EFFECT OF CIMETIDINE ON DEOXYRIBONUCLEIC ACID BIOSYNTHESIS IN GASTROINTESTINAL MUCOSA OF RATS

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Abstract—Using oxyntic and duodenal rat tissues, we investigated the effects of cimetidine, an antisecretagogue and a H2-receptor antagonist, on deoxyribonucleic acid (DNA) biosynthesis, determined according to the 3H-thymidine incorporation into DNA fraction. Serum gastrin concentration and DNA biosynthesis in the tissues increased significantly in the fed rats, and administration of cimetidine significantly increased both serum gastrin concentration and the DNA biosynthesis in the oxyntic tissues. There was a significant correlation between the oxyntic DNA biosynthesis and the serum gastrin concentration. Cimetidine in concentrations of 0.01 to 1.0 mM, added to the incubation mixture, had no stimulatory effect on the DNA biosynthesis in the oxyntic tissues. These results support the hypothesis that DNA biosynthesis in oxyntic mucosa of rats may be regulated by the circulating gastrin.

It has been hypothesized that gastrin is a trophic hormone for certain tissues of the gastrointestinal tract (1). Endogenous gastrin which is released by ingestion of food, alcohol etc., is necessary for normal growth of the oxyntic glands and for the mucosa of the duodenum (2). Accordingly, ingestion of food seems to be essential to maintain a physiological function of the gastrointestinal mucosa (3). The injection of pentagastrin or gastrin in doses comparable to those which stimulate maximal acid secretion stimulate DNA biosynthesis in rats and dogs. Recent evidence indicates that big gastrin and little gastrin have 24 to 36 times the potency of pentagastrin, on an equimolar exogenous dose basis, in the stimulation of DNA biosynthesis (4).

Cimetidine, an histamine H2-receptor antagonist is a potent inhibitor of gastric acid secretion in humans and experimental animals (5, 6), and after ingestion of food by humans, serum gastrin concentration is higher after a cimetidine injection than after placebo (7).

We investigated the effects of cimetidine on DNA biosynthesis in gastrointestinal mucosa and serum gastrin concentration in the fed and food deprived rats.

MATERIALS AND METHODS

Male Wistar rats, weighing between 150 and 200 g were randomly divided into two groups. The animals in the first group were deprived of food for 40 hr, but had free access to water. Animals in the other group were provided an ad libitum diet of rat chew (Oriental Co., Ltd.) and water.
Half the number of animals in each group were given i.p. either 60, 120, 180 or 240 mg per kg of cimetidine, prepared according to Blimblecombe et al. (8), 16 and 2 hr (at 6:00 p.m. and 8:00 a.m.) before the animals were sacrificed. The animals were anesthetized with ethyl ether, the heart was exposed by ventral incision and blood was withdrawn from the ventricle. Blood specimens were left at 0°C for 30 min and then centrifuged at 2,500 rpm for 5 min. The supernatant serum was stored at -20°C until use. Johnson’s method (9) with some modifications was used for the measurement of DNA biosynthesis in vitro. Small pieces of tissue from the oxyntic gland area of the stomach and from the duodenum were quickly removed, washed with ice cold phosphate buffer saline, then minced to about 1 mm thickness and weighed. Approximately 200 mg of the tissues per each vessel were preincubated for 3 min at 37°C in 1.0 ml of Eagle’s minimal essential culture medium (MEM), pH 7.4, and then 1 μCi ³H-thymidine (³H-Methyl, 52 Ci/mmole, Radiochemical Center, Amersham) was added and incubated for 30 min at 37°C. The reaction was halted with an equal volume of 0.4 N perchloric acid containing carrier thymidine at 5 mM concentration and centrifuged. The supernatant was decanted and the precipitate was washed with 5 ml of 0.2 N perchloric acid containing 2.5 mM thymidine. The washed precipitate was homogenized in 5 ml of 0.2 N perchloric acid and allowed to stand for 30 min in an ice bath, and then centrifuged at a rate of 2,500 rpm for 5 min. The precipitate was washed three times with 5 ml of ice chilled 0.2 N perchloric acid and then washed with 5 ml of ethyl ether : ethanol=1 : 3 mixture, and finally washed with 5 ml of ethyl ether. The resulting DNA containing pellet was suspended in 6 ml of the scintillation medium (equal volumes of toluene, dioxane, ethanol containing 0.5% of 2,5-diphenyloxazole, 0.005% of 1,4-bis-(4-methyl-5-phenoxyzole 2-yl)benzene and 8% of naphtalene. The radioactivity was estimated by the Packard scintillation counting system.

In some experiments, the mucosal layer of the oxyntic gland area and the proximal portion of duodenum was separated from other layers by a slide glass, weighed and incubated in Eagle’s MEM with ³H-thymidine for 30 min at 37°C.

Serum gastrin concentration was estimated by radioimmunoassay (Dainabot Radioisotope Lab. Ltd, Tokyo, Japan) (10).

Statistical analysis Student’s t-test was used for unpaired data. The data represent the mean±SEM and use of the word significant indicates a P value of less than 0.05.

RESULTS

When the activity of DNA biosynthesis was expressed per gram tissue weight, the activity in the separated oxyntic mucosa was 15–19 times higher than that in the tissue with all layers of the oxyntic wall. This indicates that most of the DNA biosynthetic activity appears in the mucosal layer of the oxyntic wall. As the mucosal layer on the gastric wall showed a significantly large variation in the biosynthetic activity in the mucosal specimens, tissues with all layers of the oxyntic and proximal duodenal walls were used for all further experiments. In the fed rats, the DNA biosynthesis in the oxyntic gland area was about 3.8 times higher and about 5.7 times higher in duodenal tissues (Fig. 1A). Serum gastrin concentration in the fed rats was about 4.7 times higher than that in the rats deprived of food (Fig. 1B). In the fed rats, ³H-thymidine incorporation into DNA fraction in oxyntic gland area was stimulated by the administration of cimetidine, as shown in Fig. 2A. Serum gastrin concentration also increased significantly with cimetidine administration (Fig. 2B). Maximal
Fig. 1. DNA biosynthesis in oxyntic and duodenal tissues (A) and serum gastrin concentration (B) of the 40 hours non. fed (n=10) and fed (n=19) rats. Results are mean±SEM of the number of determinations in parentheses. *P<0.001 fed vs. non. fed rats.

Fig. 2. Effect of cimetidine on the DNA biosynthesis in the oxyntic and duodenal tissues (A) and serum gastrin concentration (B). Cimetidine: 60 (n=10), 120 (n=10), 180 (n=3), 240 (n=9) mg/kg administered twice i.p., 16 and 2 hr before the rats were killed. Results are mean±SEM of the number of determinations in parentheses. +P<0.02 cimetidine vs. control, *P<0.001 cimetidine vs. control.
stimulation of the DNA biosynthesis by cimetidine was seen with a dose of 180 mg/kg. In duodenal tissues, however, cimetidine did not significantly stimulate the DNA biosynthesis. The effect of cimetidine on the time course of the DNA biosynthesis and serum gastrin concentration were also investigated. A single dose of 180 mg/kg cimetidine was given i.p. and the animals sacrificed one, two, four, eight and sixteen hr after this administration. The maximal activity of the DNA biosynthesis seen at 2–4 hr after cimetidine administration was associated with an increase in serum gastrin concentration and enhancement of the DNA biosynthesis by cimetidine gradually decreased. At 16 hr after cimetidine administration, the DNA biosynthesis activity returned to the original level. The time course of serum gastrin concentration was much the same as that of the DNA biosynthesis in oxyntic tissues, as shown in Fig. 3.

There was a significant correlation in all cases between serum gastrin concentration and $^3$H-thymidine incorporation into DNA in the oxyntic tissue in all the rats, and in all the experiments (Fig. 4).

To investigate the direct effect of cimetidine on the DNA biosynthesis, 0.01 to 1.0 mM of cimetidine was added to the media and the preparation was preincubated with the minced tissues for 3 min. Incubation in the

![Graph](image)

Fig. 3. Time course of the stimulatory effect of cimetidine (180 mg/kg) administered i.p., on the DNA biosynthesis and serum gastrin concentration. Results are mean±SEM of 4 dependent experiments. +P<0.02 cimetidine vs. control, *P<0.001 cimetidine vs. control

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Fig. 4. Correlation between serum gastrin concentration and DNA biosynthesis in oxyntic tissues. Cimetidine 60 mg/kg (△), 120 mg/kg (▲), 180 mg/kg (□) and 240 mg/kg (■) administered i.p. 16 and 2 hr before the fed rats were sacrificed. Cimetidine 60 mg/kg (●) was administered i.p. 16 and 2 hr before the non-fed rats were killed. Control; non-fed (●) and fed (○) rats were given i.p. saline solution. *r=0.65, P<0.01.
Fig. 5. Effects of various concentrations of cimetidine on the DNA biosynthesis. Cimetidine; 0.01, 0.1 and 1.0 mM were added to the incubation media. Each value expressed is mean±SEM of four dependent experiments. *P<0.02 cimetidine vs. control.

presence of $^3$H-thymidine was carried out for 30 min at 37°C, and $^3$H-thymidine incorporation into DNA was estimated. As shown in Fig. 5, no significant effect of cimetidine was seen at a concentration up to 0.1 mM. In the presence of 1 mM cimetidine, the DNA biosynthesis was inhibited significantly in oxyntic tissues of the fed rats.

DISCUSSION

Richardson et al. reported that serum gastrin concentration increased when cimetidine was administered to man (11) and we now provide evidence that gastrin has trophic effects in mucosae of the gastrointestinal tract. The dose of cimetidine which inhibits the basal acid secretion (12), increased the serum gastrin concentration to almost maximal levels. On the other hand, the DNA biosynthesis in oxyntic tissues increased dose dependently with cimetidine administration.

In both fed and food deprived rats, the DNA biosynthesis in oxyntic gland tissues correlated significantly with serum gastrin concentration, under conditions of cimetidine administration. These results confirm that the increase in endogenous gastrin by food ingestion or cimetidine administration may be an important stimulus for DNA biosynthesis in oxyntic gland tissues. In case of food deprivation, serum gastrin concentration correlated well with the DNA biosynthesis both in oxyntic and in duodenal tissues. However, in the case of increase in endogenous gastrin following the administration of cimetidine, a significant correlation between serum gastrin concentration and the DNA biosynthesis was observed in the oxyntic gland tissues, but not in the duodenal tissues. It has been reported by others that exogenous gastrin and pentagastrin injection, had no trophic effect on mucosa with significant amounts of gastrin or G-cells (13).

We examined the direct effect of cimetidine on the DNA biosynthesis in oxyntic gland tissues in vitro. As shown in Fig. 5, doses up to 0.1 mM did not influence the DNA biosynthesis. These results indicate that the stimulatory effect of cimetidine on the DNA biosynthesis might be due to increase in serum gastrin and that cimetidine probably has no direct action on the oxyntic tissues.

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