The Role of Endogenous Histamine on Gastric Acid Secretion (37224)

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Whether histamine has a role in the secretion of gastric acid is still a controversial question. Some investigators cite evidence which they interpret as demonstrating that histamine is the final local stimulator responsible for the secretion of acid from the parietal cells (1–3), while other investigators think that the evidence is insufficient to support this interpretation (4–7). A necessary piece of data which is lacking is whether histamine when released from the mucosal cells will stimulate the release of acid from the parietal cells. In the work reported here, data are presented which suggest that the secretion of acid in the frog may be stimulated either by the endogenous histamine released from the mucosa or by gastrin acting directly upon the parietal cells.

Methods. Gastric acid secretion and electrical measurement. The gastric mucosa from the adult frog (*Rana tigerina*) was stripped from the muscular layers, washed with Ringer's solution, and was then mounted between lucite chambers (8). Unbuffered saline (95 m*M* NaCl, 21 m*M* Na isethionate and 25 m*M* K_2SO_4) was placed on the mucosal side of the tissue. The rate of H^+ secretion was measured by a Beckman pH stat. The transmucosal potential difference (PD) was measured with agar–KCl bridges electrically connected via matched calomel half cells to the recording potentiometer. H^+ secretion and PD were measured both before and after the release of endogenous histamine from the gastric mucosa. The liberation of histamine was affected by (*1*) the polymer amine compound $48/_{80}$ (Sigma Chemical Co.) which selectively causes the release of histamine from the mast cells (9–11, (*2*) a nonselective histamine liberator, the nonionic detergent Triton X-100 (Rohm and Haas) which releases histamine from both mast and nonmast cells (12) and (*3*) pentagastrin (Calbiochem. Co.). Each compound was used at the final concentration of 0.2 mg/ml, 1 mg/ml, and 10^{-5} *M* respectively.

Histamine assay. Histamine content of the gastric mucosa was measured by the fluorometric method (13). The minimum concentration of histamine required for the assay is 1 μg/ml. The mucosa was divided into three comparable matched pieces. One piece was used as the control, and the content of histamine was measured immediately. The other two pieces were mounted between the lucite chambers and the rate of H^+ secretion was measured. Then, one of the histamine-releasing substances was added to the chamber, and after 60 min one of the two pieces was removed and assayed for histamine. Pentagastrin (10^{-5} *M*) was then added to the chamber of the third piece, and when the maximum rate of H^+ secretion was reached the tissue was removed for histamine assay. The volume of fluid in each side of the chambers was 25 ml. The amount of histamine released into this volume of solution was too small to be measured by the method used.

Histological localization of histamine. A fluorescent histochemical technique (14) was used on the gastric mucosa to estimate and localize the histamine content of the tissue before and after treatment with either the histamine liberators or pentagastrin. The tissue was freeze-dried and the sections exposed to *O*-phthalaldehyde vapor (Nutritional Biochemicals Corp.). Fluorescence was examined with a Leitz Orthoplan microscope, using the mercury 366 nm for excitation (UG_1 filter) and a Leitz K 430 barrier filter.

Results. Acid secretion and histamine content. Compound $48/_{80}$ reduced significantly the basal acid output from 2.1 ± 0.2 to 0.3

TABLE I. Effect of Histamine Releasing Agents on Production of Acid and the Content of Gastric Histamine.

(1) Releasing agent	(2) No. of expts	(3) Control		(4) 20 min after releasing agent		(5) 60 min after releasing agent		(6) 40 min after[b] Pentagastrin	
		H^+ secretion[a]	Histamine[a] content	H^+ secretion		H^+ secretion	Histamine content	H^+ secretion	Histamine content
Compound $48/_{80}$	6	2.1 ± 0.2	24 ± 3.0	1.8 ± 0.2		0.3 ± 0.1[c]	23 ± 3.0	2.6 ± 0.1[d]	16 ± 2.0[d]
Triton X-10	6	2.0 ± 0.4	26 ± 3.0	2.9 ± 0.5[c]		1.1 ± 0.2[c]	11 ± 3.0[c]	2.4 ± 0.6[d]	10 ± 3.0[c]

[a] Data are expressed as μequiv/cm^2/hr (mean ± SE) for H^+ secretion and μg/gm mucosa (mean ± SE) for endogenous histamine content.
[b] Pentagastrin is given after the histamine releasing agent has been tested.
[c] Difference from corresponding control (paired t test, $p < 0.01$).
[d] Difference from corresponding results of column (5) (paired t test, $p < 0.01$).

± 0.1 μequiv/cm^2/hr with a concomitant increase in the PD across the gastric mucosa (Fig. 1 and Table I). The addition of 10^{-5} M pentagastrin to the chambers restored the rate of secretion of acid to the mean control level (2.6 ± 0.1 μequiv/cm^2/hr). The response to pentagastrin occurred whether $48/_{80}$ remained in the solution or was replaced with fresh Ringer's solution. The amount of histamine in the gastric mucosa did not change significantly after exposure to $48/_{80}$ for 60 min. However, with the addition of pentagastrin to the solution there was a marked reduction in the histamine content of the tissue (Table I).

The output of acid was initially increased by 1 mg/ml of Triton X-100 (Table I and Fig. 2), and at the same time the major part of histamine in the tissue was released (from 26 ± 3 to 11 ± 3 μg/gm, $p < 0.001$). After 20–40 min, the secretion of acid declined gradually to a mean level of 1.1 ± 0.2 μequiv/cm^2/hr. Again, in each of the experiments, within 30 min after the addition of 10^{-5} M pentagastrin, the rate of secretion of acid had returned to the mean control level of 2.4 ± 0.6 μequiv/cm^2/hr. After pentagastrin, the histamine content of the gastric mucosa was not significantly reduced from what it was after the treatment with

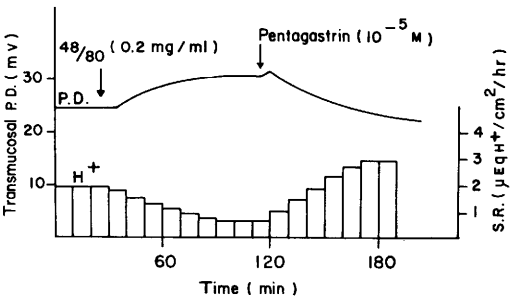


FIG. 1. The electrical and secretory characteristics of frog gastric mucosa under the influence of compound $48/_{80}$ and pentagastrin. Compound $48/_{80}$ (0.2 mg/ml) reduced the secretion of acid (bars) with a gradual increase in transmucosal potential difference (PD). Addition of pentagastrin, with or without prior removal of $48/_{80}$ from the bath media, resulted in a decrease of the PD and an increase of H^+ secretion. SR denotes secretory rate of acid, expressed in μequiv/cm^2/hr.

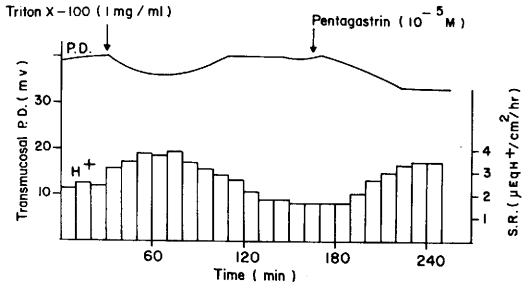


FIG. 2. The effects of compound Triton X-100 on the transmucosal potential difference and H^+ secretion. One milligram per milliliter of Triton X-100 caused an increase in the output of acid (bars) in the first 20–40 min and followed by a reduction in H^+ secretion. The transmucosal potential difference (PD) was consistent qualitatively with the direction of H^+ transport. Finally, pentagastrin restored both the secretory rate (SR) and PD to normal level.

Triton X-100 (Table I). When the histamine content of the tissue was reduced by incubation overnight and the histamine-forming capacity of the gastric mucosa inhibited with compound NSD-1024 (3-hydroxybenzyloxtamine dihydrogen phosphate; Sandev Ltd., Essex, England), with the remaining histamine content being 2.6 ± 2.1 μg/gm mucosa ($n = 4$), the addition of Triton X-100 no longer induced a stimulation of acid secretion during the first 20–40 min. However, the subsequent administration of pentagastrin or histamine (10^{-4} *M*) to the solution resulted in an increase in the production of acid with no further reduction of histamine.

Localization of histamine. The control freeze-dried sections of gastric mucosa when treated with *O*-phthalaldehyde vapor showed both yellow and bluish yellow fluorescence. Mast cells lying in the connective tissue between the gastric glands and in the submucosa showed little fluorescence. The histamine-containing enterochromaffin-like cells, a specific epithelial cell system in the gastric gland area (15), gave off a bluish yellow fluorescence (Fig. 3). The extent of the distribution of fluorescence did not change appreciably in those tissues treated with $48/_{80}$ (Fig. 3a), although the metachromatic granules of the mast cells were completely

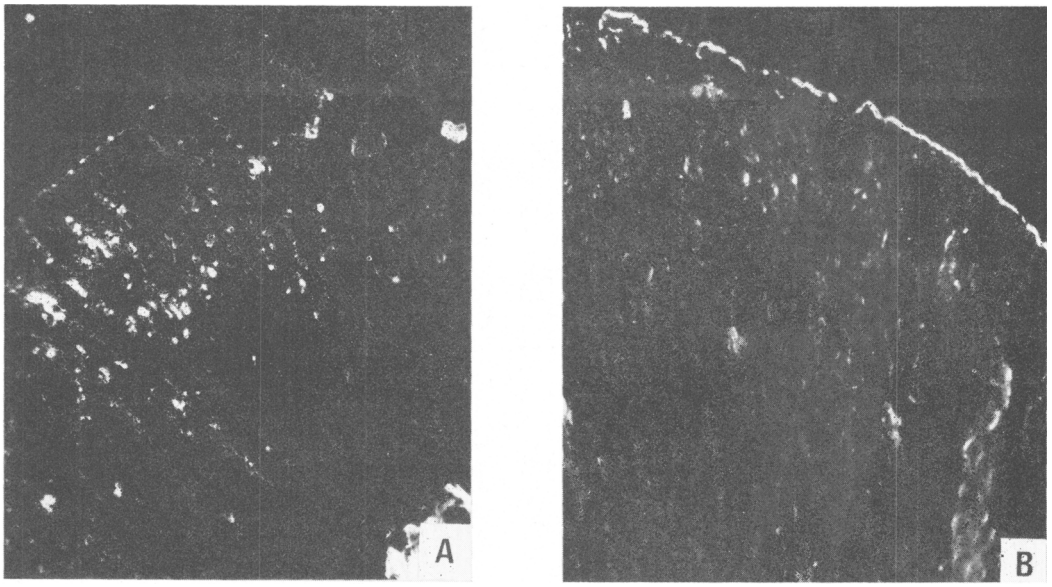
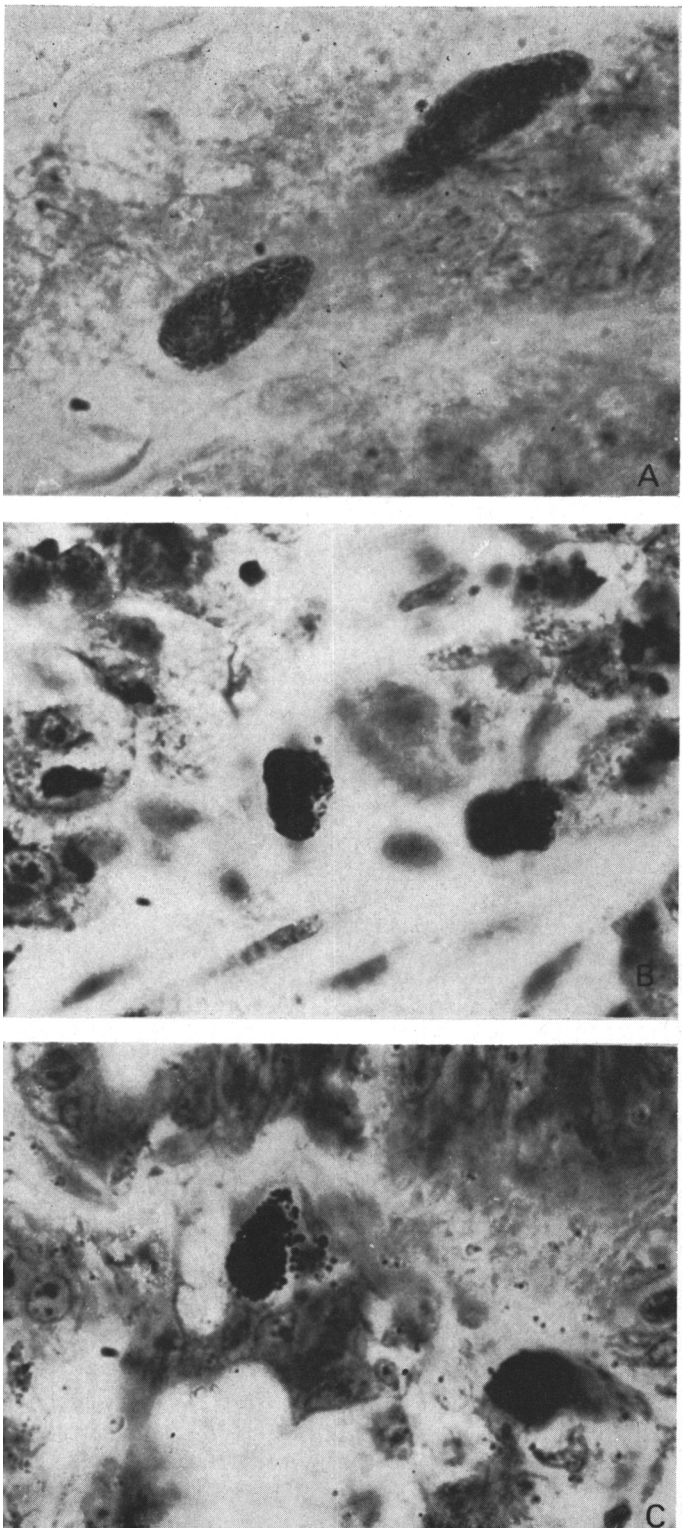


FIG. 3. Longitudinal sections through the glandular area of the stomach of *Rana tigerina* frog. The tissue was freeze-dried, and the sections were exposed to *O*-phthalaldehyde vapor at 100° for 15 min to develop fluorescence. The specific epithelial cell system in the oxyntic gland area which contained histamine gave off a bluish yellow fluorescence (here appears white). The extent of the distribution of fluorescence (or histamine) was approximately the same in the control and in those mucosae treated with $48/_{80}$ (A), but was markedly reduced in the tissues treated with Triton X-100 and pentagastrin (B) (×160.).


A
B
C

degranulated (Fig. 4b). The distribution of the fluorescence was markedly reduced in the tissues after Triton X-100 and after pentagastrin (Fig. 3b). In addition, the metachromatic granules of the mast cells were released by Triton X-100 with an apparent breakage of cell membrane (Fig. 4c).

Discussion. Compound $48/_{80}$ released very little histamine from either the gastric mucosa or the mast cells, although the mast cells were almost completely degranulated (Fig. 4). Triton X-100 and pentagastrin released more than 50% of the histamine in the tissue. Therefore, the bulk of endogenous histamine was primarily associated with the enterochromaffin-like cells which lie in close proximity to the oxyntic cells and was readily released by either Triton X-100 or pentagastrin. Its release was accompanied by an increase in the secretion of acid together with a small decrease in the transmucosal potential difference typically seen with the onset of secretion (16). The change in PD was qualitatively consistent with the direction of H^+ movement. These data suggested that the endogenous histamine, once liberated, stimulates the release of acid from oxyntic cells. In addition, the stimulating effect of Triton X-100 on the secretion of acid did not occur if the tissue was depleted of histamine and its ability to form histamine inhibited. The latter result eliminated the possibility of a direct effect of Triton X-100 on the oxyntic cells. Mast cells contain probably a relatively small amount of the total histamine present in the frog mucosa so that depletion of histamine from mast cells by compound $48/_{80}$ was not enough to either affect the oxyntic cell or markedly affect the total histamine content of the tissue. In fact, the mast cells of frogs have been reported to contain no histamine (17) in contrast to those of other species (9).

The reason for the subsequent reduction, but not cessation, in H^+ secretion after 20–40-min treatment with either $48/_{80}$ or Triton X-100 is uncertain. The integrity of the gastric gland area seemed unaffected by the histamine liberators, and the oxyntic cells could still respond to pentagastrin and exogenous histamine. One possibility is that both $48/_{80}$ and Triton X-100 have an inhibitory effect on the secretion of acid which is overcome by pentagastrin and histamine, or another possibility is that they release other amines such as serotonin and dopamine which are usually present in the metachromatic granules of mast cells and in the enterochromaffin cells of the gastric gland area (18–20). Indeed, both serotonin and dopamine have been shown to inhibit the secretion of acid in different species (15, 21–23).

Pentagastrin, when administered after $48/_{80}$, caused a significant decrease in histamine content of the tissue and a rise in the secretion of acid. The effects in the frog thus parallel those reported for the rat (20). However, even if endogenous histamine has been released by Triton X-100, pentagastrin might directly stimulate the secretion of acid without a further decrease in the content of histamine in the tissue. The histamine remaining in the mucosa was apparently not accessible to liberation by either Triton X-100 or pentagastrin. The onset of the release of acid was too rapid (within 5 min) to be due to an increase in the ability of the tissue to form new histamine. Consequently, pentagastrin seems to stimulate directly the secretion of acid as suggested by Johnson (7) in addition to its action on mucosal histamine stores to release histamine and activate histidine decarboxylase activity (2, 10). However, the endogenous histamine, once liberated in sufficient amount, can stimulate acid secretion, possibly acting on the different receptors of the parietal cells. The latter suggestion can explain why gastrin is more

FIG. 4. Frog mast cells in the connective tissue between the gastric glands and in the submucosa. Coarse metachromatic granules of varying sizes were stained with toluidine blue: (a) normal mast cells with toluidine blue-positive granules and the nuclei as the unstained areas, (b) the metachromatic granules of the mast cells were completely degranulated by compound $48/_{80}$, and (c) the granules were released by Triton X-100 with the apparent breakage of cell membrane (×1440).

potent than histamine in the stimulation of acid secretion in many species (2, 24). This explanation has been ignored frequently in assessment of the validity of histamine as the final common stimulator of the parietal cells.

Summary. Compound $48/_{80}$ decreased the secretion of acid of the frog's gastric mucosa with no significant change in the amount of histamine in the mucosa although the mast cells were almost completely degranulated. This result suggests that the mast cells of the frog contain little or no histamine. Triton X-100, a nonselective histamine liberator, released approximately 50% of the histamine in the mucosa primarily from the enterochromaffin-like cells with a corresponding increase in the secretion of acid. Pentagastrin, after either $48/_{80}$ or Triton X-100, stimulated the release of acid, although there was no further release of histamine. Therefore, pentagastrin can act directly on oxyntic cells to stimulate their secretion.

The authors are indebted to Dr. Russell A. Huggins for his helpful discussion and criticism in preparing this manuscript and to Mrs. Em-on Pramoolpol for her secretarial assistance.

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Received Nov. 8, 1972. P.S.E.B.M., 1973, Vol. 142.