Experimental Studies on Developmental Mechanism of Histamine Induced Ulcers and Ulcer Inhibitory Effects of Histamine Receptor Antagonists[*]

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ABSTRACT

Study was made of the developmental mechanism of histamine induced ulcers and inhibitory effects of receptor antagonists from the view point of gastric acidity and microvasculature of the gastric mucosa, and the following results were obtained.

1. In histamine induced ulcers, hypersecretion of gastric acid caused by histamine is not the primary developmental mechanism of the lesion. The main cause is considered to be disturbance of microcirculation in the gastric mucosa attributable to the effects of histamine on the microvasculature of the mucosa.

2. Of the histamine receptor antagonists, diphenhydramine, an H_1-antagonist, was unable to inhibit hypersecretion of gastric acid and disturbance of microcirculation of the gastric mucosa caused by histamine, and thus could not suppress the development of histamine induced ulcers, but cimetidine, an H_2-antagonist, inhibited not only hypersecretion of gastric acid, but also prevent disturbance of microcirculation in the mucosa, and consequently suppressed histamine induced ulcers.

INTRODUCTION

Since Hay et al.[7] induced histamine ulcers in various experimental animals in 1942, it has been used as a model for acute ulcers. Although it is considerd that hypersecretion of gastric acid and vascular disturbance caused by histamine effects are involved in the developmental mechanism, it has not been verified as yet which of the two plays the major role in ulcerogenesis. Recently, a new antihistamine, H_2-receptor antagonist, which is different from the hitherto H_1-type has been developed, and reports have been published on its inhibitory effects on gastric acid secretion[1,3]. Thus, this study was undertaken to review the develop-mental mechanism of histamine induced ulcers and the ulcer inhibitory effects of H_1- and H_2-receptor antagonists from the view point of gastric acidity and microvasculature of the gastric mucosa.

METHOD OF EXPERIMENT

1. Experimental animals and methods of drug administration

The experimental animals used were Wistar strain male rats weighing 200–250 g. Metal cannulas (outer diameter 0. 4 cm, inner diameter 0. 3 cm, length 2. 0 cm) were inserted into the fore stomach and antrum in accordance with the Borella method[2]. These rats with gastric fistula were bred for about 2 months. On the

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[*] 児玉　求，小川喜輝，伊藤信昭，竹内仁志，清光六郎，田中恒夫，原田光雄，児玉　治，江崎治夫：ヒスタミン潰瘍発生機序ならびにヒスタミン受容体拮抗剤の潰瘍抑制効果

day prior to the experiment, the stomach of the rats was flushed with saline to eliminate food residue, and after 24hr fasting, the experiment was carried out under nembutal anesthesia (40 mg/kg administered intraperitoneally).

The rats were classified into the following 5 groups depending on the histamine dose and type of receptor antagonist.

Control group: Saline was injected into the femoral vein.

Histamine 0.5 mg/kg injected group: As intravenous injection of 0.5 mg/kg produced maximum acid output in a preliminary experiment, this dose was injected into the femoral vein.

Histamine 5.0 mg/kg injected group: As intravenous injection of 5.0 mg/kg produced histamine ulcers at a high frequency in a preliminary experiment, this dose was injected into the femoral vein.

Diphenhydramine injected group: Diphenhydramine, an H_1-receptor antagonist, was administered intramuscularly in a dose of 10 mg/kg, 30 minutes prior to intravenous injection of 5.0 mg/kg of histamine into the femoral vein.

Cimetidine injected group: Cimetidine, an H_2-receptor antagonist, was administered intravenously in a dose of 48 mg/kg, 10 minutes prior to intravenous injection of 5.0 mg/kg of histamine into the femoral vein.

2. Observation of ulcers

Gastric fluid was collected to determine gastric acidity, 15 minutes after it reached its peak value following intravenous injection of histamine. Then, the rats were decapitated and the stomach extirpated. An incision was made along the greater curvature, and check was made for hemorrhage and erosion using a stereomicroscope.

3. Method for determination of gastric acidity

The stomach was flushed with NaOH solution (10 ml, pH 8.5) using the fore stomach cannula and gastric fluid was collected from the antrum cannula 15 minutes after intravenous injestion of histamine. The total acid volume was determined with an automatic titrator using 0.005 N NaOH solution with the final pH set at 7.0.

4. Method for observation of gastric mucosal microvasculature and red blood cell distribution within gastric mucosal capillaries

The microvasculature was observed by slowly injecting 1.0 ml of 10% FITC-dextran (mean

molecular weight 40,000) into the femoral vein 15 minutes after intravenous injection of histamine. The generalized circulation of FITC-dextran was confirmed by yellow staining of the conjunctiva, after which the stomach was extirpated. An incision was made along the greater curvature and immersed in 20% formalin for 5-6 hours for fixation. Gastric fundus sections of 2-3 mm thickness were cut and paraffin block were prepared. These were sliced into 30 μ thickness and observed under a fluorescent microscope. The distribution of RBC was observed by H-E staining of 3 μ slices taken from areas adjacent to the site from where sections had been removed for observation of the microvasculature.

RESULTS OF EXPERIMENT

1. Incidence of ulcers

There was no development of ulcers in any of the 9 rats administered 0.5 mg/kg of histamine, while the incidence was 77% or 7 out of 9 rats in those administered 5.0 mg/kg (Table 1). The ulcers were multiple, with hemorrage and erosion restricted to the fundus area, and did not develop in the antrum nor duodenum (Fig. 1).

The incidence of ulcers in the histamine receptor antagonist groups was 83% or 7 out of 8 rats in the diphenhydramine administered

Table 1. Incidence of gastric hemorrhage and erosion after intravenous injection of histamine

Group	n	hemorrhage and erosion	%
Histamine 0.5 mg/kg	9	0	0
Histamine 5.0 mg/kg	9	7	77
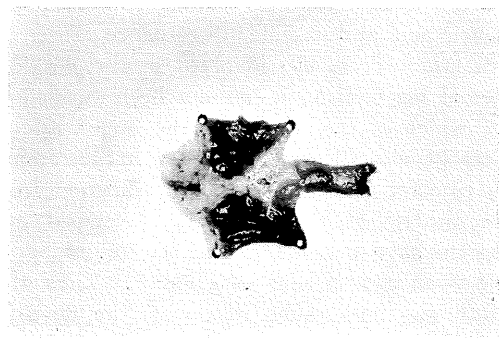
Fig. 1. Macroscopic finding of histamine induced ulcers

rats. The histamine ulcers could not be inhibited and ulcers developed in the gastric fundus as in the case of the 5.0 mg/kg dose group. The incidence in the cimrtidine administered group was 13% or 1 in 8 rats, showing that histamine induced ulcers were markedly inhibited, and the site of the one rat in which ulcers developed was the gastric fundus (Table 2).

Table 2. Effect of H_1 and H_2-receptor antagonist on histamine induced gastric hemorrhage and erosion

Group	n	hemorrhage and erosion	%
Histamine 5.0 mg/kg	9	7	77
Diphenhydramine (10mg/kg i. m.)	8	7	83
Cimetidine (48 mg/kg i. v.)	8	1	13

2. Change in gastric acidity

The gastric acid of the histamine administered group was 5.8±1.1 μEq/ml (M±SD, n=9) in the 0.5 mg/kg administered group and 5.7± 1.1 μEq/ml (M±SD, n=9) in the 5.0 mg/kg administered group. These values were significantly higher than the value of 3.2±0.5 μEq/ ml (M±SD, n=18) for the control group (p< 0.01), but there was no significant difference (N. S) between the two histamine administered groups (Table 3).

Table 3. Gastric acid concentration after intravenous injection of histamine 0.5 mg/kg and 5.0 mg/kg

Group	acid concentration (μEq/ml)
Control	3.2±0.5 (n =18)
Histamine 0.5 mg/kg	5.8±1.1* (n = 9)
Histamine 5.0 mg/kg	5.7±1.1* (n = 9)

* p<0.01 (M±SD)
* Significance of the difference between means of the control value and histamine induced value.

The gastric acidity of the histamine receptor antagonist administered groups was 5.3±1.5 μEq/ml (M±SD, n=8) for the diphenhydramine group and 1.2±0.8 μEq/ml (M±SD, n=8) for the cimetidine group. The diphenhydramine group showed a significant increase over the controls (p<0.01) presenting a state of hypersecretion of gastric acid, while the value

of the cimetidine group was significantly lower than that of the controls (p<0.01), indicating that the hypersecretive action of histamine was inhibited (Table 4).

Table 4. Effect of H_1 and H_2-receptor antagonist on gastric acid after intravenous injeston of histamine 5.0 mg/kg

| Group	acid concentration (μEq/ml)	n
Histamine 5.0 mg/kg	5.7±1.1	9
Diphenhydramine (10 mg/kg)	5.3±1.5	8
Cimetidine (48 mg/kg)	1.2±0.8*	8

* p<0.01 (M±SD)
* Significance of the difference between means of the diphenhydramine pretreated group and cimetidine pretreated group.

3. Findings of the microvasculature and distribution of the red blood cells within the capillaries of the gastric mucosa

Findings of the microvasculature of the control group showed capillaries which had branched from the arterioles of the submucosa and run tortuosly upward within the mucosa, venous capillaries which were connected horizontally like braids on the mucosal surface and collecting venules which flowed vertically into the venules of the submucosa. These were all clearly visualized by the yellow fluorescence of FITC–dextran, while study of the red blood cell distribution within the capillaries showed only a small number of red blood cells within the venous capillaries (Fig. 2a, b).

The microvasculature of the histamine 0.5 mg/kg administered group was clearly visualized with FITC–dextran and only a small number of red blood cells were present as in the control group, while in the histamine 5.0 mg/ kg administered group, the vasculature was poorly visualized and the venous capillaries and collecting venules were filled with red blood cells, also in some areas the rouleau formation of red blood cells were observed indicating disturbance of microcirculation due to stasis of blood flow (Fig. 3a, b and 4a, b, c).

Finding sof the microvasculature and distribution of the red blood cells of the histamine receptor antagonist administered groups showed that in the diphenhydramine group there was disturbance of microcirculation due to stasis of
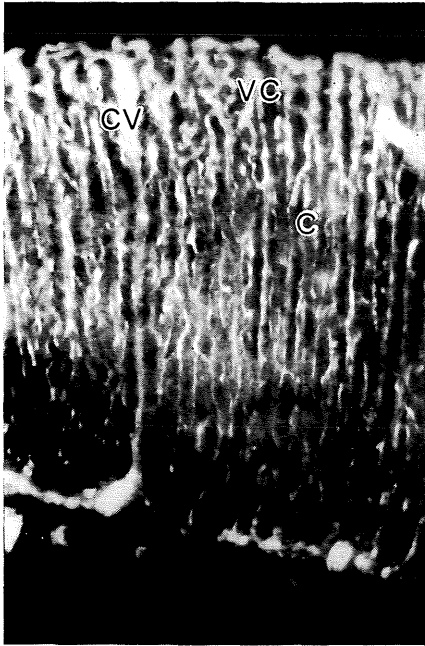
Fig. 2a. Microangiograph in the gastric mucosa of the control group. The capillary system are clearly visualized. Capillaries (C), Venous sapillaries (VC), Collecting venules (CV) ×100
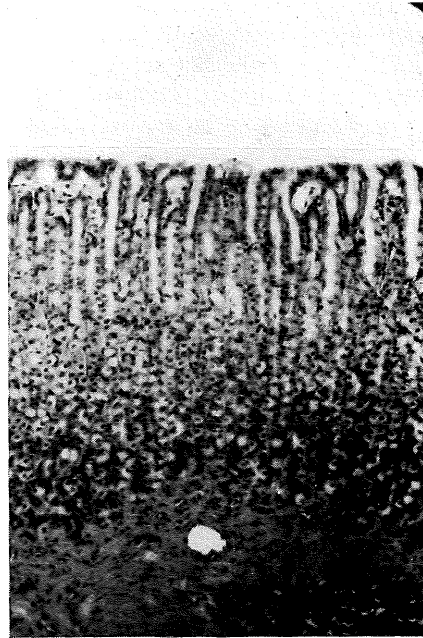
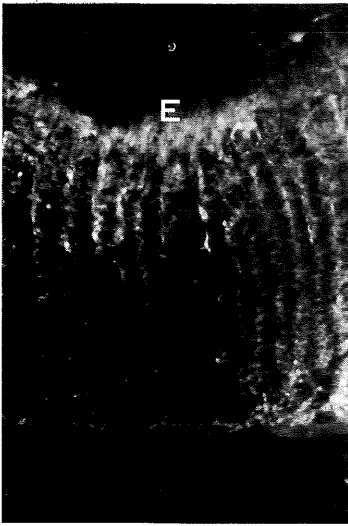**Fig. 2b.** Section of the control group. A few RBC can be seen in the venous capillaries. H–E ×100
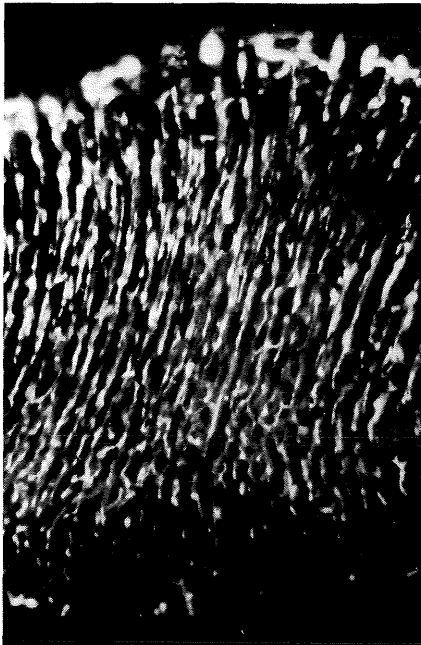
Fig. 3a. Microangiograph of the histamine 0.5 mg/kg administered rats. The capillary system are clearly visualized. ×100
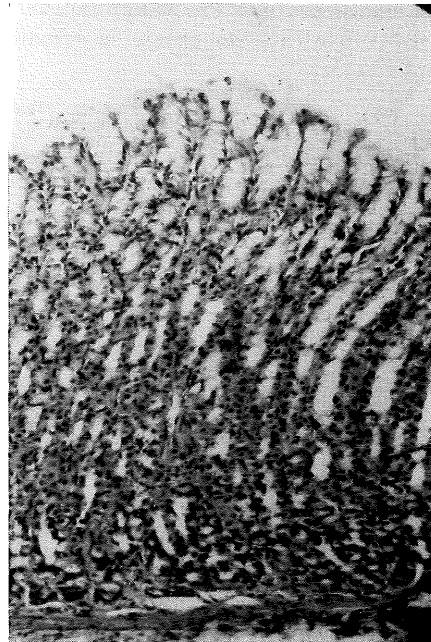
Fig. 3b. Section of the histamine 0.5 mg/kg administered rats. A few RBC can be seen in the venous capillaries. H–E ×100

Fig. 4a. Microangiograph of the histamine 5.0 mg/kg administered rats. The capillaries can not be visualized. Only erosion can be seen. Erosion (E) ×100
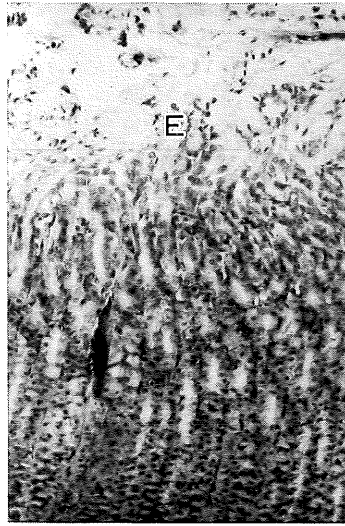
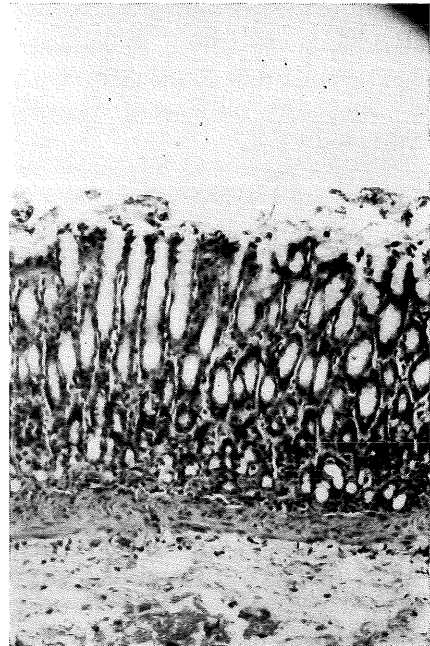**Fig. 4b.** Section of the histamine 5.0 mg/kg administered rats. Dilatation of the capillaries and RBC stasis can be seen. H–E ×100
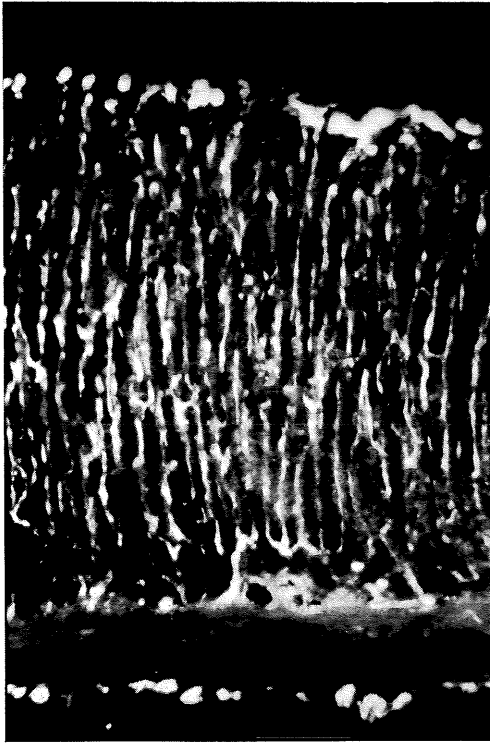
Fig. 4c. Higher magnification of an area of Fig. 4b. The capillaries with rouleau formation can be seen. ×400
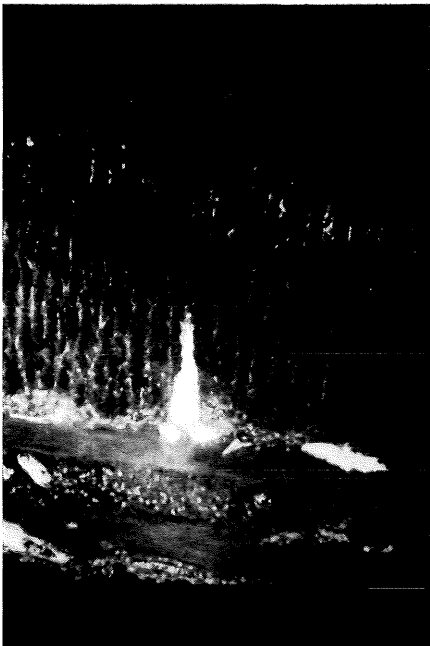
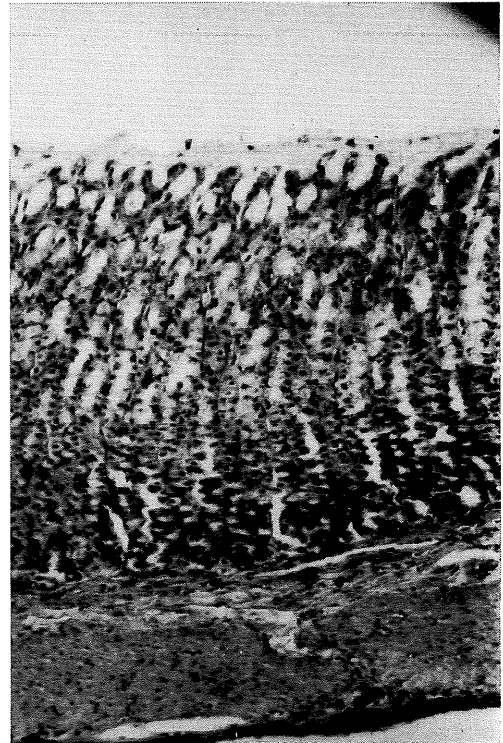

Fig. 5a. Microankiograph of the diphenhydramine administered rats. The capillaries can not be visualized. ×100

Fig. 5b. Section of the diphennydramine administered rats. The RBC stasis can be seen. H–E ×100

Fig. 6a. Microangiograph of the cimetidine administered rats. The capillary system can be clearly visualized. ×100

Fig. 6b. Section of the cimetidine administered rats. The RBC stasis can not be seen. H–E ×100

blood flow as in the 5.0 mg/kg histamine administered group, while in the cimetidine group no such circulatory disturbance was observed (Fig. 5a, b and 6a, b).

DISCUSSION

Histamine induced ulcers can be produced by a variety of method such as intramuscular injestions of histamine mixed with bees wax[7], intraperitoneal injection and intramuscular injections of histamine solution[4]. Histamine has such pharmacological effects as 1) Stimulation of gastric acid secretion, 2) Enhancement of capillary permeability and dilatation of arterioles, 3) Contraction of smooth muscles. Opinions are divided as to the mechanism responsible for the development of histamine induced ulcers, that is, some claim that the main cause in the invasion of the gastric mucosa by a large volume of H^+ created by the histamine stimulation of gastric acid secretion, while others feel it is devitalization of the gastric mucosa due to disturbance of microcirculation caused by vascular action. The experimental results of Eagleton

et al.[4] are noteworthy from this point of view. In other words, histamine phosphate solution was used in the experimental induction of histamine ulcers in guinea pigs, and although hypersecretion of gastric acid was not induced with one intraperitoneal injection of 5.0 mg/kg of histamine, gastric ulcers did develop, whereas hypersecretion of gastric acid occurred with 0.25 mg/kg doses of histamine injected intramuscularly 8 times at 30 minute intervals, inducing primarily duodenal ulcers. Thus the development of gastric ulcers was due to the effects of histamine on the blood vessels, while duodenal ulcers were caused by hypersecretion of gastric acid.

The results of the authors intravenous injection of histamine in relation to gastric acidity and hemorrhage and erosion, indicated that there was a significant increase in gastric acidity in the 0.5 mg/kg and 5.0 mg/kg histamine injected groups as compared with the controls ($p < 0.01$), but there was no significant difference between the two histamine administered groups (N. S). Hemorrhage and erosion are not in-

duced in any of the 9 animals given a dose of 0.5 mg/kg, but are induced in 7 out of 9 (77%) of the 5.0 mg/kg group, and all hemorrhage and erosions occurred in the gastric fundus with none occurring in the duodenum. This suggests that the induction of hypersecretion of gastric acid by histamine is not the primary mechanism for causing gastric lesions in histamine ulcers. The fact that no lesions occurred in the duodenum in our study is presumed to be because the state of hypersecretion of gastric acid resulting from a single intravenous injection was transient as compared to Eagleton's objective which was to maintain long period gastric acid secretion by means of frequent intramuscular injestions.

Another important factor in the histamine ulcer induction mechanism is the effect of histamine on blood vessels. The microvasculature and red blood cell distribution in capillaries of the gastric mucosa showed that in the 0.5 mg/kg histamine administered group, no disturbance of the microcirculation could be observed, whereas in the 5.0 mg/kg group, stasis of blood was noted, which caused disturbance of microcirculation, and a correlation between ulcer development could be observed. It has been reported that the effects on microcirculation of substances which stimulate secretion of gastric acid including histamine cause increase in gastric mucosal bood flow, and histamine also causes contraction of the portal vein, hepatic vein and venules of the extirpated intestinal tract. Further, Kowalewski[6] who reviewed histamine induced ulcers based on the vascular effects of histamine, reported that the histamine ulcer inhibiting effects of vasopressin which reduces blood flow in the digestive tract, did not affect histamine induced gastric acid secretion, but inhibited ulceration. Thus, he consideres that vasopressin inhibits ulcers by suppression of histamine induced blood flow increase in the mucosa. From the avove, it is considered that when a dosage of histamine large enough to induce ulcers is administered, it causes dilatation of the arterioles which permits an excessive flow of the blood into the gastric mucosa and also contracts the venules. These together with hyperpermeability of the capillaries causes stasis of blood in the gastric mucosa. Thus, it is felt the gastric mucosa lapses into a state of hypoxia causing devitaliza-

tion of the mucosa and disruption of the superficial mucosal capillaries.

Next, study of the histamine ulcer inhibition effects of histamine receptor antagonists was made, and it was found that diphenhydramine had no inhibitory effects, but cimetidine demonstrated significant effects. Study was made of the mechanism involved in the inhibition of histamine ulcers. As is well known, H_1-receptor antagonist had no inhibitory effects on histamine induced hypersecretain of gastric acid, and in our study diphenhydramine failed to demonstrate any such effects, while for H_2-receptor antagonist there are many reports including those of Black et al.[1] and our findings which have demonstrated an inhibitory effect on secretion. Diphenhydramine failed to demonstrate any preventive effects towards histamine induced disturbance of microcirculation in the gastric mucosa, while cimetidine did show such an effect. Owen et al.[7] and Pawlik et al.[8] studied the effects of histamine on the basis of the mesenteric arteriole of the histamine receptor, and reported that of the dilatory effects of histamine, those during the early stage take place through the H_1-receptor, while continuous dilatation is carried out through the H_2-receptor. Although diphenhydramine can inhibit the early dilation of vessels with an ulcer producing dose of histamine, it cannot inhibit the subsequent continuous dilation, and thus it cannot inhibit the excessive flow of blood into the gastric mucosa nor prevent disturbance of microcirculation caused by stasis of blood. On the other hand, cimetidine inhibits continuous dilation of the arterioles and suppress excessive blood flow into the gastric mucosa, and thus is considered to maintain microcirculation in the gastric mucosa and prevent the development of ulcers.

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