. Animals were fasted overnight before sacrifice in order to aid in the collection of colon microsomes. The overnight fast did not affect colon microsomal drug metabolism activities. Each of the hormones was dissolved in 0.9% NaCl solution for injection, although some of the hormones required special procedures for preparation of stable solutions. Pentagastrin solutions for injection were prepared fresh each day. To prepare the pentagastrin stock solution, pentagastrin was brought into solution in a small amount of 0.9% NaCl solution by adding 0.1 N NH₄OH dropwise until all the pentagastrin

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dissolved. The solution was titrated back to pH 7.5 with 0.1 N HCl. The stock solution was further diluted with 0.9% NaCl solution before i.p. injection. Pentagastrin-treated rats received one daily injection of 250 µg pentagastrin per kg body weight for 3 days unless otherwise stated. Secretin was dissolved in 0.9% NaCl solution containing cysteine HCl, 1 mg/ml, as a reducing agent. Secretin-treated rats received one daily s.c. injection of 75 units of secretin (27 µg) per kg body weight for each of 3 days. Cholecystokinin octapeptide was prepared as a concentrated stock solution of 0.5 mg/ml in 0.5 N NaHCO3 and diluted into 0.9% NaCl solution before use. Cholecystokinin-treated rats received one daily s.c. injection of 20 µg cholecystokinin per kg body weight for each of 3 days. Cycloheximide was dissolved with 0.9% NaCl solution.

In a similar fashion, the tissue substances used in this study were also dissolved or diluted in 0.9% NaCl solution. 16,16-Dimethylprostaglandin E2 was dissolved in ethanol and diluted to volume with 0.9% NaCl solution. Prostaglandin-treated rats received 2 daily i.p. injections of 0.4 mg of 16,16-dimethylprostaglandin E2 per kg body weight for each of 3 days. Serotonin (5-hydroxytryptamine) was dissolved in water and diluted in 0.9% NaCl solution. Serotonin-treated rats received 2 daily i.p. injections of 25 mg of serotonin per kg body weight for each of 3 days. 5-Hydroxy-L-tryptophan was dissolved in 0.01% ascorbic acid and 0.1 N HCl as a stock solution and diluted into 0.9% NaCl solution. 5-Hydroxy-L-tryptophan-treated rats received 2 daily i.p. injections of 50 mg of 5-hydroxy-L-tryptophan per kg body weight for each of 3 days. Reserpine, a plant alkaloid, was dissolved with acetic acid and diluted in 0.9% NaCl solution. Reserpine-treated rats received one daily i.p. injection of 25 mg of reserpine per kg body weight for each of 3 days. All animals were sacrificed on the fourth day, and livers and colons were removed. Microsomes were prepared from these tissues as described previously (5, 9).

Assays. The rate of hydroxylation (N-demethylation) of benzphetamine and ethylmorphine by colon microsomes was measured as described previously (21) by formaldehyde liberation, according to the method of Nash (23) as modified by Cochin and Axelrod (3). Reaction mixtures contained 150 µmol potassium phosphate buffer (pH 7.7), microsomal protein, and water in a total volume of 1.5 ml. The reaction was initiated by the addition of 0.15 µmol NADPH, and mixtures were incubated at 30° for 10 min. The rate of hydroxylation (O-dealkylation) of p-nitroanisole and p-nitrophenetole was estimated in similar reaction mixtures as the rate of formation of the product p-nitrophenol according to the method of Netter and Seidel (25). The hydroxylation of benzo(a)pyrene was measured in 1.0-ml reaction mixtures containing 100 µmol potassium phosphate buffer (pH 7.7), 80 nmol benzo(a)pyrene, and 0.15 µmol NADPH by fluorometric estimation of the 3-hydroxy and other products according to the methods of Nebert and Gelboin (24), using an excitation wavelength of 386 nm and an emission wavelength of 515 nm.

Cytochrome P-450-specific content was measured from the dithionite-reduced carbon monoxide difference spectrum according to the method of Omura and Sato (26), using an extinction coefficient of 91 cm⁻¹ mmol⁻¹. NADPH-cytochrome P-450 (cytochrome c) reductase activity was measured by a modification (5) of the method of Phillips and Langdon (28) at 30° in 1.0-ml reaction mixtures containing 300 µmol potassium phosphate buffer (pH 7.7) as the rate of reduction of cytochrome c, using an extinction coefficient of 21 cm⁻¹ mmol⁻¹ at 550 nm (36).

Materials. Horse heart cytochrome c, cycloheximide, serotonin, 5-hydroxy-L-tryptophan, and reserpine were purchased from Sigma Chemical Co., St. Louis, Mo. Benzo(a)pyrene, p-nitroanisole, and p-nitrophenetole were obtained from Eastman Kodak Co., Rochester, N. Y. Benzphetamine hydrochloride was a gift from Dr. J. W. Hinman of the Upjohn Co., Kalamazoo, Mich. Ethylmorphine was obtained from Merck & Co., Inc., Rahway, N. J. Pentagastrin was a gift from Ayerst Laboratories, New York, N. Y. Secretin and NADPH were purchased from Boehringer-Mannheim Biochemicals, Indianapolis, Ind. Cholecystokinin octapeptide was a gift from Dr. L. Lichtenberger of Department of Physiology, University of Texas Medical School at Houston. 16,16-Dimethylprostaglandin E2 was the gift of Dr. J. E. Pike of Upjohn. All other chemicals were reagent grade or better.

RESULTS

Induction of Colon Microsomal Hydroxylation Activities by Pentagastrin. In order to ascertain whether the mixed-function oxidase systems respond to gastrointestinal hormones, we utilized information on structure function relationships of these hormones and the availability of synthetic polypeptide hormones. The structure, physiological properties, and actions of the major gastrointestinal hormones, gastrin, secretin, and cholecystokinin, have recently been reviewed by Grossman (11). Furthermore, Johnson (16) has shown that the synthetic gastrointestinal hormone pentagastrin exerts a trophic effect on the colon mucosa by stimulating colonic mucosal DNA synthesis leading to a rapid increase in mucosal DNA content and, hence, cell number. As shown in Chart 1, pentagastrin pretreatment of rats brings about a marked increase in the hydroxylation rate of the carcinogen benzo(a)pyrene by colon...
mucosal microsomes from treated rats in a dose-dependent fashion. Maximal stimulation of benzo(a)pyrene hydroxylation activity (160% above control) was achieved with doses between 250 and 375 μg of pentagastrin per kg body weight, which was in good agreement with the amount of pentagastrin reported to stimulate maximal DNA synthesis in the colonic mucosa (18).

As shown in Table 1, the hydroxylation of benzo(a)pyrene and a variety of drugs is stimulated by pentagastrin pretreatment but to different degrees. The rate of hydroxylation of some drugs, especially ethylmorphine, is stimulated after only a single treatment with pentagastrin. Highest activities were observed after 3 days of treatment. The hydroxylation rates of ethylmorphine and benzo(a)pyrene were most responsive to pentagastrin pretreatment. Colon cytochrome P-450 content was almost doubled and cytochrome P-450 reductase activity was increased by pentagastrin pretreatment.

Having shown the dosage and time dependence of the pentagastrin induction of mucosal drug metabolism system activities, it was important to determine whether pentagastrin induction involves the synthesis of new enzyme protein as has already been shown for phenobarbital induction of the liver drug metabolism system (7) and for the emergence of drug metabolism activities in neonatal hepatocytes (4). The inhibitor of protein synthesis, cycloheximide, was used to examine this possibility. As shown in Table 2, cycloheximide injected 45 min before pentagastrin for 3 days completely prevented the induction of drug metabolism in the colon by pentagastrin as judged by the elimination of pentagastrin-dependent increases over the untreated controls in cytochrome P-450 content, cytochrome c reductase activity, or the rate of hydroxylation of any of a variety of substrates. These data suggest a role for protein synthesis in induction of the colonic drug metabolism system by pentagastrin.

### Induction of Hydroxylation Activities by Cholecystokinin and Secretin

The data in the experiments described above show that pentagastrin, a pentapeptide hormone containing the minimum amino acid sequence required for the physiological activities of gastrin (11), markedly increases drug metabolism activities in the colon through a process involving protein synthesis. Of the other major gastrointestinal hormones, cholecystokinin has an almost identical COOH-terminal sequence with pancreatic glucagon than with gastrin. Thus, it is important to compare the actions of these gastrointestinal hormones with those of pentagastrin. As shown in Table 3, both secretin and cholecystokinin octapeptide have marked effects on the drug metabolism activities of the colon. Cytochrome P-450-specific content was increased 2.5-fold by secretin and 1.5-fold by cholecystokinin. With the exception of its effects on the rates of O-dealkylation, cholecystokinin affects the colon drug metabolism activities in the same direction as does pentagastrin. Cholecystokinin stimulates benzphetamine hydroxylation a little more and ethylmorphine hydroxylation a little less than does pentagastrin. The main point of difference, however, between these highly similar polypeptides is the marked lowering of O-dealkylation activity after treatment with cholecystokinin, whereas pentagastrin pretreatment elicits a modest increase in O-dealkylation of p-nitroanisole and p-nitrophenetol. Secretin, which is known to inhibit the physiological effects of gastrin (31), stimulates the colon microsomal hydroxylation of most substrates, with the exception of benzphetamine and ethylmorphine. Moreover, the stimulatory effect of secretin on O-dealkylation is greater than that of pentagastrin.

The effect of the gastrointestinal hormones on hepatic drug metabolism system activities is shown in Table 4. In general, some of the effects of these hormones on the liver system parallel their effects on the colon system, while others do not. O-Dealkylation activity in the liver is not significantly affected by any gastrointestinal hormone. Pentagastrin does not significantly alter ethylmorphine metabolism in liver, and cholecystokinin does not alter benzphetamine metabolism. Quantitatively, however, the liver responds to these hormones less well in terms of the percentage of increase than does the colon. Nonetheless, the control liver activities are higher than the induced colon activities.

### Effects of Tissue Substances on Colon and Liver Microsomal Drug Metabolism System Activities

Pentagastrin, secretin, and cholecystokinin show some pronounced specificity in the hydroxylation activities that they preferentially induce inter se. They also seem to affect liver and colon systems...
differentially. In order to examine further this possible specificity, we tested several tissue substances for their effects on drug metabolism activity. (Tissue substances, like hormones, have a broad range of effects but are produced by several tissues.) We chose 5-hydroxytryptamine (serotonin) and its precursor 5-hydroxytryptophan, 16,16-dimethylprostaglandin E₂ (a stable prostaglandin derivative), and reserpine (a plant alkaloid) for this study and determined the appropriate dosages. The effects of these tissue substances on colon drug metabolism activity are shown in Table 5, and the effects on liver drug metabolism activity are shown in Table 6. None of the tissue substances induces O-dealkylation in either the liver or colon systems. In fact, the lowering of activity in pretreated animals is statistically significant in most cases. Reserpine pretreatment significantly stimulates colonic ethylmorphine demethylation. 16,16-Dimethylprostaglandin E₂, on the other hand, markedly stimulates colonic benzphetamine hydroxylation (2.5-fold) but inhibits liver benzphetamine hydroxylation.

Table 3

<table>
<thead>
<tr>
<th>Hydroxylation activities</th>
<th>Control</th>
<th>Pentagastrin</th>
<th>Secretin</th>
<th>Cholecystokinin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzphetamine</td>
<td>5.28 ± 0.25</td>
<td>6.49 ± 0.41</td>
<td>5.06 ± 0.11</td>
<td>5.51 ± 0.42</td>
</tr>
<tr>
<td>Ethylmorphine</td>
<td>6.34 ± 0.56</td>
<td>8.61 ± 0.77</td>
<td>9.66 ± 0.84</td>
<td>7.36 ± 0.82</td>
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<tr>
<td>p-Nitroanisole</td>
<td>3.50 ± 0.66</td>
<td>2.05 ± 0.16</td>
<td>2.96 ± 0.13</td>
<td>1.89 ± 0.23</td>
</tr>
<tr>
<td>p-Nitrophtholone</td>
<td>3.38 ± 0.21</td>
<td>3.87 ± 0.46</td>
<td>3.37 ± 0.27</td>
<td>4.0 ± 0.82</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>130.0 ± 10.0</td>
<td>320.0 ± 40.0</td>
<td>248.02 ± 21.83</td>
<td>370.0 ± 30.0</td>
</tr>
</tbody>
</table>

System components

| Cytochrome P-450 content | 0.76 ± 0.05 | 0.91 ± 0.04 | 0.97 ± 0.03 | 0.83 ± 0.06 |
| Cytochrome c reductase   | 153.4 ± 22.9 | 276.6 ± 24.6 | 154.6 ± 5.3 | 236.3 ± 21 |

Table 4

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<td>Benzphetamine</td>
<td>0.48 ± 0.02</td>
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</tr>
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<td>p-Nitroanisole</td>
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<td>0.24 ± 0.05</td>
<td>0.27 ± 0.17</td>
<td>0.286 ± 0.02</td>
</tr>
<tr>
<td>p-Nitrophtholone</td>
<td>1.04 ± 0.17</td>
<td>0.66 ± 0.06</td>
<td>0.08 ± 0.01</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>3.50 ± 0.70</td>
<td>5.0 ± 0.80</td>
<td>4.40 ± 0.80</td>
<td>5.0 ± 0.40</td>
</tr>
</tbody>
</table>

System components

| Cytochrome P-450 content | 0.76 ± 0.05 | 0.91 ± 0.04 | 0.97 ± 0.03 | 0.83 ± 0.06 |
| Cytochrome c reductase   | 153.4 ± 22.9 | 276.6 ± 24.6 | 154.6 ± 5.3 | 236.3 ± 21 |

Table 5

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<tr>
<th>Hydroxylation activities</th>
<th>Control</th>
<th>Dimethyl-prostaglandin E₂</th>
<th>Serotonin</th>
<th>5-Hydroxy-L-tryptophan</th>
<th>Reserpine</th>
</tr>
</thead>
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Effects of tissue substances on colon drug metabolism system

Animals were pretreated with 16,16-dimethylprostaglandin E₂, serotonin, 5-hydroxy-L-tryptophan, or reserpine, and the colon microsomes were assayed for drug metabolism activities. Experimental details are described in Table 3. All activities are expressed in units described for the colon system in Table 3.
5-Hydroxytryptamine (serotonin) and 5-hydroxytryptophan have only modest effects on colon and liver drug metabolism activities. Both substances stimulate ethylmorphine hydroxylation in the colon to a slight but statistically significant extent. Benzo(a)pyrene hydroxylation is stimulated 2.5-fold in the liver and the colon by reserpine pretreatment and almost 2-fold in the liver by 16,16-dimethylprostaglandin E. In general, the action of each of these tissue substances on the liver or colon gives a more diffuse pattern of stimulation than is seen with the gastrointestinal hormones.

**DISCUSSION**

The regulation of the rate of drug metabolism by altering the amount of component enzymes present is a central issue in studies of carcinogenesis from the standpoint of activation of environmental or dietary precarcinogens, in studies of chemotherapeutic management of tumors from the standpoint of removal of activated anticancer drugs, and in the activation of some anticancer drugs (e.g., cyclophosphamide). The demonstration of drug metabolism systems in many extrahepatic tissues (22) makes the regulation of drug metabolism even more complicated by virtue of different tissue sensitivities to regulatory effectors. Our data provide the first evidence showing that the gastrointestinal hormones significantly affect the rate of drug metabolism in the colon and, to a lesser degree, the liver. The physiological action of gastrin on the stomach, increased acid output, was reported to have a peak effect of doubling the basal level after 7 days of treatment (31). Johnson (15, 16) reported that 6 pentagastrin injections within 48 hr significantly increased DNA synthesis in the colonic mucosa as well as in the oxyntic gland area, duodenum, ileum, and pancreas. The dose-response curve presented by Johnson (Ref. 15, Fig. 3) for pentagastrin-stimulated DNA synthesis in colonic mucosa is essentially identical to that shown in Chart 1 in this paper, showing the increase in benzo(a)pyrene hydroxylation specific activity after pretreatment with increasing dosages of pentagastrin. This pentagastrin-induced increase in the specific enzyme activity is dependent on new protein synthesis and, presumably, DNA synthesis as indicated by the sensitivity of induction to cycloheximide treatment. Thus, our results confirm the trophic effects of pentagastrin on colonic mucosa (15, 16) and extend those results by showing specific effects on enzyme activities.

Furthermore, both secretin and cholecystokinin octapeptide have related, but not identical, inductive effects on colon drug metabolism activities. This is somewhat unexpected, since it has been shown that, unlike pentagastrin, secretin does not stimulate colonic mucosal DNA synthesis and, when given in combination with pentagastrin, actually inhibits pentagastrin-stimulated DNA synthesis (18). Similarly, cholecystokinin octapeptide has been shown, at best, to have a weak trophic action at moderate doses in the duodenum but not in the stomach (17). Nonetheless, these hormones stimulate drug metabolism and cytochrome P-450 content in the colon and the liver. Each of these hormones has an individual pattern of effects. For instance, secretin increases p-nitrophenolte, whereas cholecystokinin increases benzphetamine metabolism in the colon (Table 3). Secretin actually appears to decrease benzphetamine metabolism. The basis of this apparent specificity may lie in the discrete pattern of P-450 cytochromes influenced by hormone pretreatment. Although the content of colonic mucosal cytochrome P-450 rises after hormone pretreatment. Although the content of colonic mucosal cytochrome P-450 rises after hormone pretreatment, we cannot yet specify the form or forms of cytochrome P-450 induced by hormone pretreatment. Although the content of colonic mucosal cytochrome P-450 rises after hormone pretreatment, we cannot yet specify the form or forms of cytochrome P-450 accounting for that rise in cytochrome content. Identification of specific hormone-responsive forms of cytochrome P-450 could aid us in understanding the discrete effects of these hormones on drug metabolism. Induction of colonic microsomal cytochrome P-450-specific content by gastrointestinal hormones ranges from 1.6-fold for cholecystokinin and 1.8-fold for pentagastrin to 2.8-fold for secretin, comparing favorably with the 3.8-fold increase elicited by phenobarbital pretreatment (9). The 8-fold induction of cytochrome P-448 content by β-naphthoflavone, however, is the largest induction of colonic cytochrome P-450 (P-448) yet reported (9).

The gastrointestinal hormones also affect hepatic drug metabolism, although the changes are not as pronounced as those in the colon. That this group of gastrointestinal hormones affects the liver as well as the colon makes effects on drug metabolism a more generalized phenomenon. This does not rule out specificity as a tissue characteristic as is seen in the lack of any stimulation of hepatic O-dealkylation activity in response to any of the hormones.

The effects of the tissue substances on drug metabolism activities in the colon and in the liver are fewer and more diffuse than are the effects of the gastrointestinal hormones. Nonetheless, the stimulatory effects on enzyme activities where present are dramatic. For instance, reserpine, which depletes stores of the biogenic amines, increases benzo(a)pyrene hydroxylation more than 2-fold in the liver and colon. Such effects are of
particular significance in light of the recent demonstration of inhibition of malignant growth of colon tumors by 5,6-dihy- 
droxytryptamine, a cytotoxic congener of serotonin (34). Ser-
otonin, on the other hand, apparently acts to stimulate cell 
proliferation in experimentally induced coloni carcino-
mas (34).

The relationship between the trophic effects of tissue sub-
stances and gastrointestinal hormones and the effects of these 
compounds on drug metabolism remains to be defined. In 
any event, the gastrointestinal hormones and tissue substances 
do affect the growth rate and drug metabolism activity of 
the colon, and now it appears that at least serotonin and related 
compounds exert cytotoxic effects on coloni tumours. Since 
the gastrointestinal hormones do increase cytochrome P-450 
content and hydroxylation of benzo(a)pyrene, it is very likely 
that the activation of other carcinogens is also favored by 
induction of the drug metabolism system by one or more of 
the hormones, as we have shown previously for phenobarbital 
or β-naphthoflavone (8).

We are unable as yet to define which of the multiple forms 
of cytochrome P-450 is(are) increased by gastrointestinal 
hormonal pretreatment. It has, however, already been shown 
that various forms of cytochrome P-450 hydroxylate benzo-
(a)pyrene to different products, and the activation of 
benzo(a)pyrene is associated with an increase in carcinogenic- 
ity and mutagenicity (37, 38). The effects of gastrointestinal 
hormones on the metabolic profile of benzo(a)pyrene catalyzed 
by rat colon is now under study as a model system. Such 
approaches applied to human systems, also under study, are 
necessary before a relationship between coloni metabolism 
of benzo(a)pyrene or other dietary and environmental carcin-
gen and human coloni cancer can be demonstrated. The 
results reported in this paper, however, are suggestive of that 
possibility.

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