Lipase in Canine Gastric Juice[1] (35096)

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Gastric lipase has been demonstrated in uncontaminated gastric juice (3, 4, 6) and within the gastric mucosa (1, 7) of man and various laboratory animals. In the present study, the effect of stimulation with histamine and Urecholine on secretion of lipase by canine Heidenhain pouches was determined.

Materials and Methods. Collection of gastric juice. Two trained female mongrel dogs, weighing 19 and 21 kg, respectively, were equipped with chronic Heidenhain pouches. The pouches remained free of infection or exudation along the metal cannula. After collection of unstimulated gastric juice for 2 hr, saline with histamine (124 μg/kg/hr) or Urecholine (88 μg/kg/hr) was infused intravenously for 3 hr at a constant rate of 11.5 ml/hr. With each drug, three experiments per dog were performed. In two experiments, stimulated juice was pooled and collected at 30-min intervals (see experiments with 30-min samples). In one experiment, all collections of the second and third hour of stimulation were pooled (see experiments with pooled gastric juice). Gastric juice was collected in cooled containers, refrigerated immediately after collection, and incubated for determination of enzyme activity within 4 hr after collection.

Determination of lipolysis. The rate of hydrolysis of trioctanoin *in vitro* was used as an estimate of the concentration of lipase in gastric juice. Octanoic acid was measured after incubation of 1 ml of gastric juice with 20 μl of trioctanoin.

The pH of gastric juice was adjusted with 0.2 *M* citrate buffer (pH 2, 3, 4, or 5), phosphate buffer (pH 5, 6, 7, or 8), tris buffer (pH 8 or 9), or by titration with 0.2 *M* NaOH or HCl. To this mixture, trioctanoin was added (20 μl/1 ml of gastric juice). Incubations were performed for 60 min in a shaking waterbath at a temperature of 37°. Due to the rapid shaking (150 shakes/min) the fat was kept in milky suspension. After incubation, the pH was measured with a glass electrode and the incubate was mixed with five times its volume of cold extration mixture containing heptane, isopropyl alcohol, and H_2SO_4 (5).

TABLE I. Concentration of Octanoic Acid (μeq/ml of gastric juice) after Incubation of Gastric Juice with Trioctanoin (20 μl/ml of gastric juice).

For each type of test two determinations in each of four pooled juices were performed.

Type of gastric juice (pH)	Octanoic acid (μeq/ml; mean ± SE)
Basal juice	
4	13.9 ± 4.38
7	15.5 ± 6.13
4, after boiling	0.5 ± 0.08
Stimulated juice	
2	0.3 ± 0.04
3	0.5 ± 0.06
4	1.4 ± 0.38
5	1.0 ± 0.21
6	0.9 ± 0.24
7	0.5 ± 0.08
8	0.3 ± 0.04
9	0.2 ± 0.04
4, after boiling	0.3 ± 0.03
4, after extraction mixture	0.4 ± 0.07

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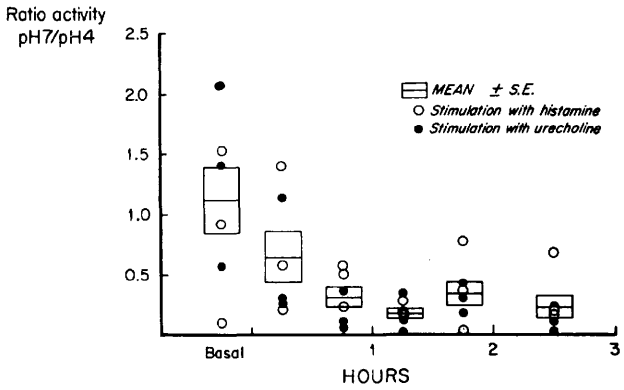

FIG. 1. Ratio of lipolytic activity pH 7/pH 4 during stimulation with Urecholine and histamine. Each point represents the ratio of lipolytic activity pH 7/pH 4 in one sample of gastric juice and the boxes represent means ± one standard error.

The concentration of free fatty acids was determined by the method of Dole (5) in all samples. In samples of two experiments, fatty acids were also determined by gas liquid chromatography (10). The only fatty acid found by this technique was octanoic acid. The concentration of octanoic acid as determined by gas chromatography was similar to the values obtained by Dole titration.

Other determinations. Pepsin concentration was measured by the method of Hunt using bovine albumin as a substrate. H^+ concentration was determined by titration to pH 8.3 with 0.01 *M* NaOH. Na^+ concentration was determined with a flame photometer. All *p* values given were determined by analysis of variance.

Results. Control experiments. Trioctanoin used for incubation experiments did not contain free fatty acids. After incubation of 20 μl of trioctanoin with 1 ml of 0.9% NaCl for 1 hr, the concentration of octanoic acid was 0.30 ± 0.022 μeq/liter (Mean ± SE). Similar concentrations of octanoic acid were found after incubation of trioctanoin with buffer solutions at pH values varying from 2 to 9 or with buffered pepsin solutions containing 0.01–0.1% pepsin (pH 2–4).

Experiments with pooled gastric juice (Table I). The activity of basal gastric juice was

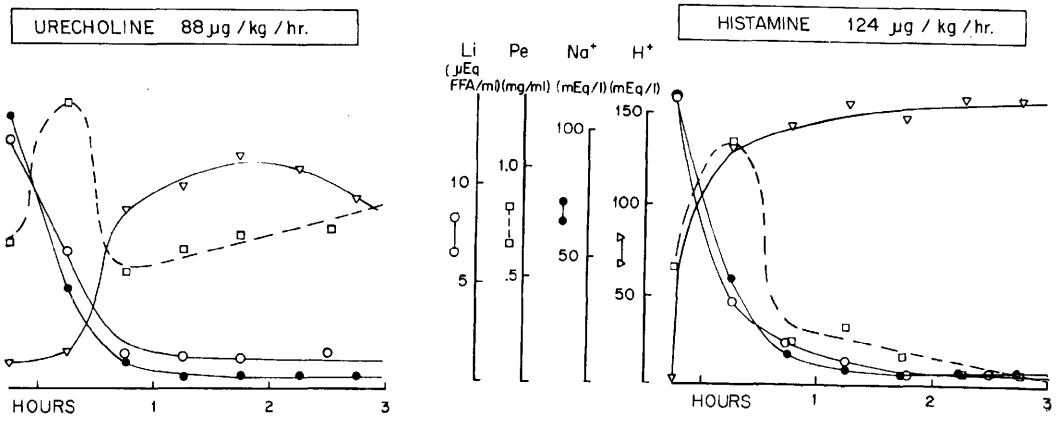

FIG. 2. Concentration of lipolytic activity (Li), Pepsin (Pe), Na^+, and H^+ in gastric juice during stimulation with Urecholine and histamine. Each point represents the mean of four experiments.

TABLE II. Output of Lipolytic Activity, Pepsin, Na^+, H^+, and Volume During Stimulation with Histamine and Urecholine.
Lipolytic activities were measured at pH 4. All values represent mean ± SE of 4 experiments.

Determination	Histamine			Urecholine		
	Basal hour	1st hour	2nd hour	3rd hour	Basal hour	1st hour	2nd hr	3rd hr
Lipolytic activity (μM FFA)	17 ± 6.8	59 ± 10.9	52 ± 10.8	42 ± 8.3	12 ± 3.4	32 ± 7.0	30 ± 5.9	27 ± 5.2
Pepsin (mg)	0.8 ± 0.43	5.1 ± 1.65	5.8 ± 2.21	1.8 ± 0.59	0.8 ± 0.25	7.5 ± 2.29	15.2 ± 4.06	15.5 ± 5.20
Na^+ (μeq)	138 ±57.6	468 ±120.8	287 ± 37.0	314 ±61.7	138 ±40.0	362 ±89.6	397 ±105.6	326 ±64.0
H^+ (meq)	0	3.3 ± 0.38	8.6 ± 1.46	10.9 ± 2.38	0	0.8 ± 0.29	2.5 ± 0.41	2.2 ± 0.52
Vol (ml)	1 ± 0	25 ± 0.3	59 ± 9.4	69 ±14.2	1 ± 0	13 ± 2.8	21 ± 3.8	20 ± 4.3

about 10 times higher than that of stimulated juice. The activity of basal juice at pH 4 was not significantly different from the activity at pH 7.

Stimulated juice had its highest activity at pH 4 and a low activity at pH 7. The difference between the two activities was significant ($p < 0.001$).

Inactivation of gastric juice was achieved either by preincubation of gastric juice for 60 min in a boiling water bath or by addition of extraction mixture before incubation instead of after incubation with trioctanoin.

Experiments with 30-min samples. Samples from six experiments were incubated both at pH 7 and 4. The ratio of activity at pH 7 and 4 is shown in Fig. 1. It was 1.1 ± 0.28 (mean ± SE) in basal juice. During the second and third hour of stimulation it fell to values ranging from 0.18 ± 0.06 to 0.34 ± 0.09. This decrease was significant ($p < 0.001$).

In eight stimulation experiments lipolytic activity was determined at pH 4. The results are shown in Fig. 2. During stimulation with either Urecholine or histamine, the concentration of lipolytic activity dropped from high basal levels to a low plateau maintained during the second and third hour of stimulation. A similar pattern was seen with Na^+ concentrations. In contrast, the concentration of H^+ rose from low basal values to high levels during stimulation. The concentration of pepsin rose during the early phase of stimulation. The subsequent decrease of pepsin concentration was more marked during stimulation with histamine than in experiments with Urecholine.

The output of lipolytic activity is shown in Table II. A slightly higher rise ($p < 0.05$) was observed with histamine than with Urecholine stimulation. The output of pepsin was more stimulated by Urecholine than by histamine ($p < 0.001$).

Discussion. Lipase in gastric juice was first demonstrated more than 50 years ago (8), but sources such as salivary glands and pancreas could only be excluded by recent studies in pylorus ligated rats and Heidenhain pouches of dogs (3, 6).

In the present study, gastric juice from canine Heidenhain pouches produced pH-dependent hydrolysis of trioctanoin. Lipolysis was abolished by denaturation of proteins or by changes in pH (Table I). Bacteria, leukocytes, and serum were unlikely sources for this activity of gastric juice, and it was therefore assumed that canine gastric juice contained lipase which was produced by the gastric mucosa.

The output of lipase was increased by infusions of histamine or Urecholine. These stimulants also increased the output of H^+, Na^+, and pepsin (Table II). The responses of lipase and pepsin to stimulation differed in several respects. The output of pepsin was more stimulated by Urecholine than by histamine. The concentration of pepsin responded to stimulation with an early rise above basal level and a subsequent fall. This pattern of pepsin secretion is characteristic of the dog (9). Lipase showed a different type of response. Histamine was a strong stimulant of lipase output, even somewhat stronger than Urecholine. The concentration of lipase fell steadily from a high basal level to a low plateau in response to both stimuli. A similar pattern was seen with the concentration of Na^+ (Fig 2.).

From these observations it is not possible to determine the origin of gastric lipase. Nonparallel transport of two enzymes or of enzymes and electrolytes does not exclude the possibility that they are secreted by the same type of cell (2, 11). However, the following differences between lipase and pepsin would favor two different production sites. In newborn rats, the peptic activity of the gastric mucosa dropped by more than 50% as compared to the prenatal activity whereas the lipolytic activity continued to rise (7). Histochemical studies in various animals failed to demonstrate lipases in zymogen cells but showed some enzymatic activity in surface epithelial cells (1). In view of these observations, the parallel changes in output and concentration of Na^+ and lipase during gastric stimulation may be the expression of a common source in the surface epithelial cell.

An interesting finding of the present study was a qualitative change of lipase activity during gastric stimulation. Lipase of stimulated gastric juice had optimal hydrolytic activity at pH 4. In basal juice, but not in stimulated juice, strong activity could also be demonstrated at pH 7 (Fig. 1). This change in the pH optimum of lipase during stimulation may be due to a structural alteration of lipase in the presence of acid. Alternatively, at least two lipases responding differently to stimulation are secreted by the stomach.

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