Kinetic Changes in Mucosal Ornithine Decarboxylase Activity during Azoxy methane-induced Colonic Carcinogenesis in the Rat

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ABSTRACT

In the azoxymethane (AOM)-treated rat model of colonic carcinogenesis, serial injections of the carcinogen lead to the eventual development of colonic tumors. However, the precise nature of carcinogenesis events in this commonly used model is not well defined, and the occurrence of classic initiation and promotion phases after serial injections is uncertain. Since increases in ornithine decarboxylase (ODC) activity have been associated with promotion in other tumor models, we measured mucosal ODC during AOM carcinogenesis in the rat. We gave male Fischer 344 rats ten weekly injections of AOM at a dose of 3 mg/kg (Wk 1–10) and measured colonic mucosal ODC activity during the entire 25-wk course. Colonic tumor incidence at Wk 25 was 0% in controls and 48% in AOM animals (15 of 31). Mucosal ODC in AOM animals was significantly increased over controls. The time course of changes in mucosal ODC was similar throughout the entire colon and differed generally in magnitude only. Distinct and prolonged increases in ODC activity occurred within 4 h of a single injection of carcinogen and persisted for at least 14 days. With a second AOM injection on Day 7, there was another distinct and prolonged increase in ODC over the persistently elevated activity. Over the entire 25 wk, the increase in ODC was distinctly biphasic, higher at Wk 2, 11, and 13 than at Wk 6, 17, 21, and 25. The findings indicate that AOM induces an increase in mucosal ODC during colonic carcinogenesis, and they suggest that this carcinogenesis model, with ten weekly injections of carcinogen, has the properties of a multistep process. The early peak in ODC might be associated with the early carcinogenesis (initiation) phase(s), and a subsequent second increase in ODC might be associated with late carcinogenesis (post-initiation or promotion) phase(s). These results strengthen the utility of the rat AOM colonic carcinogenesis model for further studies of the role of ODC and polyamine metabolism in neoplastic transformation.

INTRODUCTION

The present study was undertaken to determine whether changes in colonic mucosal ODC activity might elucidate the sequential events during chemical induction of colon carcinomas in the rat.

ODC converts ornithine to putrescine and is the first and rate-limiting enzyme of the polyamine pathway, which appears to play an important role in normal and neoplastic cell proliferation (2–6), including cell proliferation in the intestinal mucosa (7–11). Induction of ODC has also been suggested to play an important role in tumor promotion (12–14). Other studies have shown that tumor promoters in other organ systems induced ODC activity in their respective target tissue (15–17). ODC is also induced during colonic carcinogenesis (17–19). Several recent studies have shown that inhibition of ODC activity with the specific enzyme-activated irreversible inhibitor, difluoromethyl ornithine, can inhibit carcinogenesis (19–21). The results suggest that ODC may play an important role in carcinogenesis (19, 20).

In previous studies of ODC induction during carcinogenesis, the kinetics of the changes of ODC during the entire carcinogenesis process has not been elucidated (17, 19, 21–24). In the colonic carcinogenesis model using either dimethylnitosamine or AOM, multiple weekly injections of carcinogen are used in most studies (17–19, 21–36). A variety of promotional stimuli, including postresectional hyperplasia, increased fat intake, certain drugs, and rectal instillation of bile salts, has been shown to enhance carcinogenesis when given after exposure to carcinogen (17, 18, 22–25, 32, 34). These promotional stimuli, however, are not required for the development of tumors (17, 22–24, 34). Thus, the multistep nature of colonic carcinogenesis in the AOM model is not definitively resolved, and the multiple, serial administration of carcinogen may induce both the initiation and postinitiation (promotion) events.

In the present study, we measured the time course of changes in colonic mucosal ODC activity during both the acute and chronic phases after repeated carcinogen administration. We show a dramatic pattern of changes in ODC activity which may characterize the multistep nature of AOM colonic carcinogenesis in the rat.

MATERIALS AND METHODS

Animals and Diets. Specific-pathogen-free male Fischer 344 rats were obtained at 7 wk of age from Harlan Breeding Laboratories, Walkersville, MD. Arriving animals were monitored with collection of fecal samples for the detection and identification of parasites. Animals were quarantined in our facilities for 3 wk before experimentation, at which time their weight was 175–200 g. Animals were housed in plastic cages in a light (12 h/day)- and temperature (24°C)-controlled rodent colony with daily care. The rats were weighed weekly to provide weight gain curves. Animals were given water andRalston Purina 5002 rat chow diet ad libitum (28, 29).

Carcinogen Procedures. All procedures involving carcinogen were reviewed and approved by the Johns Hopkins Medical Institutions Safety Department. AOM was purchased from Ash Stevens, Detroit, MI. Carcinogen was diluted with pyrogen- and preservative-free sterile water for injection to a concentration of 5 mg/ml. AOM was aliquoted into serum vials sealed with Teflon-coated rubber diaphragms and kept stored at 4°C in the dark until use. Animals were given 10 weekly s.c. injections of AOM at a dose of 3 mg/kg (wk 1–10). Injection procedures were carried out in a chemical fume hood with an air flow of 150 linear ft/min. Animals receiving injections were left in the hood for 1 day to eliminate any evaporating or exhaled carcinogen. Bedding and excreta were then incinerated.

Tissue Preparation. Sets of 3 animals were killed 4, 8, 16, and 24 h after one injection of AOM and daily for 2 wk thereafter for one set of

Received 1/31/86; revised 5/8/86; accepted 5/15/86.

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1 Supported in part by Grants CA-37789, CA-37606, and CA-34453 from the NIH and by the Clayton Fund. Portions of this work were presented in abstract form at the American Gastroenterological Association annual meeting, May 1982, Chicago (1).

2 Recipient of a Faculty Research Award from the American Cancer Society and an American Gastroenterological Association/Robins Research Scholar Award. To whom requests for reprints should be addressed, at Room 2-127, The Oncology Center, The Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21205.

3 The abbreviations used are: ODC, ornithine decarboxylase; AOM, azoxymethane.
COLONIC MUCOSAL ODC IN AOM CARCINOGENESIS

Tumor Incidence. The tumor incidence, assessed in rats killed at wk 25, was 48%. Fifteen of 31 animals had colonic tumors, with a mean of 2.3 tumors per tumor-bearing animal.

Induction of Colonic Mucosal ODC Activity. From 4 h after a single injection of AOM, the mucosal ODC activity in the ascending colon was increased approximately 20-fold (Fig. 1). This marked increase was evident whether the results were expressed per gram wet weight of mucosa or per milligram of mucosal protein. By 16–24 h, the ODC activity had decreased, to about 3- to 4-fold basal level. Colonic mucosal ODC activity then gradually increased over the next 3 or 4 days to about 15-fold basal levels, persisting for at least 2 wk after the single AOM injection (Fig. 1). Age-matched control animals showed no significant changes in colonic mucosal ODC activity during the same time period.

A second AOM injection administered 7 days after the first resulted in an incremental increase in ODC activity from the already persistently elevated levels (Fig. 1). The increase was about 60% at 8 h after AOM, from about 17-fold basal levels to 27-fold basal levels. This additional increase in ODC activity was observed within 4–8 h after the second injection. The mucosal ODC activity then declined to the elevated levels just prior to the second injection. Thereafter, mucosal ODC activity was persistently elevated, at a level of about 17- to 20-fold basal levels (Fig. 1). Age-matched control animals showed no significant changes in colonic mucosal ODC activity during the same time period.

With continued weekly administration of AOM, mucosal ODC activity was persistently elevated (Fig. 2). However, a distinctly biphasic elevation pattern was observed, since the ODC activity was markedly increased in Wk 2, decreased to about 2- to 3-fold basal levels by Wk 6, and was again more than 20-fold elevated by Wk 11. This elevation persisted to Wk 13. Then the activity gradually declined between Wk 21 and 25 (Fig. 2). Age-matched control animals showed no significant changes in colonic mucosal ODC activity from Wk 0 control levels during this entire time period.

Colonic Tumor ODC Activity. In 5 colonic tumors obtained from AOM animals at Wk 25, ODC activity was 76 times the mucosal ODC activity found in age-matched control animals that received no AOM (Table 1). In the normal-appearing mucosa, obtained at least 5 cm away from colonic tumors in AOM animals, the mucosal ODC activity was also elevated, being about 18 times the control value (Table 1).

RESULTS

Fig. 1. Acute effects of administration of the carcinogen azoxymethane on rat colonic mucosal ornithine decarboxylase activity. Fischer 344 rats were given one or 2 s.c. injections, 1 wk apart of AOM at a dose of 3 mg/kg. Animals were sacrificed 4, 8, 16, and 24 h after injection and daily thereafter. Colonic mucosal scrapings were homogenized for measurement of ODC activity. Although the ascending colon had the highest ODC activity, the kinetics of changes in mucosal ODC activity was similar for the transverse and descending colon, and only the results for the ascending colon are shown. Age-matched control animals showed no significant changes in colonic mucosal ODC activity from 0-h control values. Results are expressed as pmol of 14CO2 released per h per mg of protein, but they are not significantly different if expressed per g of wet weight. Each injection of AOM; O, mucosal ODC activity during the 14-day period after one single AOM injection on Day 0; ●, mucosal ODC activities between Days 7 and 14 after 2 AOM injections on Day 0 and Day 7, respectively. Points, mean from 3 animals; bars, SE.

Fig. 2. Changes in rat colonic mucosal ornithine decarboxylase activity during azoxymethane induction of colonic carcinogenesis. Fischer 344 rats were given 10 weekly s.c. injections of AOM at a dose of 3 mg/kg. Fifteen rats of 31 had colon tumors by Wk 35. Animals were sacrificed just before the time of AOM injection at the indicated times. Colonic mucosal scrapings were homogenized for measurement of ODC activity. Although the absolute values of ODC were highest in the ascending colon, the kinetics of changes in mucosal ODC activity was similar for the transverse and descending colon, and only the results for the ascending colon are shown. Age-matched control animals showed no significant changes in colonic mucosal ODC activity from wk 0 control values during the entire time period. Results are expressed as pmol of 14CO2 released per h per mg of protein, but they are not significantly different if expressed per g of wet weight. Each injection of AOM; O, colonic mucosal ODC activity. Points, mean from 2-5 animals; bars, SE.

DISCUSSION

The rodent colonic carcinogenesis model clearly involves multistep characteristics (17, 22-24, 32, 34). However, the...
multiple, serial administration of a single carcinogen, dimethylhydrazine or AOM, can induce tumors with an incidence and a time course similar to that obtained with a carcinogen followed by promotional agents (32). The multistep nature of this carcinogenesis model has not been resolved and the timing of events for the initiation and postinitiation phases has not been delineated.

Previous studies in carcinogenesis models have suggested the important role of ODC induction in tumor promotion. Studies in the mouse skin model have clearly documented the importance of ODC induction for tumor formation (12, 13, 20, 40). In the rodent colon carcinogenesis model, the specific irreversible enzyme-activated inhibitor of ODC, difluoromethyl ornithine, can suppress tumor formation (19, 21). However, initiation and promotional phases in colon carcinogenesis have not been clearly delineated. Also, the precise timing for ODC induction and its role(s) at key stages of colonic mucosal cell transformation and tumor formation have not been investigated.

We have shown that induction of ODC is essential for the processes of DNA synthesis, protein synthesis, and tissue growth during intestinal maturation (8) and with mucosal hyperplasia in response to various stimuli including resection, lactation, and starvation-refeeding (9–11). Some of these stimuli have been reported to modulate the experimental carcinogenesis process. For example, mucosal hyperplasia induced by intestinal resection (34, 36) and bacterial inoculation (25) enhances colon tumor induction by carcinogen. We have also shown that mucosal ODC activity is progressively increased in normal-appearing colon mucosa, adenomas, and adenomas with high grades of dysplasia from familial polyposis family members at high risk for developing colorectal carcinoma (37). We have subsequently found that colorectal carcinomas contained even higher mucosal ODC activity than the adenomas with high grades of dysplasia.4 These results have been confirmed by other laboratories (19). Thus persistent elevation of mucosal ODC activity seems to be a marker for increased cell proliferation and possibly also for the carcinogenesis process, in both the experimental rodent chemical carcinogenesis model and the development of adenomas and adenocarcinomas in humans. The current results suggest that the rat carcinogenesis model is an appropriate model for the study of the role of ODC and polyamines in the carcinogenesis process. The results further suggest that this rodent model of colon carcinogenesis might be divided into early and late phases (which may correspond to initiation and promotion phases), on the basis of the distinct biphasic pattern of elevation in ODC activity. This approach may allow a better way of studying and eventually modulating the multistep nature of the carcinogenesis process.

ACKNOWLEDGMENTS

We thank Dr. Stephen B. Baylin, Dr. Albert H. Owens, Jr., Dr. John H. Yardley, and Dr. Benjamin M. Baker for advice, encouragement, and support, and Sandra Lund for secretarial support.

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