Effect of Histamine on Gastric Secretion in the Pylorus Ligated Rat. (30211)

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Histamine is a powerful stimulant of gastric secretion in man(1), and in dogs(2) but its effect on rat gastric secretion is controversial. Some authors have noted augmentation of rat gastric secretion with histamine (3-10) while others(11-14) have been unable to document any effect. Recent publication of a dose-response curve following histamine administration(15) indicated that as much as 40 mg/kg was required to get a maximal secretory response to a test meal in the rat. Most workers have used doses of 10 mg/kg or less in such studies.

The present study was planned to investigate the effect of adequate histamine doses on rat gastric secretion. During the experiments we discovered that failure to demonstrate stimulation of rat gastric secretion in the past may have been due in part to the use of the pylorus ligated (Shay) rat as the assay animal. These observations form the basis for this report.

Materials and methods. One hundred and fifty-one male Sprague-Dawley rats weighing between 200 and 300 g were maintained on a standard diet (Purina chow and water *ad libitum*) until 48 hours before the study when food was withdrawn and water only was continued.

On the day of testing the rats were weighed and transferred to individual cages with wide, wire mesh floors. Under ether anesthesia the gastro-duodenal region was exposed through a small upper midline incision. The pylorus was ligated using the method of Shay, Sun and Gruenstein(16), the stomach emptied by gastric intubation, washed with water until the return was clear, and left empty. The abdomen was closed in one layer with 00 black linen, care being taken to ensure apposition of the muscles, and the wound was painted with collodion to prevent ingestion of blood. Animals were then allotted to the various groups and treated as outlined below after which they were returned to their cages to rest quietly. After either 2 or 4 hours (see below), each rat was anesthetized again, the abdomen reopened and the lower end of the esophagus ligated. The stomach, thus isolated with its accumulated secretions intact, was excised and the animal sacrificed by exsanguination.

The outside of each stomach was gently rinsed with water, the stomach carefully incised, its contents collected, centrifuged, and the volume of supernatant recorded. Twelve rats with blood or feces in the stomachs were excluded from the study at this stage. Acidity of the gastric juice was determined by titration against a standard solution of sodium hydroxide on a Radiometer TTT1 automatic titrator to an end point of pH 7.0 and the acid content of the sample of gastric juice was calculated from the volume and acidity. The pH of each sample of juice was determined using the automatic titrator just prior to the titration. Volumes were recorded as milliliters per 100 g rat weight because of the known relationships of parietal cell mass(17) and gastric secretion(18) to body weight in the rat. Acidity was recorded both in pH units and as milliequivalents per liter of gastric juice as recommended by James(19) and acid output was recorded as milliequivalents of acid secreted per 100 g of rat weight per 2 or 4 hours.

Rats were divided into 4 control and 4 test groups. In the first series of experiments, 24 control rats which received no histamine and 27 rats which received histamine were killed 2 hours after histamine injection and pylorus ligation. In a second series of experiments, 28 control rats and 29 rats which received histamine were killed 4 hours after pylorus ligation and histamine injection. The third group of rats (11 control and 11 histamine injected animals) were given histamine 2

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TABLE I. Gastric Secretion After Histamine Injection.

	No.	Vol (ml/100 g)	H^+ content (pH)	Acidity (mEq/l)	Acid output (mEq/100 g)
0-2 hr study					
Control group	24	1.31* (.86–1.83) †	1.70 (1.40–2.05)	62 (40– 79)	.078 (.047–.108)
Histamine "	27	1.33 (1.04–1.68)	1.20 (1.20–1.40)	92 (74–103)	.112 (.080–.143)
Significance of difference (P)		.93	.0003	.0002	.0008
2-4 hr study					
Control group	11	1.08 (.83–1.48)	2.05 (1.85–2.15)	59 (53– 61)	.063 (.053–.087)
Histamine "	11	.71 (.48– .77)	2.50 (2.00–4.00)	37 (26– 47)	.022 (.015–.035)
Significance of difference (P)		.01	.07	.01	.0006
0-4 hr study					
Control group	28	1.88 (1.48–2.71)	1.40 (1.20–1.60)	79 (64– 95)	.149 (.106–.212)
Histamine "	29	1.60 (1.33–2.11)	1.40 (1.20–1.50)	71 (64– 90)	.118 (.093–.167)
Significance of difference (P)		.27	.81	.60	.19

* Median. † Interquartile range.

hours before and were killed 2 hours after pylorus ligation, thus enabling measurement of secretion over the third and fourth hours following histamine. The results from these 3 series of experiments are shown in Table I. The fourth series of experiments was designed to study whether effects observed after histamine were due, in fact, to the drug and not to the volume of fluid injected. Eleven rats given histamine were compared with 10 rats given isotonic saline in the same volume and by the same route. The injections were given at the time of pylorus ligation and the animals were killed 2 hours later. The results from this group are shown separately in Table II. Histamine as histamine acid phosphate 2.75 mg/ml† was used in all experiments and was given by multiple subcutaneous injections in a dose of 50 mg (18 ml) per kg rat weight.

Rats from a control and a test group were tested alternately and several groups were tested each day to minimize variations between days or batches of rats. The distributions of results were not Gaussian but were skewed towards the lower values. The groups have therefore been identified by their medians and interquartile ranges rather than by mean and standard deviation. All statistical calculations were done using a normal approximation of the rank sum test which is more suitable for non-normal distributions than the t test(20).

Results. Histamine did not result in significant alteration in volume, gastric acidity or acid production in rats killed 4 hours after pylorus ligation. The probability levels derived from these results are shown with the results in Table I.

In contrast, there was significant evidence of histamine induced gastric secretory stimulation in stomachs removed from rats killed 2 hours after injection (Table I). Median acid output was 0.112 mEq/100 g/2 hr in the histamine treated rats and 0.078 mEq/100 g/2 hr in the controls. This difference is significant ($P = .0008$). The pH of the juice was significantly lower in the histamine treated rats (1.20 *cf* 1.70 pH units; $P = .0003$) and titratable acidity was significantly higher (92 *cf* 62 mEq/liter; $P = .0002$). Vol-

† Eli Lilly & Co., ampoules type 269 equivalent to 1 mg histamine base.

TABLE II. Effects of Equal Volumes (18 ml/kg) of Subcutaneous Histamine and Isotonic Saline on Gastric Secretion 0-2 Hours.

	No.	Vol (ml/100 g)	H^+ content (pH)	Acidity (mEq/l)	Acid output (mEq/100 g)
Saline injected	10	.90* (.39–1.25)†	1.80 (1.60–2.50)	64 (36– 76)	.040 (.023–.082)
Histamine injected	11	1.26 (1.14–1.39)	1.25 (1.20–1.32)	101 (87–109)	.117 (.100–.136)
Significance of difference (P)		.03	.0008	.001	.002

* Median. † Interquartile range.

umes were identical in the 2 groups (1.31 ml/100 g/2 hr).

When the results of gastric secretion in the third and fourth hours after histamine were examined a third pattern of response was observed. In this instance gastric secretion was less in the histamine treated group than in the controls. The histamine group had significantly less volume (0.71 *cf* 1.08 ml/100 g/2 hr, P = .01), titratable acidity (37 *cf* 59 mEq/l, P = .01) and acid output (.022 *cf* .063 mEq/100 g/2 hr, P = .0006) than sham treated controls.

Finally, the results shown in Table II demonstrate that the stimulation of gastric secretion seen at 2 hours was in fact due to histamine and not to a non-specific effect of injected fluid. There was significantly more secretion by the histamine injected rats as regards volume (P = .03), pH (P = .0008), titratable acidity (P = .001), and acid output (P = .002) than in the saline treated animals.

Discussion. These studies show that subcutaneous histamine significantly stimulated gastric juice acidity and acid secretion by the pylorus ligated rat stomach within 2 hours, the first time such an effect has been shown with this assay method. This initial period of stimulation was followed by significant net secretory depression over the next 2 hours. Because of this, there was no apparent effect due to histamine when assays were made over 4 hours, the period usually recommended for studies using the Shay rat technique(16). Friedman(9) reported that histamine was ineffective as a gastric secretory stimulant in the rat, but his results show an increase in acidity and total acid output at 1½ and 2 hours. Most workers who have shown stimulation of rat gastric secretion by histamine have estimated secretion immediately or within 2 hours of injection(6,8,9); while investigators who have failed to demonstrate any effect usually employed a longer collection period(12,13). Thus we have been able to confirm both sets of results and show that the differences reside in the experimental procedures used.

It is known that saline given to dogs subcutaneously will augment gastric secretion (21) and it was necessary to examine the possibility that the injected fluid and not the histamine was causing the increase in gastric secretion. This volume of fluid was considerable (18 ml/kg) and to date there are no reports available regarding the effects of such an injection on gastric secretion in the rat. Our studies, designed specifically to examine this possibility, showed significantly greater secretion in histamine treated rats than in rats given an equal volume of isotonic sodium chloride and we have concluded that the augmented gastric secretory function was, in fact, due to histamine.

It appears, as suggested by Lane *et al*(8), that the pylorus ligation technique as originally described(16) is unsuitable for study of the effects of histamine. This technique utilizes vagally innervated total gastric "pouches" from which there is known to occur a constant interdigestive secretion dependent on vagal stimuli(9,22). Distension of vagally innervated gastric pouches over a limited period leads to increased gastric acid secretion(23,24). We believe that this mechanism may explain the gradually increasing secretory rates seen in our control rats. Because of this, it is now our practice to limit collection to 2-hour periods when assaying

gastric secretion by the pylorus ligation technique.

While histamine exerts an immediate stimulating effect and might be followed by continuing secretion due to gastric distension, the very low secretory rates seen during the latter part of the 4-hour collection period seem to be due to relative secretory refractoriness following a supreme gastric secretory response to maximal histamine stimulation. In addition, it is known that there is significant insorption of hydrogen ions from juice in the gastric lumen(25,26). It is possible that the early increased acid output in the histamine treated rats made possible more loss of hydrogen ions during the collection period than from the controls. Rats with gastric fistulae or 2-hour pylorus ligated rats minimize the time gastric juice remains subject to loss of hydrogen ions by insorption and may be more sensitive than the classical 4-hour Shay rat preparation.

Many of the previous reports on the effect of histamine in the rat used what we now recognize to be very small doses of histamine. Valberg and Witts(15) published a dose-response curve for histamine on rat gastric secretion and suggested that 50 mg/kg given subcutaneously was an appropriate dose in the rat to produce maximal stimulation. Our study, which employed a dose of this magnitude, produced a significant stimulation of acid production. Nevertheless, this effect was as transient as those previously observed with smaller doses(6,8,9,27).

Summary. The effects of single large subcutaneous doses (50 mg/kg) of histamine acid phosphate on gastric secretion were studied in pylorus ligated rats. Significant increases in gastric acidity and acid production were observed 2 hours after histamine injection. No differences between control and histamine injected rats were found 4 hours after injection. Study of the secretory patterns indicated that histamine caused early increased secretion of acid followed by a period of secretory inactivity while, in control rats, the secretory rate increased with time so that by 4 hours gastric secretion was equal in all groups. The gastric secretory stimulation was shown to be due to the histamine and not to the fluid in which it was contained. These studies help reconcile the differing results previously reported concerning the effects of histamine on the rat stomach and suggest that the standard 4-hour pylorus ligated rat preparation may not be suited for experiments utilizing histamine.

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Release of Histamine and Slow-Reacting Substance Activities from Guinea Pig Lung.* (30212)

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The addition of specific antigen *in vitro* to the lung of a sensitized guinea pig results in the release of histamine and a slow-reacting substance (SRS)(1,2). This release can be blocked by certain enzyme inhibitors(3,4). Cobra venom *(Naja naja)* added to the lung of a non-sensitized guinea pig also causes the release of histamine and an SRS(5). The possibility arises, therefore, that the antigen-antibody reaction activates an enzyme which is similar to one present in the cobra venom and which effects the release of histamine and SRS. That a phospholipase A might be the enzyme involved in the release by venom is suggested by the observation of Högberg and Uvnäs(6) that bee venom phospholipase A effected the release of histamine from rat mast cells. The present study, indeed, suggested that of the many enzymes in cobra venom, the phospholipase A could account for the release of histamine and SRS activities by the venom. Since phospholipase A catalyzes the hydrolysis of lecithin to lysolecithin, the effect of lysolecithin on release of histamine and SRS activities from guinea pig lung was also studied. In addition, the effect of non-specific tissue damage on release of histamine and SRS activities was evaluated.

Materials and methods. All materials to be tested were made up in Tyrode's solution, added to fresh chopped lung (or other tissues in one experiment) of non-sensitized guinea pigs in a ratio of 1 ml of solution to 0.1 g tissue, the suspension incubated at 37°C for 20 minutes unless otherwise stated, centrifuged, and the liquid phase removed and assayed for histamine and SRS activities using the guinea pig ileum as described previously (7). When any of the venom preparations were studied, this liquid phase was heated at 100°C for 15 minutes in order to inactivate the venom, which when unheated has a contractile action on the guinea pig ileum(7). At the alkaline pH of Tyrode's solution, this degree of heating has been reported to destroy all venom enzyme activity measured, while at neutral pH most of the phospholipase A activity survives(8,9). All of the venom solutions were added to the lung in a final concentration of 0.5 mg venom per ml Tyrode's solution. Samples to be compared were added to aliquots of the same guinea pig lung. A sample of the lung in plain Tyrode's solution served as a control for each run.

The hemolytic ability of various of the materials tested was estimated by making samples to a final volume of 2 ml containing a 1% suspension of human or sheep red blood cells washed 3 times with and made up in Tyrode's solution; this suspension was incubated at 37°C for one hour, centrifuged, and the supernatant diluted with water and its absorbance measured in a Klett-Summerson colorimeter using a 540 mμ filter.

Two preparations of lysolecithin, one made from beef brain lecithin and the other from beef serum lecithin, and one preparation of

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