Evidence That Histamine is Not the Mediator of Acid Secretion in the Rat (34903)

LEONARD R. JOHNSON AND DOROTHEA AURES
(Introduced by E. D. Jacobson)

Department of Physiology and Biophysics, University of Oklahoma Medical Center, Oklahoma City, Oklahoma 73104; and Psychopharmacology Research, Veterans Administration Hospital, Sepulveda, California 91343

In 1964 Kahlson and co-workers (1) showed that the injection of gastrin or the refeeding of fasted rats resulted in the release of gastric mucosal histamine and a marked elevation in the avitivity of mucosal histidine decarboxylase (EC 4.1.1.22). Insulin and 2-deoxy-D-glucose also induced a severalfold increase in the histidine decarboxylase activity of the rat oxyntic gland mucosa (2). This information is the basis of a model (Fig. 1), developed by Kahlson and other investigators, defining a physiological role for histamine in the stimulation of gastric acid secretion. According to the model, gastrin, insulin, and feeding release histamine from the gastric mucosa. The intramucosal histamine stimulates gastric secretion, and the decrease in mucosal histamine stores triggers an increase in histidine decarboxylase activity to replenish the histamine used during the stimulation of secretion. A number of compounds which inhibit gastrin-stimulated secretion have little or no effect on that promoted by histamine. These results have been interpreted as support for the model outlined above, for it is suggested that the inhibitor acts to prevent the release of histamine by gastrin or other secretagogues (3, 4), either by depleting mucosal histamine or by blocking its release. The basic assumption in this reasoning is that histamine release does not take place during the inhibition of acid secretion. The current work examines this assumption during the inhibition of acid secretion by the hormone, secretin.

Methods. Male Sprague-Dawley rats, weighing between 160 and 220 g, were fasted for 48 hr but allowed water *ad libitum*. Twelve rats were randomly divided into three groups. The first or control group received an intraperitoneal injection of 150 m*M* NaCl. The second group was injected intraperitoneally with a dose (125 μg/kg) of pentagastrin (ICI 50, 123) shown to produce maximal gastric acid secretion in the rat (5). The third group received the same dose of pentagastrin plus 75 units/kg of secretin (G. I. H. Laboratory, Karolinska Institute, Stockholm, Sweden). This dose of secretin had previously been shown to completely inhibit the acid secretory response to 125 μg/kg of pentagastrin in the rat (5).

The rats were killed by a blow on the head 90 min after injection. The stomach was

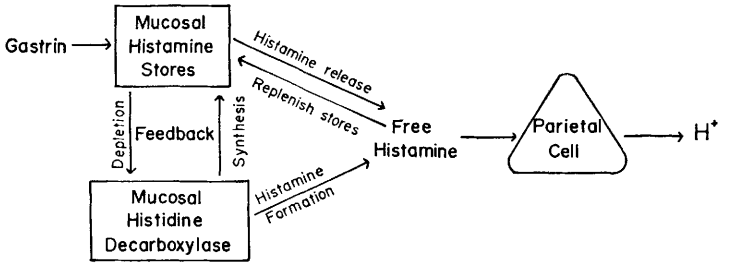

FIG. 1. Diagram of a model for the involvement of histamine in acid secretion: The only target for the hormone gastrin is mucosal histamine stores. Released histamine stimulates the parietal cells and depletion of the stores stimulates an increase in histidine decarboxylase.

removed through a midline incision and quickly trimmed to isolate an area containing only oxyntic gland mucosa. This tissue sample was weighed and immediately immersed in 10% (w/v) TCA (trichloroacetic acid), ground with powdered glass in a mortar and extracted as outlined by Code and McIntire (6). The histamine content of the final tissue extracts was determined by assay using an isolated terminal segment of guinea pig ileum suspended in atropinized Tyrode's solution (7). All results are expressed as micrograms of histamine base per gram of stomach. The experiment was repeated once so that results are means of values from 8 rats in each group.

Results. Figure 2 shows that pentagastrin caused an approximate 50% decrease in the histamine content of the stomach wall. The same effect was found in the animals which received secretin along with pentagastrin. There is essentially no difference between these two groups and each is significantly different from the saline injected controls ($p<0.001$).

Discussion. The hypothesis proffered by MacIntosh (8) in 1938 that gastric mucosal

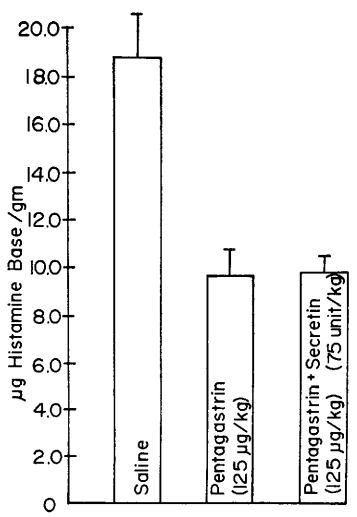

FIG. 2. Histamine content of the glandular area of rat stomachs at 90 min following saline, pentagastrin, or pentagastrin plus secretin injections: Results are μg of histamine base per gm of stomach. Means and standard errors of the means for eight rats in each group.

histamine might be a local common mediator for other gastric secretagogues is still being examined today. In his last review of the subject Code (9) concluded that stimulation of gastric secretion was a physiological function of histamine and that no other mediator comes between histamine and the parietal cell. On the other hand, Grossman (10) believes that in species other than the rat there is no firm evidence linking endogenous histamine to gastric secretion. There is relative agreement in the case of the rat because changes in mucosal histamine content and histidine decarboxylase activity accompany gastric secretion stimulated by refeeding, gastrin, and vagal activation. Most attempts to demonstrate similar changes in other species have failed (10, 11).

The Kahlson hypothesis as outlined in the introduction and in Fig. 1 is the strongest argument for a physiological role for endogenous histamine in the stimulation of gastric secretion. This model is based on several well substantiated facts but depends on two unproven assumptions. The primary assumption is that endogenous histamine actually does stimulate secretion after it is released within the mucosa. Another assumption is that the release of histamine triggers the increased activity of histidine decarboxylase, resulting in a feedback between mucosal histamine content and enzyme activity. In 1964 Kahlson *et al.* (1) wrote, "the crucial test of mucosal histamine as a final link in the excitation of the parietal cells awaits the discovery of a means to inhibit completely the release and synthesis of mucosal histamine." Levine *et al.* (12) then reported that NSD-1055 (4-bromo-3-hydroxybenzyloxamine) was a powerful inhibitor of specific histidine decarboxylase *in vitro* and histamine synthesis *in vivo*. After showing that NSD-1055 was a potent inhibitor of acid secretion stimulated by reserpine, insulin, gastrin, bethanechol, or pylorus ligation but not histamine Levine (3) concluded that this was strong evidence in favor of the hypothesis that histamine is the chemical mediator of gastric acid secretion. These results are, however, not that easy to interpret for Maudsley and Kobayashi (13) have re-

cently shown that the metabolic effects of NSD-1055 are complex, inhibiting the enzyme, diamine oxidase, which destroys histamine as well as the one which forms it.

Similar groups of experiments have been done using semicarbazide and pyridoxal-deficient diets to inhibit histidine decarboxylase and deplete mucosal histamine. In these experiments, however, gastrin remained as effective a stimulus of acid secretion in histamine-depleted animals as in normal control rats (9). Although these results indicate a dissociation between mucosal histamine, histidine decarboxylase activity, and gastric secretion, they do not rule out histamine as the final mediator of acid secretion for, as Code (9) pointed out, the supply of mucosal histamine and levels of the enzyme forming it could greatly exceed that needed for secretion in the normal animal.

The experiments (12, 3, 9) described above, designed to prove the relationship between mucosal histamine content and gastric acid secretion, suffer from two inadequacies, unknown effects of metabolic inhibitors and the inability to completely deplete mucosal histamine. We have approached these problems by examing the effects of physiological inhibitors of gastrin-stimulated acid secretion on histidine decarboxylase activity and mucosal histamine, reasoning that if these substances inhibit secretion by preventing mucosal histamine release, as has been suggested (3, 4), there should be no change in mucosal histamine content or enzyme activity in the presence of gastrin and one of these inhibitors.

Extensive experiments in the dog have shown that the two hormones originating from the duodenum, secretin and cholecystokinin (CCK), are potent inhibitors of gastrin-stimulated acid secretion (14, 15) but have little or no inhibitory effect on histamine-stimulated secretion (16, 17). However, while secretin strongly inhibited gastrin-stimulated secretion in the dog, its effect on gastrin-stimulated secretion in the cat was much less (18). Since species differences in the actions of secretin on acid secretion exist, it was necessary to examine its effects in the rat. The actions of secretin in the rat were found to parallel those in the dog, for it completely inhibited the acid response to a maximal dose of gastrin (5) and had no effect on histamine-stimulated secretion (19).

In the current experiments a dose of secretin (75 units/kg) which completely inhibited the acid response to a maximal dose of pentagastrin (125 units/kg) failed to inhibit the release of mucosal histamine stimulated by an identical amount of pentagastrin. In this instance, therefore, acid secretion was inhibited even though histamine release took place. Since the same dose of secretin that was used in these experiments failed to inhibit histamine-stimulated secretion in the rat (19), we must conclude that the histamine released from the mucosa by gastrin does not stimulate acid secretion. Further evidence that gastrin-stimulated histamine release is not blocked by secretin is that gastrin still activates histidine decarboxylase in the presence of secretin (20). This, of course, assumes that the release of histamine triggers the increase in histidine decarboxylase (HD) activity and that this activation is not stimulated directly by gastrin (21).

It has recently been suggested that gastrin activates HD directly (21). This concept has been supported by Rosengren and Svensson (22) who further concluded that HD then provides histamine for stimulation of gastric secretion. This idea, however, is also untenable with the finding that secretin fails to inhibit the gastrin-induced activation of HD (20).

Figure 3 is a diagram summarizing the actions of gastrin and secretin within the gastric mucosa. This model is based on our findings that gastrin-stimulated acid secretion is inhibited by secretin while histamine release and histidine decarboxylase activation are not. In the diagram gastrin stimulates acid secretion directly; this action, 1, is blocked by secretin. Gastrin acts on mucosal histamine stores to release histamine; this action, 2, is not blocked by secretin. The release of histamine triggers an increase in histidine decarboxylase activity, which, 3, is not blocked by secretin. Therefore, due to both release and synthesis, endogenous histamine enters the mucosa and is either de-

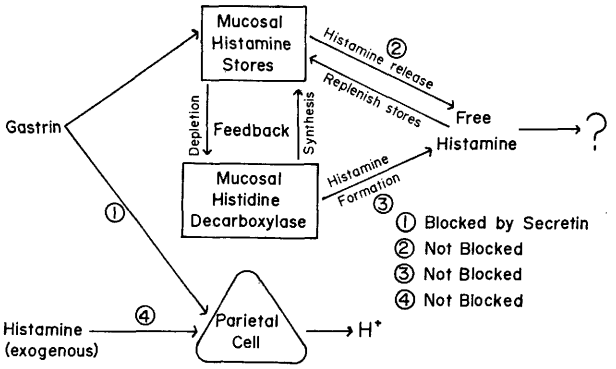

FIG. 3. Diagram of the effects of gastrin on histamine release and secretion in accordance with the actions of secretin: Gastrin has two targets, mucosal histamine stores and the parietal cell itself. Endogenous histamine does not stimulate acid secretion.

stroyed or carried away by the blood stream. Extremely high doses of exogenous histamine are required to stimulate the parietal cell, and this action, 4, is not blocked by secretin.

This model explains all the observations seen with combinations of gastrin and secretin, and seems to be the only reasonable way to account for these results. The results force one to make the assumption that exogenous histamine reaches the parietal cells while endogenous histamine does not. At first this may seem unlikely; however, there is other evidence that this indeed may be the case. Recently it has been shown that in the rat gastric histamine is localized in structures at the base of the gastric gland which are unrelated to parietal cells (23, 24). It is, therefore, possible that after being released, histamine could be destroyed or removed from the mucosa without stimulating secretion.

There are compounds other than secretin whose actions do not fit the Kahlson hypothesis outlined in Fig. 1. Antigastrin (SC 15396) inhibits gastrin-stimulated acid secretion but not histidine decarboxylase activity (20). In fact, when administered alone to a fasted rat, antigastrin actually stimulates histidine decarboxylase activity (20). Cholecystokinin has the same effect as antigastrin on histidine decarboxylase activity (20), and also inhibits gastrin-stimulated secretion in the rat (5). In the case of CCK, however, the implications are not as clear cut, for this hormone is a partial agonist and weakly stimulates secretion when given by itself in small doses (25).

In summary, the results obtained with the physiological regulators of acid secretion are incompatible with the hypothesis that endogenous histamine is the final common mediator of acid secretion. To explain the fact that secretin inhibits gastrin-stimulated acid secretion even though histamine is released from the mucosa, we have concluded that gastrin stimulates the parietal cells without intervention by histamine.

Summary. Fasted rats were injected with either saline, pentagastrin (125 μg/kg) or pentagastrin plus secretin (75 units/kg) and killed 90 min later. Pentagastrin and the combination of pentagastrin and secretin released 50% of the histamine contained in the wall of the oxynitc gland area of the stomach. Therefore, secretin does not inhibit gastrin-stimulated secretion by inhibiting histamine release as has been hypothesized. Since this dose of secretin completely inhibits acid secretion stimulated by 125 μg/kg of pentagastrin in rats but has no effect on a comparable amount of histamine stimulated secretion, we conclude that gastrin stimulates the parietal cell without the intervention of histamine.

We are grateful to Susan Snyder and Alvin C. K. Chang for expert technical assistance and Dr. Morton I. Grossman for advice. We thank Mr. B. Mollov and Dr. Robitscher of Ayerst Laboratories for the gift of pentagastrin. This study was support-

ed by USPHS Grant R01-AM-11073-03 and by the G. A. Manahan Trust Fund.

1. Kahlson, G., Rosengren, G. E., Svahn, D., and Thunberg, R., J. Physiol. (London) **174**, 400 (1964).

2. Kahlson, G., Rosengren, G. E., and Thunberg, R., J. Physiol. (London) **190**, 455 (1967).

3. Levine, R. J., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **24**, 1331 (1965).

4. Ivy, A. C., and Bachrach, W. H., *in* "Handbook of Experimental Pharmacology" (O. Eichler and A. Farah, eds.), Vol. 18, p. 810. Springer-Verlag, New York/Berlin (1966).

5. Tumpson, D. B., and Johnson, L. R., Proc. Soc. Exp. Biol. Med. **131**, 186 (1969).

6. Code, C. F., and McIntire, F. C., *in* "Methods of Biochemical Analysis" (D. Glick, ed.), Vol. 3, p. 49. Interscience, New York (1956).

7. Barsoum, G. S., and Gaddum, J. H., J. Physiol. (London) **85**, 1 (1935).

8. MacIntosh, F. C., Quart. J. Exp. Physiol. **28**, 87 (1938).

9. Code, C. F., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **24**, 1311 (1965).

10. Grossman, M. I., "Handbook of Physiology. Alimentary Canal," Sect. 6, Vol. II, Chapt. 47, p. 835. Am. Physiol. Soc., Washington, D. C. (1967).

11. Blair, E. L., *in* "Gastrin, Proceedings of a conference," (M. I. Grossman, ed.), p. 255. Univ. of California Press, Berkeley (1966).

12. Levine, R. J., Sato, T. L., and Sjoerdsma, A., Biochem. Pharmacol. **14**, 139 (1965).

13. Maudsley, D. V., and Kobayashi, Y., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 353 (1969).

14. Johnson, L R., and Grossman, M. I., Amer. J. Physiol. **217**, 1401 (1969).

15. Johnson, L. R., and Grossman, M. I., Amer. J. Physiol. **218**, 550 (1970).

16. Johnson, L. R., and Grossman, M. I., Amer. J. Physiol. **215**, 885 (1968).

17. Johnson, L. R., and Grossman, M. I., Amer. J. Physiol. **216**, 1176 (1969).

18. Stening, G. F., Johnson, L. R., and Grossman, M. I., Gastroenterology **56**, 687 (1969).

19. Johnson, L. R., and Tumpson, D. B., Proc. Soc. Exp. Biol. Med. **133**, 125 (1970).

20. Caren, J. F., Aures, D., and Johnson, L. R., Proc. Soc. Exp. Biol. Med. **131**, 1194 (1969).

21. Aures, D., Johnson, L. R., Way, L. W., and Clark, W. B., Fed. Proc., Fed. Amer. Soc. Exp. Biol **28**, 353 (1969).

22. Rosengren, E., and Svensson, S. E., J. Physiol. (London) **205**, 275 (1969).

23. Thunberg, R., Exp. Cell. Res. **47**, 108 (1967).

24. Häkanson, R., Owman, C., and Sjöberg, N. O., Life Sci. **6**, 2535 (1967).

25. Stening, G. F., Johnson, L. R., and Grossman, M. I., Gastroenterology **57**, 44 (1969).

Received Mar. 30, 1970. P.S.E.B.M., 1970, Vol. 134.