Enhancement by Vasoactive Intestinal Peptide of Experimental Carcinogenesis Induced by Azoxymethane in Rat Colon

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ABSTRACT

The effects of vasoactive intestinal peptide (VIP) on the incidence and histology of colonic tumors induced by azoxymethane (AOM) were investigated in Wistar rats. Rats were given 20 μg/kg body weight of VIP every other day for 12 weeks and from experimental week 3, were given 10 weekly injections of 7.4 mg/kg body weight of AOM. The administration of VIP before and during AOM treatment resulted in a significant increase in the incidence of colonic tumors in week 40. Furthermore, it caused a significant increase in the labeling index of the colonic mucosa during AOM treatment. These findings indicate that VIP enhanced the development of colonic tumors. This effect may have been related to its effect in increasing proliferation of cells in the colonic mucosa during administration of the carcinogen.

INTRODUCTION

VIP was initially isolated from the gut (1) and later found in the central and peripheral nervous system (2, 3). It has important roles in the physiology and pathophysiology of the gastrointestinal tract (4), and its biological effects seem to be mediated via cAMP production (5–10). These effects have been shown to be due to its stimulation of adenylate cyclase activity in the plasma membranes of epithelial cells. Specific receptors or binding sites for VIP have been identified and characterized on hepatocytes (11), enterocytes (12) and human colonic cancer cells (10) and pancreatic acinar cells (13). There is much evidence that cAMP is involved in the control of growth, differentiation, and transformation of various cell types (14–17), and that its level is altered in malignant tumor cells (18). Moreover, the cAMP content of adenocarcinomas of the human colon is reported to be lower than that of adjacent uninvolved mucosa (19). These findings suggest that VIP has an effect on growth of colonic tumors. However, further work is needed to demonstrate that VIP receptors in the colon are not only of physiological relevance but also can influence the development of colonic tumors (20). Therefore, in the present work, we examined the effect of treatment of rats with VIP from before the time of injection of a carcinogen on the development of colonic tumors.

MATERIALS AND METHODS

A total of 84 young male Wistar rats weighing 80–90 g were randomly divided into four groups and treated as follows.

Group 1 (30 rats) was given VIP every other day for 12 weeks at a dose of 20 μg/kg body weight. VIP (porcine; Peptide Institute Inc., Osaka, Japan) was injected as a suspension in olive oil to prolong its effect. Injections were given s.c. in a volume of 2 ml/kg body weight, between 2 and 3 p.m. on each day, various sites of injection being chosen. From experimental week 3, animals were also given 10 weekly s.c. injections of 7.4 mg/kg body weight of AOM (Sigma, St. Louis, MO) in 0.9% NaCl solution. Group 2 (30 rats) was given the vehicle olive oil instead of VIP, and from week 3, AOM for 10 weeks in the same way as group 1. Group 3 (12 rats) was given VIP only in the same way as group 1, and was not treated with AOM. Group 4 (12 rats) was given olive oil only in the same way as group 2, and was not treated with either VIP or AOM.

The experimental groups were kept in different cages with a wire mesh bottom under identical conditions during the experiment, and had free access to tap water and regular chow pellets (Oriental Yeast Co., Tokyo, Japan).

Rats were killed when they became moribund, and surviving animals were killed at the end of week 40. Rats that were killed during the experimental period were autopsied. The large intestine was opened, pinned flat on a cork mat, and fixed with buffered picric acid-formaldehyde solution (21). The fixed colon was cut into five segments of equal length, which are referred to hereafter as part 1 (adjacent to the anal orifice to part 2) (adjacent to the cecum). Tumor-bearing areas and areas suspected of having lesions were dissected, and embedded in paraffin. Semiserial sections (average, 30 per block) of 5-μm thickness were made to expose the central part of the tumor or the stalk, when present, and stained with H & E. In addition to tumors, flat mucosa of the fixed colon with no visible tumors from each segment of the colon was cut into two strips of 3-mm width, which were embedded in paraffin. Five thin sections from each block were prepared, and were inspected microscopically for tumor foci. Sections were examined without knowledge of the treatment.

The crypt column lengths and labeling indices of the colonic mucosa in parts 2 and 4 were examined in weeks 7 and 40. For these examinations, five rats each in groups 1 and 2, and six rats each in groups 3 and 4 were killed in week 7, and six rats each in all groups were killed in week 40. For measurement of the crypt column length (22), only sections that showed the entire crypt column length from its base to the mouth of the crypt were used. On two slides from each segment of the colon, the number of cells from the bottom to the mouth of the crypt was counted in 20 separate crypt columns, care being taken to avoid adenomas and CIS. The labeling index of the colonic mucosa was measured with an immunohistochemical kit for analysis of BrdUrd incorporation (23, 24) from Becton Dickinson Immunocytometry System (Mountain View, CA), by the modified method described by Tada et al. (25). In brief, rats received an i.p. injection of 20 mg/kg body weight of BrdUrd and 1 h later were killed with ether. The colon was then excised, fixed in 70% ethanol for 4 h and embedded in paraffin. Sections of 3-μm thickness were immersed in 2 N HCl solution for 30 min at room temperature, and then in 0.1 M Na2B4O7 to neutralize the acid. Slides were immersed in methanol containing 3% H3O2 for 30 min, and then treated with 10% porcine serum. The specimens were stained with anti-BrdUrd monoclonal antibody (dilution 1:100) for 2 h at room temperature, washed, stained with biotin-conjugated horse anti-mouse antibody (at a dilution of 1:200) for 2 h, and stained with avidin-biotin-peroxidase complex for 30 min. The reaction product was located with 3,3'-diaminobenzidine-tetrahydrochloride. Cells that contained BrdUrd were identified by the presence of a dark pigment over their nuclei. For analysis of the labeling index, the numbers of BrdUrd-labeled cells were counted in 25 glands on each slide from two different blocks of each segment of the colon without knowledge of the treatment. Care was taken to avoid areas of adenoma and CIS in making counts. The labeling index was expressed as the number of labeled nuclei per gland.
RESULTS

Incidence and Number of Colonic Tumors. In week 40, there was no significant difference in the body weights of the four groups. In groups 1 and 2, three and one rat, respectively, died of respiratory infection before week 28. No tumors were found in these animals, which were excluded from effective numbers. The first tumor of the colon was found in a rat in group 2 that died in week 28, so animals that survived for more than 28 experimental weeks were included in effective numbers. In groups 1 and 2, five and six rats, respectively, died between weeks 28 and 40. The average (±SE) times of these early deaths in weeks 1 and 2 were 34 ± 1 (range: 31–36) and 35 ± 1 (range: 31–39) weeks, respectively.

The incidences and numbers of colonic tumors per tumor-bearing animal in each group are summarized in Table 1.

In group 2 (AOM and olive oil), colonic tumors were found in 15 (62.5%) of 24 rats examined. The average number of colonic tumors per tumor-bearing rat being 1.3 ± 0.1. In group 1 (AOM and VIP) the incidence of tumors and number of tumors per tumor-bearing rat were significantly higher than those in control group 2. No colonic tumors were found in groups 3 (VIP alone) or 4 (olive oil alone).

Histological Types of Colonic Tumors. Colonic tumors were classified histologically into adenomas, CIS, and adenocarcinomas (28). The adenomas were benign, with mild or moderate epithelial atypism. CIS showed severe atypia of the epithelium but no penetration through the muscularis mucosae. Adenocarcinomas were frank malignant tumors showing invasion through the muscularis mucosae. The adenocarcinomas were further classified as well-differentiated and mucinous carcinomas. In the former, tumor cells were found in acinar clusters simulating the glandular crypts of normal colon mucosa. In the latter, mucin secretion was active, resulting in mucous nodules containing large amounts of extracellular mucin with only a few isolated groups of tumor cells. The tumors had a glandular structure with regularly arranged cells.

Table 2 shows the histological types of the 65 colonic tumors in groups 1 and 2. In control group 2, only 5 (26.3%) of the 19 tumors were adenocarcinomas, but in group 1 the incidence of adenocarcinomas was significantly greater, being 65.2%. Table 2 also shows the distribution of histological types of adenocarcinomas induced in AOM-treated rats. In group 2 (AOM and olive oil), 60.0% of the adenocarcinomas were of the mucinous type, whereas in group 1 (AOM and VIP), 20.0% were of the mucinous type, but the difference in their incidences in these two groups was not statistically significant.

There were no significant differences between groups 1 and 2 in the incidences of metastases of colonic carcinomas to the peritoneum and/or lymph nodes, ear duct tumors or tumors of the small intestine.

Labeling Indices and Crypt Column Lengths of Colonic Mucosa. Table 3 summarizes the data on the labeling indices and crypt column lengths of colonic mucosa in each group in experimental weeks 7 and 40.

In experimental week 7, AOM treatment of group 1 (AOM and VIP) and group 2 (AOM and olive oil) resulted in significant increases in the crypt column length and labeling index in both parts 2 and 4 of the colonic mucosa. Administration of VIP during AOM treatment in group 1 caused a significant increase in the crypt column lengths and labeling indices of parts 2 and 4 of the colonic mucosa. Treatment with VIP alone (group 3) had no influence on the crypt column length or labeling index of part 2 or 4 of the colonic mucosa.

In experimental week 40, there was no significant difference in the column lengths or labeling indices of the colonic mucosa in the four groups.

DISCUSSION

An interesting problem is whether VIP regulates normal and malignant cell growth in the gut mucosa. Murakami and Masui (29) reported establishment of a human colon carcinoma cell line in nude mice and found that VIP had little or no stimulatory effect on growth of these cells in serum-free medium. Moreover, Kobori et al. (30, 31) examined the effects of many gastrointestinal hormones on growth of cell line BV9, derived from a rat gastric carcinoma, and found that VIP had little or no influence on growth of these cells. However, there are no reports on the effects of VIP on experimental gastrointestinal carcinogenesis. In the present work, we found that administration of VIP resulted in significant increase in the incidence and number of colonic tumors in rats.

For prolonging the period of their absorption, hormones are

Table 1 Incidences and numbers of colonic tumors and body weights in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body weight</th>
<th>Effective no. of rats</th>
<th>No. of rats with colonic tumors (%)</th>
<th>No. of colonic tumors per tumor-bearing rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial 40W</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>AOM + VIP</td>
<td>86 ± 1</td>
<td>354 ± 12</td>
<td>22</td>
<td>21 (95.5)%</td>
</tr>
<tr>
<td>2</td>
<td>AOM + olive oil</td>
<td>82 ± 1</td>
<td>341 ± 13</td>
<td>24</td>
<td>15 (62.5%)</td>
</tr>
<tr>
<td>3</td>
<td>VIP alone</td>
<td>84 ± 2</td>
<td>364 ± 9</td>
<td>6</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>4</td>
<td>Olive oil</td>
<td>85 ± 1</td>
<td>350 ± 5</td>
<td>6</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

* AOM + VIP: Rats were given 20 μg/kg of VIP for every other day for 12 weeks, and from week 3, were given 10, weekly injections of 7.4 mg/kg of AOM. AOM + olive oil: Rats were given the vehicle, olive oil, only for 12 weeks, and from week 3, were given 10, weekly injections of 7.4 mg/kg of AOM. VIP alone: Rats were given 20 μg/kg of VIP for 12 weeks, with no carcinoma. Olive oil alone: Rats were given olive oil only for 12 weeks, with no carcinoma.
* The difference between the values for groups 1 and 2 was significant at P < 0.05.
* The difference between the values for groups 1 and 2 was significant at P < 0.01.

Table 2 Histological types of colonic tumors and incidences of metastases and extracolonic tumors in AOM-treated rats

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of colonic tumors</th>
<th>Adenoma</th>
<th>CIS</th>
<th>Total no.</th>
<th>Well differentiated (%)</th>
<th>Mucinous (%)</th>
<th>Effective no. of rats</th>
<th>Metastases of colonic carcinomas to the peritoneum and/or lymph nodes (%)</th>
<th>Ear duct tumor (%)</th>
<th>Small intestinal tumor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM + VIP</td>
<td>46</td>
<td>3</td>
<td>13</td>
<td>30</td>
<td>24 (80.0%)</td>
<td>6 (20.0%)</td>
<td>22</td>
<td>1 (4.5)</td>
<td>6 (27.3%)</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>2</td>
<td>AOM + olive oil</td>
<td>19</td>
<td>2</td>
<td>12</td>
<td>5</td>
<td>2 (40.0%)</td>
<td>3 (60.0%)</td>
<td>24</td>
<td>3 (12.5)</td>
<td>8 (33.3%)</td>
<td>2 (8.3%)</td>
</tr>
</tbody>
</table>

* For explanation of treatments, see Table 1.
* The difference between the incidences of adenocarcinomas in groups 1 and 2 was significant at P < 0.01.

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often dissolved in dehydrolyzed gelatin (32, 33) or soybean oil with and without beeswax (34, 35). For this purpose, in the present work, we used VIP as a suspension in olive oil, because in previous studies on the effect of tetragastrin as a suspension in olive oil on gastric carcinogenesis in rats, we found that its administration in this depot form caused significant increase in acid secretion for more than 2 days.  

There is much evidence that CAMP is involved in the control of growth and differentiation of various cell types (14–17), and recent results have suggested that CAMP may inhibit cell growth (36). However, under certain experimental conditions, CAMP is a positive rather than a negative signal for hepatocyte growth (37). Moreover, adenylate cyclase activity has been reported to be higher in rapidly proliferating crypt cells of the intestinal mucosa than in slowly growing superficial cells (38). Johnson (39) reported that VIP did not stimulate DNA synthesis of normal colonic mucosa. In the present work, however, we found that VIP increased the labeling index and crypt column length of the colonic mucosa of rats in week 7 during carcinogen treatment. We also found that the responses to VIP of colon epithelial cells with and without carcinogen treatment differed. Therefore, although other possibilities cannot be excluded, it seems likely that this effect of VIP is seen only when hyper-reactivity induced by carcinogen treatment is evident. The exact mechanism of the effect of VIP is unknown and needs further investigation, but VIP may influence rapidly proliferating cells and promote the appearance of genetic damage and eventual tumor formation.

REFERENCES


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