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Molecular Forms of Gastrin in Antral Mucosa and Serum of Dogs (38848)

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Many peptide hormones occur in two or more molecular forms of different size (1). Frequently a larger molecular form may be converted to a smaller form with greater biological activity by cleavage by enzymes with trypsin-like specificity, the point of cleavage generally consisting of two consecutive basic amino acids (1).

Several molecular forms of the antral hormone gastrin have been described (2–4). They differ in peptide chain length, and the single Tyr residue which may be either sulfated (G-II) or unsulfated (G-I). The largest form chemically characterized is big gastrin which has 34 amino acids and is, therefore, designated G-34 (3). It consists of an amino terminal pentadecapeptide linked by two Lys residues to the C-terminal biologically active heptadecapeptide amide (G-17) (3). By analogy with other hormones G-34 can be considered a biosynthetic precursor of G-17. G-34 predominates in serum of patients with gastrinoma (5) and in normal-fed subjects (6), whereas G-17 is the major form extracted from antral mucosa and gastrinoma tissue (3). A larger form of gastrin immunoreactivity, eluting in the void volume on Sephadex G-50 chromatography and called big-big gastrin, has been found to predominate in normal fasting serum (7). Small amounts of the C-terminal tridecapeptide of G-34 (G-13) also have been found in tissues and in serum (4, 8). The C-terminal tetrapeptide amide sequence of all these molecular forms is identical; since this sequence contains the residues necessary for full biological activity (9), it is not surprising that all the molecular forms described above are biologically active. Based on circulating molar concentrations required for a given response, G-17 is about 5 times more potent than G-34 and about 2.5 times more potent than G-13 as a stimulant of acid secretion in dogs (10, 11). Sulfated and nonsulfated forms appear to have equal biological activity. However, the half-life of G-34 is about 5 times greater than that of G-17 and the plateau blood levels are correspondingly greater after infusion of G-34 than after infusion of G-17. Thus, when exogenous doses of these gastrins are compared, G-17 and G-34 are nearly equipotent (10, 11). Because of these differences in biological activity, the factors governing the relative concentrations of these different forms of gastrin in the circulation are of particular physiological interest.

We report here on the relationships between G-34 and G-17 concentrations in antral mucosa and blood of dogs during stimulation of antral gastrin release.

Methods. Four dogs weighing 20–25 kg were prepared with denervated antral pouches and gastric fistulas. Experiments were begun at least 3 wk after operations. Two dogs were studied on two occasions and the other two on a single occasion. Gastrin release was stimulated by instillation of liver extract (15% w/v) into the antral pouch under 30 cm pressure. Gastric secretion was measured continuously from the gastric fistula. Biopsies of antral mucosa were obtained by means of a suction biopsy instrument before and 3 hr after continuous stimulation of the antral pouch. Serum samples of peripheral venous blood were obtained before stimulation, and 5 min and 3 hr after instillation of liver extract. Biopsy specimens were boiled immediately in water for 3 min (10 mg tissue/ml), centrifuged (2000*g* for 5 min), and the supernatant stored. The molecular forms of gastrin in serum and in biopsy extracts were separated on Sephadex G-50 superfine columns (1 × 100 cm). Gastrin in the column eluates was measured by radioimmunoassay by use of antisera with specificity for the C-terminal region of G-17 (12). Concentrations of G-17 and G-34 were estimated by use of pure human G-17-I and G-34-I standards, and applied to the

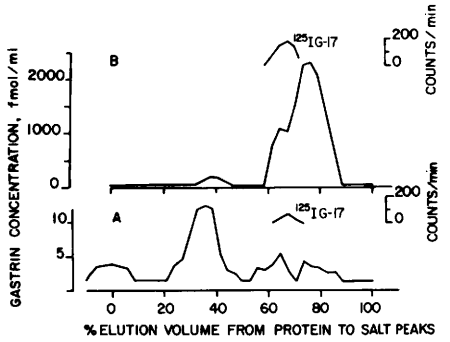

FIG. 1. A. Sephadex G-50 elution profile of serum taken after 5 min stimulation of the antral pouch with liver extract. The sample (1.0 ml) was applied to the column with 1000 cpm monoiodo ^{125}I G-17. Fraction number is expressed as percentage elution volume from protein peak (determined from absorbance at 280 nm) to salt peak (determined from conductivity). Column: 1 × 100 cm; buffer: 0.02 *M* sodium barbital, pH 8.4, containing 0.2 g/liter sodium azide; flow rate: 6 ml/hr; fraction volume: 1 ml. B. Sephadex G-50 elution profile of an extract of antral biopsy taken immediately before stimulation of antral pouch with liver extract. The sample corresponded to 1.0 mg tissue. Column conditions as in A.

peaks eluting in the same regions as standard G-34 and G-17 used to calibrate the Sephadex columns.

Results. Minor peaks of immunoreactivity were identified in eluates of serum which emerged in the void volumes of the columns and corresponded to "big big gastrin" (BBG) (Fig. 1) (7). The apparent concentration of BBG did not change during stimulation. In all four dogs, BBG was present in the fasting serum specimen. In two dogs it was the only immunoreactive component identified in the fasting state, while the other two dogs also had small peaks (less than 10 fmoles/ml) corresponding to G-34 and G-17 in the fasting serum. The biological significance and chemical nature of BBG are unknown. We have found that the binding of labeled G-17 to the antibody (1296) used in this study is inhibited by concentrations of purified bovine or human serum albumin similar to those present in serum. Thus, some of the BBG activity in dog serum found under our conditions of measurement can be explained by nonspecific effects of protein which elutes in the void volume of Sephadex G-50 columns.

Figure 1 shows that 5 min after instillation of liver extract into the antral pouch, the predominant form of gastrin in the serum had gel filtration properties of G-34 (35–45% elution volume). There were also two minor peaks (elution volumes 60–66 and 70–77%) which eluted from the Sephadex column on either side of a marker of ^{125}I G-17. Pure canine gastrins were not available for calibration, but the two minor peaks had elution volumes similar to human G-17-II and G-17-I and, therefore, were compatible with the corresponding hectadecapeptides previously isolated from extracts of dog antral mucosa (13). After 3-hr stimulation, G-17 concentration increased 21% over the 5-min value and G-34, 76% (Table I). Assuming steady-state release of antral gastrin during stimulation, this greater increase of G-34 at the later time can be explained by its longer half-life (15 min) compared with G-17 (3 min) and thus the greater time required for G-34 to reach equilibrium.

Two main peaks of immunoreactive gastrin were identified in biopsy extracts corresponding to G-17-II and G-17-I. In addition, there was a minor peak of gastrin immunoreactivity in the region typical for G-34 (6, 8, 12). There was no alteration in the relative concentrations of G-34 and G-17 in tissue after stimulation of the antral pouch. A small decrease in total immunoreactive gastrin activity after stimulation was not statistically significant (Table I).

Discussion. Assuming that G-17 and G-34 are released into the blood in the same ratio as they occur in antral mucosal extracts, it is possible to predict their relative concentrations in serum during steady stimulation by application of the known ratio of half-lives (10) or clearance rates. The ratio predicted differs from observed values in that the proportion of circulating G-17 at 3 hr is only about half as great as expected (Table I). The discrepancy between observation and prediction is greater at 5 min than at 3 hr. A similar discrepancy exists between observed ratios of G-17 and G-34 in blood of gastrinoma patients and those predicted from tumor ratios (14). Three suggestions have been discussed which might account for these discrepancies (14): (1) ultrarapid conversion of G-34 to G-17 during tissue extraction; (2) differences in the half-lives of

TABLE I. CONCENTRATIONS OF G-34 AND G-17 IN SERUM AND ANTRAL MUCOSAL BIOPSIES OF ANTRAL POUCH DOGS BEFORE, DURING, AND AFTER STIMULATION OF ANTRAL GASTRIN RELEASE BY LIVER EXTRACT. PREDICTED VALUES IN SERUM WERE ESTIMATED FROM ANTRAL MOLAR RATIOS OF G-17/G-34 AND HALF-LIVES OF G-17 (3 MIN) AND G-34 (15 MIN). MEAN ± SEM; SIX EXPERIMENTS, FOUR DOGS.

		Stimulated	
	Basal	5 min	3 hr
Antrum			
Total gastrin immunoreactivity (nmoles/g)	10.6 ± 2.7		8.4 ± 2.2
Relative concentrations:			
G-17	0.89		0.90
G-34	0.11		0.10
Serum			
Total gastrin immunoreactivity (fmol/ml)	15.0 ± 3.0	110 ± 12	164 ± 18
Concentrations (fmoles/ml):			
G-17		38 ± 7.0	46 ± 7.0
G-34		58 ± 13	102 ± 15
Relative concentrations			
G-17 observed		0.39	0.31
G-17 predicted		0.85	0.64
G-34 observed		0.61	0.69
G-34 predicted		0.15	0.36

exogenously administered and endogenously released hormones; (3) nonparallelism of storage ratios and secretion ratios of G-34 and G-17 of antral mucosa. To these may be added a fourth possible explanation which is that G-17 but not G-34 may be selectively removed by the liver. It has recently been reported that in dogs with portocaval transposition acid responses to G-17 were lower when it was infused by way of the portal vein (i.e., into a hind leg vein) than when it was infused into a systemic vein (front leg vein) (15).

Since for a given molar serum concentration G-17 is about five times more bioactive than G-34 (10), and since the molar ratio of G-17 to G-34 at 3 hr was about 1 to 2, G-17 would be contributing about 5/7ths (71%) of the bioactivity even though it represented only about one third of the total molar concentration of the two gastrins. The serum concentration of G-17 at 3 hr expressed as a multiple of its D50 (dose needed for half-maximal response) was about 1.7 whereas that of G-34 was about 0.7. The sum of these two bioactivities, about 2.4 D50 units, would be expected to give about 71% of the maximal response based on Michaelis–Menten kinetics. This prediction agrees reasonably well with observation. Thus, the highest acid response from the gastric fistula recorded during antral stimulation was 3.9 meq/10 min and was about 66% of the maximal response to pentagastrin, 5.9 meq/10 min.

The dose of exogenous G-17 that would be required to give a plateau serum level like that observed at 3 hr during endogenous release by liver extract (46 fmoles/ml) is about 150 pmoles/kg-hr (10). Taking this as an estimate of the rate of release of G-17 from the antrum, during 3 hr 450 pmoles/kg would be released or 9000 pmoles or 9 nmoles for a 20-kg dog. Assuming that the antral mucosa of a dog weighs about 10 g, the total content of G-17 in the resting antrum was about 100 nmoles. Thus the expected loss during the 3 hr would be only about 10% of the original total and it is unlikely that this degree of change could be accurately measured. It is, therefore, not surprising that no significant change in total antral gastrin content was found.

Summary. Instillation of liver extract into antral pouches produced an increase in the serum concentrations of both little (G-17)

and big (G-34) gastrins. The molar fraction of G-17 plus G-34 represented by G-17 was about 0.9 in antral mucosa and about 0.3 in serum 3 hr after initiating release with liver extract. The predominance of G-34 in serum can be accounted for only in part by its slower rate of removal from the blood so other factors probably also contribute. Although G-17 contributed only about 30% of the total molar concentration of gastrins in serum, it accounted for about 70% of the acid stimulatory activity because on a molar basis it is about five times more bioactive than G-34.

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