Comparison of the Effects of Human and Porcine Gastrin on Isolated Gastric Mucosa of the Bullfrog* (33521)

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Porcine gastrin and its synthetic analogues are potent stimulants of HCl secretion in the intact animal (1) and isolated gastric mucosa (2, 3). It has been shown that all of the physiological activities of gastrin, a heptadecapeptide, reside in the *C*-terminal tetrapeptide, *Try·Met·Asp·Phe·NH*$_2$ (1). Recently, human gastrin has been isolated, characterized and synthesized (4–6). It differs from porcine gastrin only in a single amino acid substitution (*leucine* in place of *methionine*) in the apparently nonessential portion of the molecule (5) (Table I).

The purpose of this paper is to report the action of synthetic human gastrin on isolated bullfrog gastric mucosa, and to compare its activity to that of porcine gastrin and pentagastrin, a synthetic gastrin-like pentapeptide (Table I).

Materials and Methods. Fasted bullfrogs (*Rana catesbiana*) were stored in running tap water at room temperature. Frogs were killed by decapitation and pithing. The gastric mucosa was separated from the muscular layers of the stomach and mounted in an incubation chamber. The serosal side was bathed with an amphibian bicarbonate Ringer's solution, and the secretory or mucosal side was bathed with the same solution containing an isosmotic quantity of NaCl in place of $NaHCO_3$. The serosal side was gassed with 95% O_2, 5% CO_2 and the secretory side with 100% O_2. Acid formed on the secretory side was titrated continuously and automatically to pH 5.5 with 0.1 *N* NaOH using a Radiometer pH meter and autoburet. All incubations were carried out at room temperature, 21–25°. These studies were performed in August and September when frog secretory responses were optimal. Porcine gastrin was prepared by the method of Gregory and Tracy (7) except that the final product was not separated into gastrins I and II. Synthetic human gastrin was purchased from the American Gastroenterological Association Gastrin Committee (Dr. C. F. Code, Chairman) and from the Imperial Chemical Industries, Ltd., London. Pentagastrin was a gift from Dr. J. D. Fitzgerald, Imperial Chemical Industries. The hormones, dissolved in 0.7% NaCl solution made slightly alkaline with NH_4OH, were added to the serosal solution bathing the gastric mucosa following a 1.5–3 hr period of spontaneous or basal acid secretion.

Results. The response of a typical mucosa to human gastrin and pentagastrin is shown in Fig. 1. Following addition of human gastrin or pentagastrin, peak rates of acid secretion were observed in 30–45 min with a slow decline thereafter. Washing the mucosa by exchanging the serosal solution returned the rate of secretion to basal levels so that the responses to several doses of hormone could be compared in the same mucosa.

Dose response curves for human gastrin, porcine gastrin, and pentagastrin are shown in Fig. 2. The secretory responses are expressed as a ratio of the peak stimulated rate to the spontaneous or basal secretory rate just prior to the addition of the stimulant. Although the dose response curves are not strictly parallel, visual inspection and comparison of the nearly linear portions of curves indicate that porcine gastrin is about 100 times more potent than pentagastrin and about 200 times more potent than human

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TABLE I. Structure of Porcine and Human Gastrin and Pentagastrin.[a]

Porcine gastrin I

$\sqsubset$Glu–Gly–Pro–Try–Met–(Glu)$_5$–Ala–Tyr–Gly–*Try.Met.Asp.Phe.–NH$_2$*

Human gastrin I

$\sqsubset$Glu–Gly–Pro–Try–Leu–(Glu)$_5$–Ala–Tyr–Gly–*Try.Met.Asp.Phe.–NH$_2$*

Pentagastrin

t-butyloxycarbonyl-β-Ala.*Try.Met.Asp.Phe.–NH$_2$*

[a] Gastrin II differs from gastrin I only in the substitution of tyrosylester sulfate in place of tyrosine. This substitution does not alter the physiological properties of the molecule (7). The italicized *C*-terminal tetrapeptide has been shown to possess all of the actions of the entire molecule.

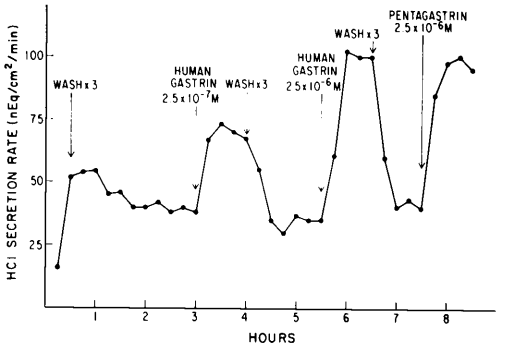

FIG. 1. Response of bullfrog gastric mucosa to two doses of human gastrin and one dose of pentagastrin.

gastrin on a molar basis. The dose–response curves for pentagastrin and human gastrin are very nearly parallel. Pentagastrin is about twice as potent as human gastrin on a molar basis.

The maximal secretory response was achieved with 2.5×10^{-6} *M* porcine gastrin, 2.5×10^{-6} *M* pentagastrin, and 2.5×10^{-6} *M* human gastrin. Doses of human gastrin greater than 2.5×10^{-6} *M* were precluded by the limited supply of this synthetic hormone.

Discussion. Detailed dose response curves comparing human and porcine gastrin have recently been described in studies of gastric fistula dogs (8). In these animals, porcine gastrin was approximately twice as potent as human gastrin on a molar basis. In our studies, the observation that porcine gastrin is about 200 times more potent than human gastrin was an unexpected finding. This difference in molar potency is caused by a single amino acid substitution in the portion of the bastrin molecule which is not essential for acid stimulation. We conclude from these findings that minor changes in what was previously considered to be the nonessential portion of the gastrin molecule may greatly change activity, perhaps by altering association with or binding to oxyntic cell receptor sites.

Summary. Synthetic human gastrin stimu-

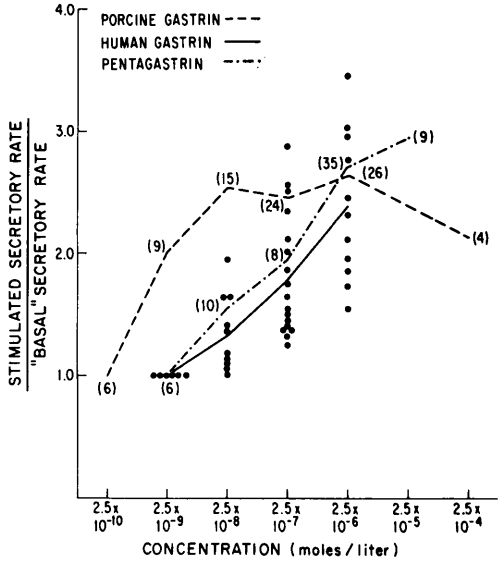

FIG. 2. Response of 78 mucosae to 45 doses of human gastrin, 84 doses of porcine gastrin, and 68 doses of pentagastrin. Each dot represents an individual response to human gastrin at the indicated dose level; the continuous line connects the mean responses at each dose level; numbers in parentheses indicate the number of doses of pentagastrin or porcine gastrin.

lates HCl secretion by the isolated gastric mucosa of the bullfrog. On a molar basis, human gastrin is about ½ as potent as pentagastrin and about 1/200 as potent as porcine gastrin. Maximal secretory response to human gastrin, pentagastrin, and porcine gastrin occurred at 2.5×10^{-6}, 2.5×10^{-6} or 2.5×10^{-5}M, and 2.5×10^{-8} *M*, respectively.

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Retardation of the Emergence of Isoniazid-Resistant Mycobacteria by Phenothiazines and Quinacrine (33522)

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Sevag and his co-workers (1–5) have reported that Atabrine (quinacrine hydrochloride) in combination with an antibiotic prevents the emergence of antibiotic-resistant strains of bacteria from a normal population. They have described the use of such combinations in the prevention of emergence of antibiotic-resistant populations of *Staphylococcus aureus* and *Escherichia coli*. When they tested an antibiotic-resistant organism, they found the quinacrine–antibiotic combination to be ineffective in delaying the onset of growth.

Warren *et al.* (5) have found that with isoniazid-sensitive strains of *Mycobacterium tuberculosis* and *M. bovis* resistance to isoniazid (INH) is much less apparent in the course of six subcultures if sublethal concentrations of quinacrine are added. They have also found that quinacrine in combination with dihydrostreptomycin sulfate or para-aminosalicylic acid (PAS) eliminated the emergence of resistance to these antituberculous drugs.

In the light of these investigations, it was thought that the measurement of the effect of various heteroanthracenes on the growth rates of various mycobacteria in the presence of marginally or minimally inhibitory amounts of INH would be worthwhile. Comparison of the effects of various heteroanthracenes to those of PAS, ethionamide, and ethambutol on development of resistance to INH was made. The following report compares the various systems as measured photometrically.

Materials and Methods. M. tuberculosis strain $H_{37}Ra$ and *M. intracellulare* strain P-55 ("Battey" mycobacterium, Runyon Group III) were grown in Middlebrook 7H9 broth (Difco) enriched with oleate–dextrose–albumin complex (Difco) and with 0.5% Tween 80 (Atlas Chemical Co.) for dispersed growth. Test organisms were grown on slants of Lowenstein-Jensen medium and were then inoculated into flasks of 7H9 broth. Each tube of broth (9.5 ml) used in the test was then inoculated with 0.5 ml of a 72–96 hr