Introduction of Ornithine Decarboxylase Activity in Mouse Urinary Bladder by L-Tryptophan and Some of Its Metabolites

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ABSTRACT

The responses of female noninbred mouse urinary bladder ornithine decarboxylase (EC 4.1.1.17) (ODC) and S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) (SAMD) activities to L-tryptophan feeding and to topical intravesical administration of L-tryptophan and some of its urinary metabolites were studied. Mice fed a diet containing 1% L-tryptophan demonstrated significant increases in vesical ODC and SAMD activities as early as 2 weeks after the commencement of the diet. By the end of the third week, ODC and SAMD activities reached peak values of 7- and 3-fold, respectively, significantly greater than the control levels (p < 0.05 and p < 0.01, respectively). Then, enzyme activities gradually decreased but remained at levels significantly higher than those of the control mice until the end of the sixth week when the study was terminated. Topical application by urethral catheter of some urinary tryptophan metabolites was followed by a rapid, transient induction of urinary bladder ODC activity within 5 hr. Statistically significant differences between vehicle controls and xanthurenic acid (p < 0.01), DL-kynurenine (p < 0.01), L-kynurenine (p < 0.01), anthranilic acid (p < 0.01), and quinolact acid (p < 0.05) were observed. However, no significant differences were seen with L-tryptophan, 3-hydroxy-DL-kynurenine, the 8-methyl ether of xanthurenic acid, or D-kynurenine or with 3-hydroxyxanthanlanilic, kynurenic, quinolinic, picolinic, and nicotinic acids. Bladder SAMD was not elevated significantly by most of these directly applied tryptophan metabolites. ODC inducibility by active compounds was followed by mucosal hyperplasia within 7 days. These data suggest that certain L-tryptophan metabolites may be involved in two-stage urinary bladder carcinogenesis in a manner similar to that shown to occur in murine skin tumor systems by other chemicals.

INTRODUCTION

The urinary bladder epithelium is susceptible to a wide variety of chemical carcinogens (4-6, 11, 12, 31, 32). L-Tryptophan, an essential amino acid, is extensively metabolized (Chart 1), resulting in urinary excretion of several metabolites, some of which have demonstrated urinary bladder carcinogenic activities in experimental systems (4-6, 9). A relationship between elevated urinary tryptophan metabolite levels and bladder cancer in humans was proposed (3, 4, 32, 40). Recent data (11, 12, 16, 21, 34) suggest that tryptophan or its urinary metabolites may be cocarcinogens or promoters of bladder neoplasia. We first proposed (4-6, 9, 40) that bladder carcinogenesis was a multifactorial process that might involve stages of induction and promotion analogous to those of skin tumorigenesis (1, 39). This proposal has been confirmed and extended by others (11, 12, 16, 17, 21).

Evidence that polyamines and the enzymes responsible for their biosynthesis play a significant role in carcinogenesis, tumor promotion, and cellular hyperplasia has been presented (1, 19, 28, 35, 39). Data have been presented that ODC induction by 12-O-tetradecanoylphorbol-13-acetate in mouse skin, although not sufficient (20, 27), is an important component of the mechanism of tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (1, 28, 38). ODC induction also was reported to occur in carcinogenesis of liver (30, 36), lung (13), and colon (37), as well as urinary bladder (22). Single topical i.u. administration of the vesical carcinogens FANFT and ANFT resulted in early, exaggerated ODC activity in rodent urinary bladders (22). We report here the induction of mouse urinary bladder ODC and SAMD activities following systemic administration of L-tryptophan and following i.u. administration of some tryptophan metabolites.

MATERIALS AND METHODS

Chemicals. The following chemicals were purchased: reagent grade dimethyl sulfoxide and L-tryptophan (Aldrich Chemical Co., Milwaukee, Wis.); anthranilic acid and 3-hydroxyxanthanlanilic acid (Sigma Chemical Co., St. Louis, Mo.); ANFT (Saber Laboratories, Morton Grove, III.); DL-[1-14C]ornithine hydrochloride (specific activity, 52.5 mCi/mmol) and S-adenosyl-L-[carboxyl-14C]methionine (specific activity, 52.3 mCi/mmol) (New England Nuclear, Boston, Mass.). The tryptophan metabolites nicotinic acid; picolinic acid; quinic acid; quinolinic acid; kynurenic acid; D- and L-kynurenine; 3-hydroxy-DL-kynurenine; the 8-methyl ether of xanthurenic acid; and xanthurenic acid were generously provided by Dr. R. R. Brown (University of Wisconsin Clinical Cancer Center, Madison, Wis.). Chemical identity and purity of all test compounds were checked by melting point, IR and UV absorption spectrophotometry, and paper and high-performance liquid chromatography (3).

Animals and Treatment. Female noninbred Swiss albino mice (Sprague-Dawley, Madison, Wis.), 12 to 14 weeks old, received pelleted diet (Wayne Lab Blox; Allied Mills, Inc., Chicago, Ill.) and water ad libitum and were killed between 1 and 3 p.m. in order to avoid circadian rhythm variations.

For the 6-week feeding trial with L-tryptophan, the following protocol was used: L-Tryptophan (50 g; 1% w/w) was mixed mechanically with...
40 g of anhydrous dextrose and 4910 g of ground diet and was stored in a refrigerator at 4–10° until used (15). This diet was fed to 180 mice for 0 to 6 weeks. Forty control mice were fed unmedicated ground diet for 0 to 6 weeks. Mice were weighed at the start of the study and when killed. Food consumption was determined at weekly intervals. Eight groups (3 mice/group) of L-tryptophan-treated mice were killed at weekly intervals; 4 groups (3 mice/group) of control animals were killed at the start of the study and at the end of the third and sixth weeks. For histological study, 6 mice fed the L-tryptophan-containing diet and 3 mice fed unmedicated ground diet were killed at weekly intervals. Urinary bladders were appropriately inflated with and fixed in 10% buffered formalin, and sections were stained with hematoxylin and eosin as described previously (15, 37).

Chemicals applied by i.u. instillations directly into the vesical lumen were solubilized in an appropriate solvent (Table 1), diluted with distilled water to the final indicated solvent percentage, and administered in a volume of 0.10 ml as described previously (22). For histological study, 3 or 6 mice were killed at 3, 5, 7, 10, 12, 24, 48, 120, and 168 hr after instillation of tryptophan metabolites.

**Tissue Preparation and Enzyme Assays.** Mice were killed at predetermined times by cervical dislocation. Whole bladders from 3 mice were pooled, homogenized, and centrifuged, and ODC and SAMD activities and protein content of the soluble bladder extracts were determined by methods described previously (22).

**RESULTS**

**Effects of L-Tryptophan Feeding on Urinary Bladder ODC and SAMD Activities.** No significant differences in diet consumption and growth rates were found between experimental and control groups. The daily dose of L-tryptophan in the experimental group was about 50 mg/mouse. Urinary bladder ODC (Chart 2A) and SAMD (Chart 2B) activities, measured at

![Chart 1. Metabolic scheme of the conversion of L-tryptophan to niacin, illustrating structures of metabolites studied. Compounds underlined exhibited significant murine bladder carcinogenicity by the intravesical pellet implantation technique (4).](chart)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Chemical</th>
<th>Dose (µmol)</th>
<th>Enzyme activity 5 hr after treatment (pmol CO2/30 min/mg protein)</th>
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<tr>
<td>1</td>
<td>L-Tryptophan Vehicle control (0.9% NaCl solution)</td>
<td>1.0</td>
<td>Groups ODC Groups SAMD</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>29 ± 8*</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>35 ± 8</td>
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<tr>
<td>2</td>
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<td></td>
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<tr>
<td></td>
<td>Quinolic acid</td>
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<td>Vehicle control (0.2% dimethyl sulfoxide)</td>
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*Mean ± S.E. for duplicate assay.

† p < 0.01.

‡ 0.05 > p > 0.01.

§ A few drops of 7.16 N NH4OH diluted with 2 × 10⁻² M phosphate buffer (pH 7.4).
activities. Following the instillation of 1 jumol of xanthurenic acid topphan Metabolites on Urinary Bladder ODC and SAMD Ac

groups at indicated weeks: *, 0.05 > p > 0.01 ; • •, p < 0.01 ; * • •, p < 0.001. S.E.; asterisks, statistically significant differences for treatment versus control

combined bladder extracts prepared from 3 mice for each group determination; bars, S.E. Points, mean of 4 (control groups) or

8 (experimental groups) duplicate determinations of enzyme activities from combined bladder extracts prepared from 3 mice for each group determination; bars, S.E.; asterisks, statistically significant differences for treatment versus control groups at indicated weeks: *, 0.05 > p > 0.01 ; **, p < 0.01 ; ****, p < 0.001.

weekly intervals after the start of the 1% L-tryptophan diet, increased significantly by 3 weeks and reached peak values 7- and 3-fold, respectively, greater than the control levels. Then, the activities gradually decreased but remained at significantly higher levels compared with the control levels through the sixth week. By 2 weeks, the epithelial mucosa of some mice fed L-tryptophan became 4 to 5 cells thick (normally 3 cells thick) in focal areas. This epithelial change did not appear progressive or involve more than 1 or 2 mice at each time period studied. No epithelial changes were evident in control mice.

Effects of Direct Intravesical Administration of Some Tryptophan Metabolites on Urinary Bladder ODC and SAMD Activities. Following the instillation of 1 jumol of xanthurenic acid into the bladder, the ODC activity increased rapidly to a significant (p < 0.01) maximum at 5 hr, 2-fold over the control level, and quickly decreased to control levels by 24 hr (Chart 3). This kinetic pattern was similar to that obtained after treatment with ANFT (22) except that the magnitude of ODC induction was much greater with ANFT treatment (Table 1). Nicotinic acid treatment did not activate ODC significantly compared with the vehicle control at any time (Chart 3). Other tested metabolites (Table 1) showed the same kinetic patterns as that of xanthurenic acid or nicotinic acid.

Peak ODC and SAMD activities at 5 hr following i.u. administration of L-tryptophan and some of its metabolites are presented in Table 1. Xanthurenic acid, DL-kynurenine, anthranilic acid, and quinaldic acid showed significant inductions of ODC but not of SAMD when compared with the matched vehicle controls. L-Kynurenine activated ODC significantly whereas D-kynurenine did not. Furthermore, the racemic compound DL-kynurenine resulted in a value of ODC activity intermediate between those of the L and D isomers. L-Tryptophan 3-hydroxy-

Bladder ODC Induction by Tryptophan Metabolites

Effects of Tryptophan Metabolites on Urinary Bladder Morphology. Following tryptophan metabolite or vehicle treatment, some bladders showed mucosal hyperemia, congestion, and focal hemorrhage as early as 3 hr, but these changes were not observed consistently with any treatment. By 24 hr following i.u. treatment with xanthurenic acid, DL-kynurenine, L-kynurenine, anthranilic acid, or quinaldic acid, the urothelial cells demonstrated positional irregularity and crowding, and by 48 hr mitotic figures were frequently observed. By 7 days, mild, multifocal hyperplasia appeared as the epithelial mucosa became 4 to 5 cells thick. This hyperplastic effect of the tested tryptophan metabolites was parallel to their ODC inducibility.

DISCUSSION

Since the report of Dunning et al. (14) that added dietary tryptophan enhanced the rat bladder carcinogenicity of 2-acetylaminofluorene, a number of studies have been conducted in an effort to ascertain if the essential amino acid L-tryptophan or its urinary metabolites are bladder carcinogens (3-9). Several of these metabolites exhibited bladder oncogenicity when tested by the intravesical pellet implantation technique (4, 9), although the systemic administration of tryptophan or some of its metabolites did not induce bladder cancer (4, 21, 25, 29, 34). Recent studies (11, 12, 16, 21, 34) suggested that tryptophan or its urinary metabolites might be cocarcinogens or promoters of bladder neoplasia. Promoting activity of dietary DL-tryptophan for the bladder was demonstrated in rats (12) and dogs (34) following initiation by FANFT or arylamines, respectively. Dietary L-tryptophan was shown to promote bladder tumor formation in mice (21) and rats (16) following initiation by FANFT. It also significantly increased the labeling and hyperplastic indices of mouse bladder urothelium (21).
Although the initiation-promotion concept was developed from tumorigenicity investigations with mouse skin, recent studies have suggested that tumor promotion may be operative in some other organs including the urinary bladder (4-6, 9, 11, 12, 16, 17, 21, 34). Several properties of initiation and promotion have been characterized experimentally in the murine skin cancer model (1, 28, 39). Most initiating agents are mutagenic in various short-term in vitro assays, whereas promoting agents are not necessarily mutagenic or have only weak mutagenic activity. Promoting agents usually have the ability to induce hyperplasia of target tissues without initiation, but this alteration does not progress toward cancer formation unless the tissues first have been treated with initiating agents. Tryptophan and several of its metabolites were not mutagenic by the Ames test (2). However, focal hyperplasia or cellular proliferation following tryptophan feeding was reported in mice (21), rats (25), and dogs (33).

We now have demonstrated that mouse urinary bladder ODC and SAMD activities were stimulated by feeding L-tryptophan, the method generally selected for experimental induction of bladder neoplasia by this chemical (12, 16, 21, 25, 34), that direct application of several tryptophan metabolites to the mouse urinary bladder led to a rapid, transient induction of urinary bladder ODC activity within 5 hr, and that the metabolites which activated ODC also induced a hyperplastic reaction of the urothelium following their topical application. Xanthurenic acid, DL-kynurenine, L-kynurenine, anthranilic acid, and quinaldic acid showed significant increases of bladder ODC activity; all these compounds except anthranilic acid were active carcinogens by the bladder pellet implantation technique (4, 9). Conversely, L-tryptophan or D-kynurenine, 3-hydroxy-DL-kynurenine, and 3-hydroxyanthranilic acid displayed bladder carcinogenicity (4, 9). The in vitro induction of ODC activity in rat urinary bladder carcinoma cells by 3-hydroxyanthranilic acid was recently reported (18). The relationship of this observation (18) utilizing an in vitro rat neoplastic cell system to the in vivo study of nonneoplastic mouse urothelial cell responses reported here remains to be determined. Previous studies demonstrated that, by the methods of i.u. administration used, L-tryptophan (26), 3-hydroxy-L-kynurenine, 3-hydroxyanthranilic acid (8), or the 8-methyl ether of xanthurenic acid (7) easily traversed the bladder wall and thus afforded urothelial cell contact. L-Kynurenine, a natural metabolite of L-tryptophan, showed a significant induction of bladder ODC activity, but D-kynurenine, an optical isomer of L-kynurenine, failed to stimulate bladder ODC activity. The stereoisomeric specificity of bladder ODC induction suggests that this phenomenon is not simply the result of physical or chemical irritation but is due to rather specific biological or biochemical processes.

The results of the present study provide additional evidence that some L-tryptophan metabolites have properties consistent with those of a promoter for urinary bladder carcinogenesis. Although urinary metabolites of tryptophan were not measured in this study, it was previously shown (9) that, following a single dose of 25 mg of L-tryptophan to mice, increased quantities of several metabolites were in urine. Several of these metabolites are excreted in augmented quantities in the urine of as many as 50% of patients with bladder neoplasia (3). Additionally, patients with certain elevated urinary tryptophan metabolites exhibited a significant tendency towards the development of heterotopic bladder recurrences following removal of initial low-stage, low-grade bladder neoplasia (40). Because metabolism of L-tryptophan is dependent on the bioavailability of vitamin B6 (pyridoxine), the metabolism of tryptophan to some of these promoting or cocarcinogenic metabolites can be minimized by pyridoxine administration. Of substantial interest is the report (10) that pyridoxine was significantly better than placebo in preventing recurrence of Stage I bladder cancer in those patients not developing recurrence within a 10-month period, although quantitative urinary tryptophan metabolism was not measured in these patients.

ACKNOWLEDGMENTS

We thank Drs. R. K. Boutwell and A. K. Verma of the McArdle Laboratory for Cancer Research, University of Wisconsin, for assistance with method development and critical discussions; D. Lueder for preparation of histology sections; and S. Pertzborn and K. Blomstrom for aid with manuscript preparation.

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