[CANCER RESEARCH 51, 6150–6152, November 15, 1991]

Inhibition by the Dopamine Antagonist Haloperidol of Experimental Carcinogenesis Induced by Azoxymethane in Rat Colon[1]

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ABSTRACT

The effects of the dopamine agonist bromocriptine and antagonist haloperidol on the incidence and histology of colon tumors induced by azoxymethane and on the labeling index of colon mucosa were investigated in Wistar rats. Rats received weekly s.c. injections of 7.4 mg/kg of body weight azoxymethane for 10 weeks and s.c. injections of 2 mg/kg of body weight bromocriptine or 2 mg/kg of body weight haloperidol, in depot form, every other day until the end of the experiment in week 30. Administration of haloperidol resulted in a significant decrease in the incidence of colon tumors. It also caused a significant decrease in the incidence of adenocarcinomas, with 75% of the tumors being adenomas, and in the labeling index of the colon epithelial cells. In contrast, bromocriptine had no influence on the incidence or histology of colon tumors or the labeling index of the colon mucosa. These findings indicate that the dopamine antagonist haloperidol inhibits colon carcinogenesis and that this effect may be related to its effect in decreasing the proliferation of colon epithelial cells.

INTRODUCTION

Dopamine is an important enteric neruromodulator. In the gut, it has a wide spectrum of actions, including effects on gastric, pancreatic, and duodenal secretions, gastric, intestinal, and colonic motility, and gastric and intestinal mucosal blood flow (1). Recently, we found that administration of the dopamine agonist bromocriptine[3] every other day significantly increased the incidence and number of gastric cancers and the labeling index of the gastric antral mucosa. These findings indicate that gastric carcinogenesis is regulated by a dopaminergic mechanism.[4] Consistent with these findings, Scemama *et al.* (2) reported the presence of dopamine receptors in a human colon adenocarcinoma cell line (HT 29). Therefore, it seemed likely that dopamine might affect colon carcinogenesis. To test this possibility, we examined the effects of a dopamine agonist, bromocriptine, and an antagonist, haloperidol, on the incidence, number, and histological type of colon tumors induced by AOM in rats.

MATERIALS AND METHODS

Animals. Seventy-five 6-week-old male Wistar rats were purchased from SLC (Shizuoka, Japan). The animals were housed in suspended cages with wire mesh bottoms, in a room controlled at 21 ± 1°C and 40 ± 10% humidity, with a 12/12 light/darkness cycle. Regular chow pellets (Oriental Yeast, Tokyo, Japan) were available *ad libitum*.

Treatments. The rats were randomly divided into three groups, of 25 rats each, and given weekly s.c. injections of 7.4 mg/kg of body weight AOM (Sigma, St. Louis, MO), in 0.9% NaCl solution, for 10 weeks. From the start of administration of AOM, the animals also received s.c. injections of olive oil (Group 1), 2 mg/kg of body weight bromocriptine (Group 2), or 2 mg/kg of body weight haloperidol (Group 3). Bromocriptine (Sigma) and haloperidol (Sigma) were suspended in olive oil and were injected in a volume of 1 ml/mg of body weight every other day between 2 and 3 p.m. until the end of the experiment, with various sites of injection being chosen. Group 1 received s.c. injections of 1 ml/kg of body weight of the vehicle, olive oil, only every other day.

The doses of bromocriptine and haloperidol were based on the results of our previous experiment. We recently found that administration of 1 or 2 mg/kg of body weight bromocriptine significantly increased the incidence of gastric cancers induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in rats, but 1 or 2 mg/kg of body weight haloperidol had little or no influence on gastric carcinogenesis. Therefore, in the present study, we used 2 mg/kg of body weight bromocriptine and haloperidol.[4]

Histological Observations. Since the first tumor of the colon was found in a rat in Group 1 that died in week 27, animals that survived for more than 27 experimental weeks were included in the effective numbers. Rats were killed when they became moribund during the experiment, and surviving animals were killed in week 30. All rats were autopsied and their internal organs were carefully examined. 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 5 (adjacent to the cecum). Tumor-bearing areas and areas suspected of having lesions were excised and embedded in paraffin. In addition to tumors, flat mucosa from each segment of the fixed colon, with no visible tumors, was cut into two strips of 3 mm width, which were embedded in paraffin.

Classification of Colon Tumors. Colon tumors induced by AOM were classified histologically into adenoma, carcinoma *in situ*, and adenocarcinoma (3). The adenocarcinomas were further classified as either well differentiated or mucinous carcinomas.

Measurement of Labeling Index and Mitotic Index of Colon Mucosa. The labeling index of colon mucosa was measured in weeks 9 and 30, with an immunohistochemical analysis kit for assaying BrdU incorporation (Becton-Dickinson, Mountain View, CA) (4, 5), by the modified method described by Tada *et al.* (6). For this, five rats in each group were fasted for 12 h and then treated s.c. with 1 ml/kg olive oil (Group 1), 2 mg/kg bromocriptine (Group 2), or 2 mg/kg haloperidol (Group 3). One h later the animals received an i.p. injection of 20 mg/kg BrdU, and after another hour they were sacrificed with ether. The colon was removed and fixed in 70% ethanol for 4 h. Parts 2 (distal colon) and 4 (proximal colon) of the colon were then excised and embedded in paraffin.

For determination of the labeling index of colon mucosa, the numbers of BrdU-labeled and unlabeled cells in the zone of proliferating cells were counted without knowledge of which treatment group the samples were from. All cells below the highest labeled cell in each crypt column were regarded as being within the zone of proliferating cells (7). From these measurements, we calculated the labeling index (number of BrdU-labeled cells/total number of cells within the zone of proliferating cells).

The mitotic index of colon mucosa was also measured in weeks 9 and 30 by the method of Cameron *et al.* (8). The mitotic index was defined as the mean number of metaphase figures per midaxial crypt section in the 30 crypts. A midaxial (longitudinal) crypt section was considered complete if the lumen was exposed from the top to the base of crypt.

Received 5/14/91; accepted 9/9/91.

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[1] This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare for a Comprehensive 10-Year Strategy for Cancer Control, Japan.

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[3] The abbreviations used are: bromocriptine, 2-bromo-α-ergocryptine methanesulfonate; AOM, azoxymethane; BrdU, bromodeoxyuridine; haloperidol, [4-[4-(4-chlorophenyl)-4-hydroxy-piperidino]-4'-fluorobutyrophenone].

[4] H. Iishi, M. Baba, M. Tatsuta, S. Okuda, and H. Taniguchi. Dopaminergic influence on gastric carcinogenesis induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in Wistar rats, submitted for publication.

Statistical Analysis. Data were analyzed by the χ^2 test or Fisher's exact probability test (9) or by one-way analysis of variance with Dunn's multiple comparison (9–11). Data are shown as means ± SE. Differences were regarded as significant when the calculated P value was <0.05.

RESULTS

Incidences and Numbers of Colon Tumors and Body Weights. Five rats in each group were killed in week 9 for determination of the labeling index of the colon mucosa. In addition, three rats in Group 1 and one in Group 3 were killed before week 27, because they became moribund. No tumors were found in any of these animals, which were excluded from the effective numbers.

The incidences and numbers per rat of colon tumors, and the mean body weights in each group, are summarized in Table 1. In week 30, the body weights of animals that had received bromocriptine (Group 2) and haloperidol (Group 3) were significantly more and less, respectively, than those of the controls (Group 1).

In Group 1 (olive oil only), colon tumors were found in 9 (53%) of 17 rats examined, and the average number of colon tumors per rat was 0.6 ± 0.2. Administration of bromocriptine (Group 2) had no influence on the incidence or average number per rat of colon tumors, compared with the values in Group 1. Administration of haloperidol (Group 3), however, reduced the incidence of colon tumors significantly and the average number per rat of colon tumors slightly but not significantly.

Histological Types of Colon Tumors and Adenocarcinomas. Table 2 shows the distribution of different histological types of colon tumors and the histological types and depths of involvement of adenocarcinomas in the three groups. In Group 1 (olive oil only), 73% of the colon tumors were identified as adenocarcinomas. In Group 3 (haloperidol), the incidence of adenocarcinomas was significantly less than that in Group 1, with 75% of colon tumors being adenomas. Administration of bromocriptine (Group 2) had no influence on the histological types of colon tumors. Table 2 also shows that there was no significant difference in the distribution of histological types or depths of involvement of colon adenocarcinomas in the three groups. In this series, there were no significant differences in the incidences of extracolonic tumors (ear duct tumors, small intestinal tumors, and metastasis to the peritoneum and/or lymph nodes) in the three groups.

Labeling Index and Mitotic Index of Colon Mucosa. Table 3 summarizes data on the labeling indices and the mitotic indices of the colon mucosa in weeks 9 and 30. At both times, administration of haloperidol (Group 3) significantly decreased the labeling indices and the mitotic indices of epithelial cells of the proximal and distal colon, but bromocriptine (Group 2) had no effect on the labeling index or the mitotic index of the colon mucosa at either time. In Group 3 (haloperidol), tumor-bearing rats had significantly higher mitotic indices of the proximal and distal colon than non-tumor-bearing rats (1.9 ± 0.3 *versus* 0.9 ± 0.2 in the proximal colon, $P < 0.05$; 1.6 ± 0.4 *versus* 0.6 ± 0.2 in the distal colon, $P < 0.05$).

Table 1 *Incidences and numbers of colon tumors, and body weights, in AOM-treated rats*

Group	Treatment[a]	Body weight (g) Initial	Body weight (g) 30 weeks	Effective no. of rats	No. of rats with colon tumors (%)	No. of colon tumors/ tumor-bearing rat
1	Olive oil	107 ± 1	370 ± 10	17	9 (53)	1.2 ± 0.1
2	Bromocriptine	110 ± 1	406 ± 6	20	8 (40)	1.4 ± 0.3
3	Haloperidol	109 ± 1	332 ± 7[b]	19	3 (16)[c]	1.3 ± 0.3

[a] Rats were given weekly s.c. injections of 7.4 mg/kg AOM for 10 weeks and s.c. injections of the vehicle (olive oil) (Group 1), 2 mg/kg bromocriptine (Group 2), or 2 mg/kg haloperidol (Group 3) every other day until the end of the experiment.
[b] $P < 0.01$.
[c] Significantly different from the value for Group 1, $P < 0.05$.

DISCUSSION

The present study showed that the dopamine antagonist haloperidol inhibited colon carcinogenesis induced by AOM in Wistar rats, resulting in a significant decrease in the incidence of colon tumors in week 30, but that the dopamine agonist bromocriptine had little or no influence on the induction of colon carcinogenesis.

The mechanism of the effect of haloperidol is unknown, but three possibilities may be considered. One is that its effect is related to decreased caloric intake. Klurfeld *et al.* (12) found that, when rats were treated with 7,12-dimethylbenz(*a*)-anthracene, a group subjected to caloric restriction that weighed 40% less than rats fed *ad libitum* had significantly lower incidences of mammary and colon tumors than the group fed *ad libitum*. Albanes (13) also found that total caloric intake is an important determinant of tumorigenesis in mice and that body weight may be a sensitive indicator of this effect. In the present study, treatment with haloperidol, but not bromocriptine, resulted in significantly lower body weights than those of control rats, which reflected lower food or caloric intake by the haloperidol-treated rats. Thus, their lower incidence of colon tumors could be explained by reduced caloric intake.

A second possibility involves a change in the intracellular cAMP level. There is much evidence that cAMP is involved in the control of growth and differentiation of various cell types (14–16). Tamaki *et al.* (17) examined the effect of dopamine on cAMP accumulation in isolated canine superficial cortical afferent arterioles and found that dopamine caused a dose-dependent increase in cAMP accumulation in afferent arterioles. Cheng *et al.* (18) also examined the effect of dopamine receptor agonists and antagonists on cAMP metabolism in opossum kidney cells, finding that dopamine stimulated cAMP production in these cells and that the stimulatory effect of dopamine was abolished by a dopamine$_1$ receptor antagonist. Their results suggested that cAMP might inhibit cell growth. However, under certain experimental conditions cAMP is a positive rather than a negative signal for hepatocyte growth (19).

A third possibility is an effect of haloperidol on the parasympathetic nervous system. Friedman *et al.* (20) found that K^+-evoked release of [^{3}H]acetylcholine from superfused slices of the striatum was enhanced by chronic treatment with haloperidol. Moreover, Miranda *et al.* (21) reported that the acetylcholinesterase activity of isolated rat vasa deferentia was significantly depressed by treatment with haloperidol, and they suggested that the ability of haloperidol to lower the acetylcholinesterase activity was the same as that of neostigmine. Acetylcholine has been demonstrated to influence cell division (22). Gurkalo and Volfson (23) examined the effect of nicotine on the development of gastric cancers induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and suggested that compounds that enhance cholinergic functions inhibit carcinogenesis. Recently, we found that prolonged administration of an inhibitor of acetylcholinesterase, neostigmine, significantly decreased the incidence and number of colon tumors (24). We also found that

Table 2 *Histological types of colon tumors and adenocarcinomas in AOM-treated rats*

		Colon tumor (%)				Adenocarcinoma (%)			
						Histology		Depth of involvement	
Group	Treatment[a]	Total no.	Adenoma	CIS[b]	Adenocarcinoma	Well differentiated	Mucinous	Submucosa	Muscle layer or deeper
1	Olive oil	11	3 (27)	0 (0)	8 (73)	2 (25)	6 (75)	3 (38)	5 (62)
2	Bromocriptine	11	6 (55)	0 (0)	5 (45)	1 (20)	4 (80)	2 (40)	3 (60)
3	Haloperidol	4	3 (75)[c]	0 (0)	1 (25)[c]	0 (0)	1 (100)	1 (100)	0 (0)

[a] For explanation of treatments, see Table 1.
[b] CIS, carcinoma *in situ*.
[c] Significantly different from the value for Group 1, $P < 0.05$.

Table 3 *Labeling indices and mitotic indices of colon mucosa in AOM-treated rats*

			Labeling index (%)		Mitotic index	
Experimental week	Group	Treatment[a]	Distal colon	Proximal colon	Distal colon	Proximal colon
9	1	Olive oil	33.1 ± 2.1	32.0 ± 2.1	3.4 ± 0.2	3.4 ± 0.4
	2	Bromocriptine	31.4 ± 2.4	33.9 ± 1.9	4.4 ± 0.2	4.0 ± 0.3
	3	Haloperidol	21.8 ± 1.8[b]	20.3 ± 1.2[c]	1.2 ± 0.4[c]	1.0 ± 0.4[d]
30	1	Olive oil	29.9 ± 1.9	30.6 ± 2.0	3.1 ± 0.2	3.8 ± 0.2
	2	Bromocriptine	30.7 ± 1.5	32.1 ± 1.4	3.6 ± 0.2	4.3 ± 0.3
	3	Haloperidol	18.2 ± 1.3[c]	21.9 ± 1.4[b]	1.3 ± 0.3[c]	1.5 ± 0.2[c]

[a] For explanation of treatments, see Table 1.
[b] Significantly different from the value for Group 1, $P < 0.02$.
[c] $P < 0.001$.
[d] $P < 0.01$.

neostigmine significantly decreased the labeling index of colon mucosa during AOM treatment. In the present study, we found that administration of haloperidol significantly decreased the labeling indices of epithelial cells of the proximal and distal colon, both during and after AOM treatment. If haloperidol reduced the blood flow to the colon, it could give the appearance of reduce BrdU incorporation. However, in the present study, we found that administration of haloperidol significantly decreased the mitotic indices of epithelial cells of the proximal and distal colon at both times examined.

Our results showed that administration of the dopamine antagonist haloperidol every other day caused significant decreases in both the incidence of colon tumors and the proliferative activity of the colon epithelial cells. Although we should combine the treatments to determine if bromocriptine would override the effect of haloperidol, our findings indicate that the development of colon tumors is regulated, through a dopaminergic mechanism, by proliferation of colon epithelial cells.

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