Effect of Serotonin on Experimentally Induced Gastric Ulcer in Rats

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ABSTRACT

Ulcerative gastric lesions in rats were produced by the procedure of Shay et al. The lesion develops uniformly in the remen, less often in the antrum, and least frequently in the body of the stomach. Administration of serotonin (8 mg/kg) was effective in preventing the occurrence of gastric lesions and the effect is distinct particularly in the group which had 48 hrs of starvation and 10 hrs of pyloric ligation. Bilateral vagotomy was completely effective and pretreatment of atropine or morphine was moderately effective in preventing the gastric lesions. The acidity of gastric juice was considerably lower, however, the mucin content was higher in the animals treated with serotonin than nontreated control animals. Histologically, mucus secretion was greater in the animals that were given serotonin. In summary, it is concluded that serotonin is effective in preventing ulceration in the stomach by its action of increasing mucin secretion and inhibitory gastric acid secretion.

INTRODUCTION

The recent literature contains reports of the action in dogs of serotonin which stimulates the mucin secretion of stomach and inhibits the acid secretion (White and Magee, 1958; White et al., 1959; Black et al., 1958; Whang et al., 1963).

In 1958 White and Magee stated that the secretion of mucin was directly due to the action of serotonin and was not due to mechanical stimulation from peristalsis or the rubbing together of mucosal surfaces. The mucin has a protective effect on the gastric mucosa, and also reduces acid secretion.

Paradoxically, however, others have reported the use of serotonin as an ulcerogenic agent (Nikodijevic and Vanov, 1960; Nikodijevic and Trakrov, 1963). Brodie et al. in 1962 found a 90% incidence of gastric ulcers in rats which had been given 8 mg of serotonin per kg. Gastric acidity was not significantly changed. Using 10 rats which had been fasted for 24 hours before the administration of serotonin we repeated Brodie's experiment, but were unable to duplicate his findings. The only change was a slight increase in the mucin content of the gastric juice.

The present experiment, using rats in which the pylorus had been closed by ligature, investigated the effect of serotonin in preventing, or protecting against, gastric ulceration.

METHODS

A hundred and twenty albino rats, weighing 200 to 300g, of both sexes were used. The animals were starved for forty-eight to ninety-six
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hours depending upon the experimental schedule. During the starvation period, water was permitted ad libitum. Throughout the experimental period the animals were housed singly in cages with raised bottoms of wide wire mesh to avoid coprophagy. The pyloric ligature was placed by the procedure described by Shay et al. (1945).

Ninty-six rats were used in the first series of experiments. The first group of 16 rats was starved for 96 hours and sacrificed. This group did not have pyloric ligation. The second group of 14 rats after starving for 48 hours had ligation of the pylorus. The animals were sacrificed 5 hours after pyloric ligation. The third group of 20 rats was starved for 48 hours and then the pyloric ligature was placed. The animals were sacrificed 10 hours after pyloric ligation. The fourth group of 30 rats was starved and the pyloric ligature placed as in the second group, but the animals were sacrificed 20 hours after pyloric ligation. The fifth group of 16 rats were starved for 72 hours and the pyloric ligature placed as with the second group, and were sacrificed 20 hours after pyloric ligation.

Daily serotonin (8 mg/kg) was given subcutaneously to one-half of the animals in each group during starvation and after pyloric ligation, while the remaining one-half of the animals in each group served as the serotonin untreated control.

Twenty-four rats were used in the second series of experiments. The first group of six rats was the control, e.g., the rats were starved for 48 hours and then the pyloric ligation was performed. Twenty hours after the pyloric ligation the rats were sacrificed. The second, third or fourth group of six rats each were starved and received the pyloric ligation as did the control group in this second experiment.

Following starvation and pyloric ligation the rats were given daily doses of atropine to the second group, morphine to the third group, and a bilateral vagotomy was done in the fourth group.

Under ether anesthesia, the rats were killed by exsanguination from the femoral artery. The stomach was removed, trimmed free of adipose tissue, and then opened by cutting along the greater curvature. The gastric juice was collected. The specimens were then washed under tap water. The volumes of solid matter and juice were noted. Free and total acid of the juice were titrated in the usual manner using Topfer's and phenolphthalein as the indicators and N/20 NaOH for titration. In some animals the gastric mucin was determined by the method of micro-Kjeldahl and pepsin activity by the method of Riggs and Stadie (1943).

RESULTS

Ulcerative gastric lesions in rats were produced by the procedure of Shay et al. (1945), which was a simple technique, namely pyloric ligation after starvation (Fig. 1). The ulceration develops uniformly in the rumen, less often in the antrum, and least frequently in the body of the stomach. The lesions are most marked in the rumen. The administration of serotonin was effective in preventing the occurrence of gastric lesions produced by pyloric ligation after starvation (Table 1). When the rats were starved for 48 hours and then the pyloric ligation left for 20 hours, the incidence of gastric ulceration was 92.9%.

Fig. 1. Typical ulcerative change in gastric mucosa of rat. The rat was starved for 48 hrs and then the pyloric ligation (left) for 20 hrs. Right shows normal gastric mucosa.
Fig. 2. Effect of serotonin on acidity of gastric contents. Note lower incidence of occurrence of gastric ulcer (b) and of free acidity (a) in the group treated with serotonin.

Fig. 3. Effect of serotonin on mucin production. The protein content is higher in serotonin group than control.
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### Table 1. Influence of serotonin on experimentally-induced gastric ulcer

<table>
<thead>
<tr>
<th>Group</th>
<th>Starved (hr.)</th>
<th>Ligated (hr.)</th>
<th>Treated</th>
<th>No. of rats used</th>
<th>No. of rats died</th>
<th>No. of rats had ulcer</th>
<th>Ulcer incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1° 2° 3° 4° Total</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>96</td>
<td>0</td>
<td>Control</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serotonin</td>
<td>8</td>
<td>3</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>48</td>
<td>5</td>
<td>Control</td>
<td>7</td>
<td>0</td>
<td>1 1</td>
<td>28.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>0</td>
<td>1</td>
<td>14.3</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>10</td>
<td>Control</td>
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<td>1</td>
<td>3 2 1 1 7</td>
<td>77.8</td>
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<td>1</td>
<td>1</td>
<td>22.2</td>
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<tr>
<td>4</td>
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<td>20</td>
<td>Control</td>
<td>15</td>
<td>1</td>
<td>2 4 5 2 13</td>
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<td>4</td>
<td>3</td>
<td>45.5</td>
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<td>5</td>
<td>72</td>
<td>20</td>
<td>Control</td>
<td>8</td>
<td>0</td>
<td>2 3 2 7</td>
<td>87.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Serotonin</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>83.3</td>
</tr>
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</table>

*1°: slight, 2°: moderate, 3°: severe, and 4°: the most severe degree of ulcer

### Table 2. Influence of atropine, morphine or vagotomy on experimentally-induced gastric ulcer

<table>
<thead>
<tr>
<th>Group</th>
<th>Starved (hr.)</th>
<th>Ligated (hr.)</th>
<th>Treated</th>
<th>No. of rats used</th>
<th>No. of rats had ulcer</th>
<th>Ulcer incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1° 2° 3° 4° Total</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>20</td>
<td>Control</td>
<td>6</td>
<td>1 1 2 1 5</td>
<td>83.3</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>20</td>
<td>Atropine</td>
<td>6</td>
<td>1 2 2 2 33.3</td>
<td></td>
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<tr>
<td>3</td>
<td>48</td>
<td>20</td>
<td>Morphine</td>
<td>6</td>
<td>2 1 3 50.0</td>
<td></td>
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<tr>
<td>4</td>
<td>48</td>
<td>20</td>
<td>Vagotomy</td>
<td>6</td>
<td>2 1 3 0 0</td>
<td></td>
</tr>
</tbody>
</table>

*1°: slight, 2°: moderate, 3°: severe, and 4°: the most severe degree of ulcer

However, pretreatment of the animals with serotonin partially prevented the gastric lesions and markedly reduced the incidence to 45.5%. Though the incidence of gastric lesions depends upon the time elapsed from either starvation or pyloric ligation to the time the animals were sacrificed, one can clearly see in every group in the first experiment that the administration of serotonin was effective in preventing the gastric lesions.

The acidity of gastric juice was considerably lower in the animals treated with serotonin than the nontreated control animals (Fig. 2). It was true particularly in the group which had 48 hours of starvation and 5 or 10 hours of pyloric ligation. Here only a few ulcerative lesions were found. The determination of gastric contents was often impossible in the group having more than 48 hours of starvation and 10 hours of ligation since extensive gastric ulceration resulted in contamination of the gastric contents with blood and mucosal debris. In the group given serotonin, there was an increase in the mucin content of the gastric juice (Fig. 3). This was noted when little ulcers were produced in rats having 48 hours of starvation and 5 hours of pyloric ligation. Mucin content could not be determined in the other groups because of the extensive gastric ulceration.

In the second experiment the bilateral gastric vagotomy was completely effective in preventing the gastric lesions usually produced by 48 hours of starvation and 20 hours of pyloric ligation (Table 2). Pretreatment using either atropine or morphine was moderately effective in preventing the gastric lesions usually produced by the same procedure.

Microscopically, the majority of the rats showed one or more of ulcers in the rumen. However, the animals in the first group that were given serotonin did not show any evidence of ulcer. The severity and the numbers of ulcers differed from one group to another but, in general in each group the rats that were given
serotonin showed less severe and less frequent ulcers than the control animals.

The first stage of the ulcer was characterized by an extreme thinning and later a breaking of the stratified squamous epithelium associated with the collection of acute inflammatory exudate (Fig. 4 & 5). A moderate to marked degree of edema was present under the mucosa. The inflammatory exudative cells appeared to hug the epithelial portions of the mucosa. The ulcers of a moderate severity were characterized by the complete loss of the superficial epithelium with more wider areas of necrosis and localized collections of polymorphonuclear leukocytes extending into the deeper portions of the gastric wall. Necrotic fibrinous materials were frequently seen over the ulcer surface. Widely scattered inflammatory cells were noted along with the edema of the neighboring tissue. The most severe degree of ulcer was characterized by necrosis of the entire wall associated with an intense inflammatory reaction. The gastric wall at these points showed gangrene. The superficial layers often were covered by blood clot.

Although there was a considerable degree of individual variation, the studies, after Periodic Acid Schiff reaction, revealed that mucus secretion was greater in the animals that were given serotonin than in the control animals (Fig. 6, 7, 8 & 9). Frequently, patches of mucus were noted in the lumen over the gastric glands.

**DISCUSSION**

Serotonin exists in particularly high concentrations in the mucosa of the stomach and duodenum and in lesser amounts in the other parts of gastro-intestinal tract (Erspermer, 1954). The presence of such greater amounts of this biogenic amine in the normal gastric mucosa has focussed an interest that serotonin may play in the regulation of normal gastric function. Black et al. (1958) reported the intravenous infusion of serotonin in the dog did not evoke acid gastric secretion but appeared to increase the production of mucus. White and Magee (1958) found that infusion of serotonin increased the secretion of mucin from the canine pyloric mucosa due to a direct action on the mucosa but not due to the increased motility. Whang et al. (1963) observed in dog that a small single dose of serotonin induced marked increase of mucin, and suggested that the effect appeared to have a nature which is closely related to the D-receptor on the basis of classification of serotonin receptors by Gaddum and Picarelli (1957).

The results reported here show that the production of gastric ulcer in rats by the procedure of pyloric ligation of Shay et al. (1945) was partially inhibited following the administration of serotonin, atropine or morphine. The mechanism of serotonin in preventing the gastric lesions may be explained by increased mucin content and decreased acidity of gastric juice in accordance with the above reports as well as ours. Furthermore, the histological studies revealed that mucus secretion was greater in the animals that were given serotonin than the control animals.

However, Nikodijevic et al. (1960, 1963) found that subcutaneous injection of a large doses of serotonin (20 mg/kg twice per day) in rats induced hemorrhage or ulcer in the glandular portion of gastric mucosa. This fact, together with our findings which showed inhibitory gastric lesions by small doses of serotonin (8 mg/kg) indicates that serotonin may have dual effects on the gastric mucosa, namely, serotonin in a small dose may protect the mucosa against gastric lesions by increased secretion of mucin and in a large amount may induce gastric lesions by the depletion of mucin. The clinical facts that carcinoid tumor contains a large amount of serotonin (Lewis, 1958) and the gastric ulcer was found in about 40% of malignant carcinoid patients also do not contradict the above view.

Kim and Shore (1963) reported that intraperitoneal injection of reserpine which releases most of serotonin in the body induced hemorrhage or ulcer in the glandular portion of the murine gastric mucosa. These lesions were prevented by vagotomy or a monoamine oxidase
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inhibitor, pheniprazine. The protective mechanism of pheniprazine against reserpine induced gastric lesions may be local action, possibly due to the presence of a high free serotonin concentration in the gastric mucosa. Their considerations together with our results indicate that not only the exogenous serotonin but also endogenous serotonin play an important role to protect the mucosa from the induced gastric lesions.

The mode of action of atropine or vagotomy on gastric lesions is well known. Morphine exerted a moderate efficacy in preventing the gastric lesions in our experiment. It is not clear whether the mechanism of the effect of morphine is similar to atropine, though morphine is a typical blocking agent of M-receptor type of serotonin.

Acknowledgements: We wish to thank to Professor Kwang Shik Min, Chairman of Surgery, for encouragement and help, and Professor Woo Choo Lee, Chairman of Pharmacology, for his critical advice in performing this experiment.

Also we are grateful to Professor Reberta G. Rice of Surgery, for her help in preparing the manuscript.

REFERENCES


LEGENDS OF FIGURES

Fig. 4. Early change of ulcer in rumen of rat (No. 3-19). The rat was starved for 96 hrs and sacrificed. Marked degree of edema under mucosa and the inflammatory exudative cells in epithelial portion with loss of the epithelium are seen. (H & E from ×100)

Fig. 5. A relatively small ulcer in rumen of rat treated with serotonin (No. 5-22). The rat was starved for 48 hrs and then the pyloric ligation left for 20 hrs. Numerous leukocytic infiltration in a local breaking of the stratified squamous epithelium is evident. Edema and scattered inflammatory exudative cells in submucosal layer are also seen. (H & E from ×100)

Fig. 6. Mucus layer in rumen of rat treated with serotonin (No. 2-13). The rat starved for 96 hrs and sacrificed. Mucosa is covered by mucus layer. Edema and inflammatory cells under the mucosa are noted. (PAS from ×100)

Fig. 7. Mucus in glandular portion of stomach of rat (No. 2-19). The rat was starved for 96 hrs and sacrificed. Mucus is noted within the lumen. (PAS from ×100)

Fig. 8. Mucus layer in glandular portion of stomach in rat treated with serotonin (No. 5-15). The rat was starved for 48 hrs and then left pyloric ligation for 10 hrs. Mucosa is covered by mucus. (PAS from ×100)

Fig. 9. Mucus layer in glandular portion of stomach in rat treated with serotonin (No. 3-13). The rat was starved for 72 hrs and then left pyloric ligation for 20 hrs. Patches of mucus was noted in the lumen. (PAS from ×100)