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ENHANCEMENT BY SULPIRIDE OF THE INHIBITORY EFFECTS OF CYSTEAMINE ON GASTRIC CARCINOGENESIS INDUCED BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE IN WISTAR RATS

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The effects of sulpiride on cysteamine inhibition of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and on the BUdR labelling index of gastric mucosa were investigated in inbred Wistar rats. After 25 weeks of oral treatment with MNNG, rats received one of the following alternate-day injections: cysteamine (2 doses), cysteamine (2 doses) plus sulpiride or sulpiride. At week 52, prolonged administration of cysteamine significantly reduced the incidence of adenocarcinomas of the glandular stomach. Cysteamine at low dose had no effect on the incidence of gastric cancers, but a combination of low-dose cysteamine and sulpiride caused a significantly greater reduction in the incidence of gastric cancers. Administration of sulpiride alone had no influence on gastric carcinogenesis. The labelling index of the antral mucosa was significantly lower in rats treated with high but not low doses of cysteamine. However, a combination of low-dose cysteamine and sulpiride significantly decreased the labelling index of the antral mucosa. Our findings indicate that cysteamine suppressed gastric carcinogenesis and that sulpiride enhanced this inhibition. Because sulpiride is a dopamine antagonist, these findings also indicate that dopamine may play an important role in cysteamine inhibition of gastric carcinogenesis.

We previously observed that long-term administration of the thiol agent cysteamine after oral treatment with MNNG for 25 weeks resulted in a significant reduction in the incidence of gastric cancers in the glandular stomach of Wistar rats (Tatsuta *et al.*, 1989*b,e,f*). We also found that cysteamine inhibits carcinogenesis in the colon (Tatsuta *et al.*, 1989*d*) and in the liver (Tatsuta *et al.*, 1989*a*). The exact mechanism of this effect is not clear.

Cysteamine is well known as the ulcerogen in experimental duodenal ulcers (Gallagher and Szabo, 1984; Szabo *et al.*, 1977). Gallagher *et al.* (1987) and Horner and Szabo (1981) have shown that the dopamine antagonist sulpiride aggravates cysteamine-induced duodenal ulcers. Therefore, in the present work, we examined the effects of concomitant treatment of cysteamine plus sulpiride on the development of gastric cancers induced by MNNG in Wistar rats.

MATERIAL AND METHODS

Animals

One hundred and fifty young (6-week-old) male inbred Wistar rats were used in this study. Animals were purchased from SLC (Shizuoka, Japan). The rats were housed in suspended, wire-bottomed metal cages in animal quarters with controlled temperature (21–22°C), humidity (30–50%), and light (12-hr cycle), and had free access to regular chow pellets (Oriental Yeast, Tokyo, Japan).

Carcinogen and treatments

The animals were given drinking water containing MNNG (50 μg/ml; Aldrich, Milwaukee, WI) for 25 weeks. The MNNG was dissolved in de-ionized water at a concentration of 2 mg/ml and kept in a cool, dark place. The stock solution was diluted to 50 μg/ml with tap water just before use. The MNNG solution was given to each rat from bottles covered with aluminum foil to prevent photolysis of MNNG, and the bottles were replenished every other day.

From week 26, the rats were given normal tap water *ad libitum* and randomly divided into 6 groups. They were injected s.c. every other day as follows until the end of the experiment: Groups 1 and 2 (30 rats each) were given 25 or 5 mg/kg body weight of cysteamine and 1 ml/kg body weight of olive oil. Groups 3 and 4 (30 rats each) were given cysteamine at the same dose as Groups 1 and 2, respectively, plus 30 mg/kg body weight of sulpiride. Group 5 (30 rats) was given 30 mg/kg body weight of sulpiride and 2 ml/kg of 0.9% NaCl solution. Group 6 (30 rats), the control group, was given only the vehicles, 2 ml/kg of 0.9% NaCl solution and 1 ml/kg of plain olive oil.

The doses of cysteamine and sulpiride were based on the results of our previous experiments (Tatsuta *et al.*, 1989*f*) and on those of Gallagher *et al.* (1987), respectively. Gallagher *et al.* (1987) reported that 30 mg/kg of sulpiride significantly aggravated cysteamine-induced duodenal ulcer.

Cysteamine (Sigma, St. Louis, MO) was given in 0.9% NaCl solution; sulpiride (Sigma) was given as a suspension in olive oil. Injections were made s.c. in various sites every other day at a volume of 2 mg/kg body weight for cysteamine and of 1 ml/kg body weight for cysteamine, between 2 and 3 P.M. each day.

Tissue sampling

Animals that survived for more than 50 weeks were included in the effective numbers because the first tumor of the glandular stomach was found in a rat from Group 6 that died in week 50. All surviving animals were killed at the end of the experiment in week 52. All rats were autopsied, and the stomach and other organs were carefully examined. The stomach was opened along the greater curvature, pinned flat on a cork mat, and fixed with Zamboni's solution (Stefanini *et al.*, 1967) for histological examination. The fixed stomach was cut into longitudinal strips 3 mm wide. The specimens were embedded in paraffin, and serial sections 5 μm thick were stained with hematoxylin and eosin. Sections were examined without knowledge of their group of origin.

Histological study

Histologically, adenocarcinomas were defined as lesions in which neoplastic glands had penetrated the muscularis mucosae to involve the submucosa or deeper layers. As previously

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Abbreviations: MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; sulpiride, N-1-[ethylpyrrolidine-2-ylmethyl]-2-methoxy-5-sulfamoylbenzamide; cysteamine, 2-mercaptoethylamine; BUdR, bromodeoxyuridine.

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reported (Tatsuta *et al.*, 1989*f*), the adenocarcinomas were classified into highly well-differentiated, well-differentiated, and poorly differentiated types.

Measurement of catecholamines in gastric wall

Norepinephrine and epinephrine concentrations in tissue of the gastric wall were determined at weeks 30 and 52 by high-performance liquid chromatography (Tatsuta *et al.*, 1983). Five rats in each group were fasted for 12 hr and received the following s.c. injections: Groups 1 and 2, cysteamine, 25 and 5 mg/kg, respectively, plus olive oil, 1 ml/kg; Groups 3 and 4, cysteamine 25 or 5 mg/kg, respectively, plus sulpiride, 30 mg/kg; Group 5, sulpiride, 30 mg/kg plus 0.9% NaCl, 2 ml/kg; and Group 6, 0.9% NaCl solution, 2 ml/kg plus plain olive oil, 1 ml/kg. Four hours later, the animals were killed by cervical dislocation and a sample of about 50 mg of the gastric wall was taken from each rat from the fundic and antral portions. In this experiment, mucosal samples were not used, because it is impossible to determine histologically the incidence and number of gastric cancers in mucosal samples over 50 mg. Each sample was homogenized with 4.0 ml of 0.4 N perchloride acid and centrifuged at 2,500 rpm for 10 min. The supernatant was mixed with 1.0 ml of 0.2 M EDTA and the mixture was adjusted to pH 6.0 with ammonium hydroxide. The mixture was then added to 300 mg of purified aluminum (Woelm Neutral Active Grade I) according to Anton and Sayre (1962), and pH was adjusted to 8.4–8.8 with ammonium hydroxide. The mixture was stirred for 5 min and centrifuged at 10,000 *g* for 10 min; the supernatant was aspirated and discarded. The precipitated aluminum was washed twice with distilled water and then shaken vigorously with 2.5 ml of 0.4 N acetate. The mixture was centrifuged and the clear supernatant transferred to a small glass tube and lyophilized for 3 hr. After the residue was dissolved in 0.5 ml of 0.2 N acetic acid, a 50-μl aliquot was injected into a liquid chromatographic column (Hitachi, Tokyo, Japan, 3011-C gel column, 2.6 × 250 mm). The material was eluted with 0.1 M KH_2PO_4 containing 0.05% H_3PO_4 at a constant flow rate of 0.5 ml/min at 45.0 ± 0.2°C. The effluent was mixed with the reagent for trihydroxyindole reaction, consisting of 0.0075% potassium ferricyanide, 0.1% ascorbic acid and 5 N sodium hydroxide. The resulting fluorescent products were examined with a highly sensitive spectrofluorophotometer.

Measurement of labelling index of gastric mucosa

The labelling index of gastric mucosa was measured at weeks 30 and 52 with an immunohistochemical analysis kit for assaying BUdR incorporation (Becton-Dickinson, Mountain View, CA) (Gratzner, 1982; Morstyn *et al.*, 1983), using the modified method of Tada *et al.* (1985). After fasting for 12 hr, 5 rats in each group received the following s.c. injections: Groups 1 and 2, cysteamine, 25 or 5 mg/kg, respectively, plus olive oil, 1 ml/kg; Groups 3 and 4, cysteamine 25 or 5 mg/kg, respectively, plus sulpiride 30 mg/kg; Group 5, sulpiride, 30 mg/kg plus 0.9% NaCl solution, 2 ml/kg; and Group 6, 0.9% NaCl solution, 2 ml/kg plus olive oil, 1 ml/kg. Three hours later, the rats received an intraperitoneal injection of 20 mg/kg of BUdR, and were killed 1 hr later with ether. The stomach was fixed in 70% ethanol for 4 hr. Sections 3 μm thick were immersed in 2 N HCl solution for 30 min at room temperature, and then in 0.1 M $Na_2B_4O_7$ to neutralize the acid. The sections were then stained with anti-BUdR monoclonal antibody (diluted 1:100) for 2 hr at room temperature, washed, stained with biotin-conjugated horse anti-mouse antibody (at a dilution of 1:200) for 30 min, and stained with avidin-biotin-peroxidase complex for 30 min. The reaction product was located with 3,3′-diaminobenzidine-tetrahydrochloride. Cells containing BUdR were identified by the presence of dark pigment over the nuclei.

To analyze the labelling index of gastric mucosa, the numbers of BUdR-labelled and unlabelled cells in the zone of proliferating cells were counted without knowledge of the treatment group of origin (Eastwood and Quimby, 1983). The zone of proliferating cells in the fundic mucosa was defined as a 250-μm rectangle between the highest and lowest labelled cells in a well-oriented section. Ten such rectangular areas were selected in each rat. In the antral mucosa, all cells below the highest labelled cell in each pit-gland column were regarded as being within the zone of proliferating cells. We selected 100 well-oriented columns of pits and glands in each rat. On the basis of these measurements, we derived the labelling index (number of BUdR-labelled cells/total number of cells within the proliferating zone).

Measurement of gastric acid secretion and serum gastrin levels

Gastric acid secretion and serum gastrin level were determined in experimental week 52.

Gastric secretions were collected for 1 hr by the method of Shay *et al.* (1954). Rats were fasted for 12 hr, and then treated as described above. Two hours later, the rats were anesthetized with ether, and the stomach pylorus was ligated. One hour later, the fluid in the gastric cavity was collected. The volume of fluid was measured, and its acid content was determined by titrating a 2-ml portion with 0.1 N NaOH to pH 7.0, using glass electrodes. Then the acid output was calculated.

To measure serum gastrin levels, rats were fasted for 12 hr and then given the above treatment. One hour later the animals were anesthetized and blood was obtained by cardiac puncture. The serum was separated and stored at −20°C for not more than 1 week. Gastrin levels were assayed with a radioimmunoassay kit from Dainabot (Tokyo, Japan) (Tatsuta *et al.*, 1977*a*). Using this kit, gastrin (1-17) and gastrin (5-17) levels were determined.

Statistical analysis

Results were analyzed by the chi-square test or by 1-way analysis of variance with Dunn's multiple comparison (Miller, 1966; Siegel, 1956; Snedecor and Cochran, 1967). Data are given as means ± SE. "Significant" indicates a calculated p value of less than 0.05.

RESULTS

Incidence, number, histological type, and depth of involvement of gastric cancers

Ten rats in each group were killed at week 30 for determination of tissue catecholamines of the gastric wall and labelling index of the gastric mucosa. Two rats each in Groups 5 and 6 died before week 50. No tumors were found in any of these animals, which were excluded from the effective numbers.

The body weights were significantly decreased in Groups 1 and 3 receiving 25 mg/kg of cysteamine with and without sulpiride, as compared with Group 6 receiving vehicles only. The body weights were slightly, but not significantly, decreased in rats with gastric cancer compared to those without gastric cancer.

The incidence, number, histological type and depth of involvement of gastric cancers are summarized in Tables I and II. In Group 6 (vehicles only), gastric cancers were found in 13 (72%) of the 18 rats examined. The incidence of gastric cancers in Group 1 (cysteamine at 25 mg/kg) was significantly lower than in Group 6. A combination of 25 mg/kg of cysteamine and sulpiride in Group 3 did not cause further reduction in the incidence of gastric cancers. Administration of 5 mg/kg of cysteamine in Group 2 had no influence on the incidence of gastric cancers as compared to Group 6, but concomitant administration of cysteamine at 5 mg/kg and sulpiride significantly reduced the incidence of gastric cancers as compared to

TABLE I – BODY WEIGHT, INCIDENCE AND NUMBER OF GASTRIC CANCERS IN MNNG-TREATED RATS

Group number	Treatment[1]	Body weight (g)			Effective number	Number of rats with gastric cancer (%)	Number of gastric cancers per rat
		26 w	52 w				
			Rats without gastric cancer	Rats with gastric cancer			
1	Cysteamine 25 mg/kg	353 ± 6	300 ± 8[4]	295 ± 8[4]	20	6 (30)[2]	0.4 ± 0.2
2	Cysteamine 5 mg/kg	362 ± 5	377 ± 7	370 ± 10	20	13 (65)	0.9 ± 0.2
3	Cysteamine 25 mg/kg + sulpiride 30 mg/kg	357 ± 7	292 ± 5[4]	280 ± 7[4]	20	6 (30)	0.4 ± 0.2
4	Cysteamine 5 mg/kg + sulpiride 30 mg/kg	355 ± 7	370 ± 6	368 ± 7	20	5 (25)[5]	0.3 ± 0.1[3,5]
5	Sulpiride 30 mg/kg	356 ± 5	388 ± 7	380 ± 11	18	11 (61)	0.7 ± 0.2
6	Vehicles only	348 ± 7	386 ± 10	382 ± 13	18	13 (72)	1.0 ± 0.2

[1]Treatment regimens: Cysteamine 25 or 5 mg/kg: 25 or 5 mg/kg of cysteamine and 1 ml/kg of olive oil were given every other day after 25 weeks of MNNG treatment; Cysteamine 25 or 5 mg/kg + sulpiride 30 mg/kg; 25 or 5 mg/kg of cysteamine and 30 mg/kg of sulpiride were given every other day after 25 weeks of MNNG treatment; Sulpiride 30 mg/kg: 30 mg/kg of sulpiride and 2 ml/kg of 0.9% NaCl solution were given every other day after 25 weeks of MNNG treatment; Vehicles only: 2 ml/kg of 0.9% NaCl solution and 1 ml/kg of olive oil were given every other day after 25 weeks of MNNG treatment.–[2–4]Significantly different from the value for Group 6: [2]$p < 0.05$; [3]$p < 0.01$; [4]$p < 0.001$.–[5]Significantly different from the value for Group 2 at $p < 0.05$.

that in Group 2 (cysteamine at 5 mg/kg). Sulpiride alone had no effect.

In Group 6, the average number of gastric cancers was 1.0 ± 0.2 per animal. It was slightly reduced in Group 2, but the difference was not statistically significant. Concomitant administration of cysteamine at 5 mg/kg and sulpiride at 30 mg/kg in Group 4 significantly decreased the number of gastric cancers as compared with that in Groups 2 and 6.

All tumors induced in the glandular stomach were histologically determined to be adenocarcinomas. In Group 6, all cancers were highly well-differentiated. However, the incidence of highly well-differentiated cancers was significantly lower in Group 1 than in Group 6. No poorly-differentiated cancers were found in this series. Table I also shows that there was no significant difference in the incidence of submucosal cancers among the 6 groups. All cancers were found in the antral mucosa, and no metastases were seen in any rat.

Tissue norepinephrine and labelling index of the gastric mucosa

Tables III and IV summarize data on norepinephrine concentrations in the gastric wall and the labelling index of gastric mucosa in weeks 30 and 52. At both times examined, cysteamine at 25 mg/kg in Group 1 significantly decreased tissue norepinephrine concentration in both the fundic and antral portions of the gastric wall. In contrast, cysteamine at 5 mg/kg in Group 2 caused a slight but non-significant reduction in the tissue norepinephrine concentration. Concomitant use of sulpiride and 25 or 5 mg/kg of cysteamine in Groups 3 and 4 had no further effect as compared with Groups 1 and 2, respectively. Sulpiride alone in Group 5 had little or no effect on tissue norepinephrine concentration. Epinephrine was not detected in any samples obtained from gastric walls of the various groups.

As shown in Table IV, at both times examined, administration of 25 mg/kg of cysteamine in Group 1 significantly decreased the labelling index of both the fundic and antral mucosae as compared to Group 6. Concomitant administration of 25 mg/kg of cysteamine and sulpiride had no further influence on the labelling index. Administration of 5 mg/kg of cysteamine in Group 2 slightly decreased the labelling indices of both the fundic and antral mucosa, but the difference was not statistically significant. However, combined administration of 5 mg/kg of cysteamine and sulpiride significantly reduced the labelling indices of gastric mucosae as compared to that in Group 2. Sulpiride alone in Group 5 decreased the labelling indices of gastric mucosae slightly but not significantly.

Gastric acid secretion and serum gastrin levels

Table V summarizes data on gastric acid secretion and serum gastrin levels in the different groups in week 52. Gastric acid secretion and serum gastrin in Group 1 after administration of 25 mg/kg of cysteamine were significantly higher and increased, respectively, compared to those in Group 6 (vehicles only). Concomitant administration of 25 mg/kg of cysteamine and sulpiride in Group 3 had no influence on the gastric acid secretion and serum gastrin levels induced by 25 mg/kg of cysteamine only. Administration of 5 mg/kg of cysteamine with and without sulpiride in Groups 2 and 4, and sulpiride alone in Group 5, had no influence on gastric acid secretion and serum gastrin level.

DISCUSSION

Our data show that sulpiride enhances the inhibitory effect of cysteamine on MNNG-induced gastric carcinogenesis in Wistar rats. After MNNG treatment, administration of 25 mg/kg of cysteamine significantly reduced the incidence of gastric

TABLE II – HISTOLOGICAL TYPE AND DEPTH OF INVOLVEMENT OF GASTRIC CANCERS IN MNNG-TREATED RATS

Group number	Treatment[1]	Number of gastric cancers	Histology (%)		Depth of involvement (%)	
			Highly well-differentiated	Well-differentiated	Submucosal layer	Muscle layer or deeper
1	Cysteamine 25 mg/kg	8	5 (63)[2]	3 (37)	6 (75)	2 (25)
2	Cysteamine 5 mg/kg	18	14 (78)	4 (22)	17 (94)	1 (6)
3	Cysteamine 25 mg/kg + sulpiride 30 mg/kg	8	8 (100)	0 (0)	7 (88)	1 (12)
4	Cysteamine 5 mg/kg + sulpiride 30 mg/kg	5	5 (100)	0 (0)	4 (80)	1 (20)
5	Sulpiride 30 mg/kg	13	11 (85)	2 (15)	10 (77)	3 (23)
6	Vehicles only	18	18 (100)	0 (0)	16 (89)	2 (11)

[1]For explanation of treatments, see Table I.–[2]Significantly different from the value for Group 6 at $p < 0.05$.

TABLE III – NOREPINEPHRINE CONCENTRATION IN THE STOMACH WALL IN MNNG-TREATED RATS

Experimental week	Group number	Treatment[1]	Norepinephrine concentration (ng/g tissue)	
			Fundic portion	Antral portion
30	1	Cysteamine 25 mg/kg	259.0 ± 17.5[2]	237.4 ± 17.2[4]
	2	Cysteamine 5 mg/kg	329.0 ± 15.9	363.6 ± 20.6
	3	Cysteamine 25 mg/kg + sulpiride 30 mg/kg	246.0 ± 16.9[3]	221.4 ± 11.3[4]
	4	Cysteamine 5 mg/kg + sulpiride 30 mg/kg	304.0 ± 23.4	295.2 ± 33.0[2]
	5	Sulpiride 30 mg/kg	379.4 ± 31.3	420.6 ± 29.0
	6	Vehicles only	366.8 ± 8.3	417.2 ± 21.1
52	1	Cysteamine 25 mg/kg	241.4 ± 16.5[3]	226.0 ± 19.6[4]
	2	Cysteamine 5 mg/kg	339.0 ± 10.0	376.2 ± 24.1
	3	Cysteamine 25 mg/kg + sulpiride 30 mg/kg	224.0 ± 22.4[4]	210.6 ± 10.4[4]
	4	Cysteamine 5 mg/kg + sulpiride 30 mg/kg	298.8 ± 14.3[3]	298.0 ± 34.0[2]
	5	Sulpiride 30 mg/kg	389.0 ± 24.5	425.8 ± 31.4
	6	Vehicles only	371.0 ± 7.8	421.0 ± 20.5

[1]For explanation of treatments, see Table I.–[2–4]Significantly different from the value for Group 6: [2]$p < 0.05$; [3]$p < 0.01$; [4]$p < 0.001$.

cancers at week 52, but treatment of the rats with cysteamine at the concentration of 5 mg/kg had little or no influence on the development of gastric cancers. However, treatment with 5 mg/kg of cysteamine plus sulpiride resulted in significant decreases in the incidence of gastric cancers compared to the group treated with 5 mg/kg of cysteamine alone.

This effect of sulpiride is not yet fully understood, but at least 3 possible mechanisms may be considered. One is an alteration in the secretion of gastric acid and gastrin. Administration of cysteamine is followed by increased acid secretion and increased serum gastrin levels (Gallagher and Szabo, 1984; Groves *et al.*, 1974; Szabo *et al.*, 1977). The trophic effects of gastrin on mucosal cells of the stomach are well established (Johnson, 1977). We previously found that prolonged administration of tetragastrin in depot form after MNNG treatment resulted in a significant increase in gastric acid secretion and a significant decrease in the incidence of gastric cancers at week 52 (Tatsuta *et al.*, 1977*b*). In the present work, we found that administration of 25 mg/kg of cysteamine caused significant increases in gastric acid secretion and serum gastrin level, but that cysteamine at the dosage of 5 mg/kg had little or no influence on these 2 parameters. Sulpiride significantly decreased serum gastrin concentration, but not gastric acidity (Caldara *et al.*, 1978, 1983; McGuigan, 1978). However, concomitant treatment with cysteamine at the concentration of 5 mg/kg plus sulpiride had no effect on the gastric acid secretion and serum gastrin levels. These findings demonstrate that the enhancing effects of sulpiride on cysteamine anticarcinogenesis are probably not mediated via secretory alterations in gastric acid or serum gastrin.

A second possible mechanism involves an effect of tissue norepinephrine on the gastric wall. Szabo *et al.* (1987) found that cysteamine caused complete depletion of norepinephrine in all tissues, including the forestomach, glandular stomach, duodenum and brain. There is evidence of neutral involvement in control of cell proliferation (Kennedy *et al.*, 1983). Recently we found that prolonged administration of nialamide, a monoamine oxidase inhibitor, caused significant increases in the norepinephrine concentration in the gastric wall, in the labelling index of the gastric mucosa, and in the incidence of gastric cancers (Tatsuta *et al.*, 1989*c*). In the present work, we found that injection of cysteamine at the concentration of 25 mg/kg, but not 5 mg/kg, significantly reduced the norepinephrine concentration in the antral portion of the gastric wall, but that its administration in combination with sulpiride had little or no influence on the norepinephrine concentration in the antral por-

TABLE IV – LABELLING INDEX OF GASTRIC MUCOSA IN MNNG-TREATED RATS

Experimental week	Group number	Treatment[1]	Labelling index	
			Fundic mucosa	Antral mucosa
30	1	Cysteamine 25 mg/kg	0.028 ± 0.007[3]	0.034 ± 0.005[3]
	2	Cysteamine 5 mg/kg	0.078 ± 0.010	0.094 ± 0.004
	3	Cysteamine 25 mg/kg + sulpiride 30 mg/kg	0.030 ± 0.007[3]	0.030 ± 0.005[3]
	4	Cysteamine 5 mg/kg + sulpiride 30 mg/kg	0.044 ± 0.004[2,4]	0.044 ± 0.004[3,6]
	5	Sulpiride 30 mg/kg	0.078 ± 0.009	0.102 ± 0.008
	6	Vehicles only	0.100 ± 0.007	0.124 ± 0.010
52	1	Cysteamine 25 mg/kg	0.032 ± 0.005[3]	0.042 ± 0.006[3]
	2	Cysteamine 5 mg/kg	0.078 ± 0.008	0.120 ± 0.013
	3	Cysteamine 25 mg/kg + sulpiride 30 mg/kg	0.026 ± 0.005[4]	0.046 ± 0.004[3]
	4	Cysteamine 5 mg/kg + sulpiride 30 mg/kg	0.040 ± 0.009[2,4]	0.058 ± 0.009[3,5]
	5	Sulpiride 30 mg/kg	0.080 ± 0.007	0.096 ± 0.018
	6	Vehicles only	0.102 ± 0.011	0.136 ± 0.006

[1]For explanation of treatments, see Table I.–[2,3]Significantly different from the value for Group 6: [2]$p < 0.01$; [3]$p < 0.001$. –[4–6]Significantly different from the value for Group 2: [4]$p < 0.05$; [5]$p < 0.01$; [6]$p < 0.001$.

TABLE V – GASTRIC ACID SECRETION AND SERUM GASTRIN LEVEL IN MNNG-TREATED RATS

Group number	Treatment[1]	Gastric acid secretion (mEg/hr)	Serum gastrin level (pg/ml)
1	Cysteamine 25 mg/kg	0.239 ± 0.032[3]	455 ± 27[2]
2	Cysteamine 5 mg/kg	0.039 ± 0.008	406 ± 54
3	Cysteamine 25 mg/kg + sulpirine 30 mg/kg	0.209 ± 0.047[2]	451 ± 42[2]
4	Cysteamine 5 mg/kg + sulpiride 30 mg/kg	0.048 ± 0.001	333 ± 23
5	Sulpiride 30 mg/kg	0.035 ± 0.005	310 ± 40
6	Vehicles only	0.044 ± 0.006	279 ± 28

[1]For explanation of treatments, see Table I.–[2,3]Significantly different from the value for Group 6: $^2p < 0.05$; $^3p < 0.01$.

tion of the gastric wall. However, Kohli and Cripe (1979) reported that sulpiride is a weak antagonist of norepinephrine. They found that sulpiride caused a progressive shift of the dose-response curve of norepinephrine to the right without inhibiting the response to KCl. Similarly, Barrett *et al.* (1982) found that sulpiride facilitates sympathetic nerve function via a preferential antagonism of alpha 2-adrenoceptors.

A third possible mechanism involves alterations in nucleic acid and protein syntheses and cell proliferation of gastric mucosal cells. Sewerynek *et al.* (1988) found that a single *in vivo* injection of cysteamine increased ^{3}H-thymidine incorporation into DNA of the organ-cultured adrenals. However, Mitznegg (1973) suggested that formation of cyclic AMP after cysteamine treatment might induce transient functional suppression of DNA synthesis, and concluded that cyclic AMP might be a second messenger of cysteamine. Takagi and Shikita (1983) reported that mitotic cells disappeared almost completely within 60 min after the addition of cysteamine to HeLa cell culture. Kalbermann *et al.* (1979) and Burdman *et al.* (1979) found that sulpiride administration stimulates DNA synthesis in the rat anterior pituitary gland. Moreover, Anton and Rozados (1976) found that daily s.c. injections of sulpiride produced increased tumor growth; they proposed that the sulpiride effect may be mediated by augmented prolactin levels in serum. In the present work, however, we found that administration of 25 mg/kg of cysteamine significantly decreased the labelling indices of the antral and fundic mucosal cells. Cysteamine at the concentration of 5 mg/kg had little or no influence on the labelling index of gastric mucosae, but concomitant treatment with cysteamine at the concentration of 5 mg/kg plus sulpiride significantly decreased the labelling indices of the antral and fundic mucosa as compared with those observed when cysteamine at the concentration of 5 mg/kg alone was administered.

Our results indicate that combined administration of cysteamine and sulpiride enhances the inhibitory effect of cysteamine on gastric carcinogenesis, and suggest that dopamine may be related to the inhibition of gastric carcinogenesis by cysteamine.

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