Action of Pancreozymin–Cholecystokinin on the Isolated Gastric Mucosa of the Bullfrog* (33405)

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Gastrin and its synthetic analogues are potent stimulants of acid secretion in the intact animal (1) and isolated gastric mucosa (2, 3). Gastrin and its analogues are also known to possess a pancreozymin-like activity in the intact animal (4). All of the physiological actions of gastrin, a heptadecapeptide, reside in the C-terminal tetrapeptide (Trp–Met–Asp–Phe–NH_2) of the gastrin molecule (4). Recently Mutt and Jorpes (5) have isolated and partially characterized the intestinal hormone pancreozymin–cholecystokinin (PZ-CCK). It shares the same C-terminal dipeptide as gastrin and most probably the same C-terminal pentapeptide (Table I). Because of this similarity of structure, we have attempted to determine whether PZ-CCK possesses acid-stimulating properties in the isolated gastric mucosa of the bullfrog and to compare its activity to that of gastrin and its synthetic analogue, pentagastrin.

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 instead 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 (2, 3). All incubations were carried out at room temperature, 21–25°. These studies were performed during the months of June to November when secretory responses of isolated bullfrog mucosa were optimal. Porcine gastrin was isolated in pure form from hog antral mucosa (6). Pentagastrin was a gift from Dr. J. D. Fitzgerald, Imperial Chemical Industries. Porcine PZ-CCK was purchased from the Gastroenterology Research Unit, Karolinska Institute, Stockholm, Sweden. The potency of the PZ-CCK was 1500 Ivy dog units of cholecystokinin or 6000 Crick, Harper, Raper units of pancreozymin/mg. Pure PZ-CCK has a potency of 3000 Ivy dog units or 12,000 Crick, Harper, Raper units/mg (7). The hormones, dissolved in 0.7% NaCl made slightly alkaline with NH_4OH, were added to the serosal solution bathing the gastric mucosa following a 1.5 to 3-hr period of spontaneous or basal secretion.

Results. The response of a typical mucosa to PZ-CCK and pentagastrin in shown in Fig. 1. Following addition of pentagastrin or PZ-CCK 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 two or more doses of hormone could be compared in the same mucosa.

Dose response curves for porcine gastrin, pentagastrin, and PZ-CCK 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. For purposes of calculation, pure PZ-CCK is assumed to have an activity of 3000 Ivy dog units and 12,000 Crick, Harper, Raper units/mg and a molecular weight of 4000 (5, 7). Although the dose response curves are not strictly parallel, visual inspection and comparison of the

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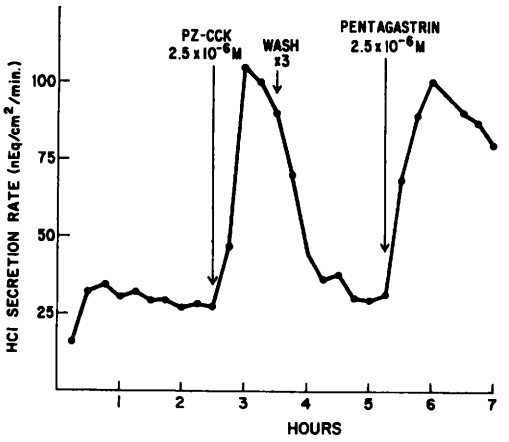

FIG 1. Response of a bullfrog gastric mucosa to PZ-CCK and pentagastrin.

nearly linear portions of the curves indicate that porcine gastrin is about 25–35 times as potent as porcine PZ-CCK, and PZ-CCK is about 2–3 times as potent as pentagastrin on a molar basis. The maximal secretory response to porcine gastrin, pentagastrin, and PZ-CCK was achieved at 2.5×10^{-8}, 2.5×10^{-6}, and 2.5×10^{-6} M, respectively.

Since the preparation of PZ-CCK used in this study was only 50% pure, the possibility of contamination by gastrin was considered. The PZ-CCK was found to contain 0.026% (by wt.) immunoassayable gastrin activity as measured by a specific radioimmunoassay (8). This small amount of gastrin activity could not account for the acid stimulating

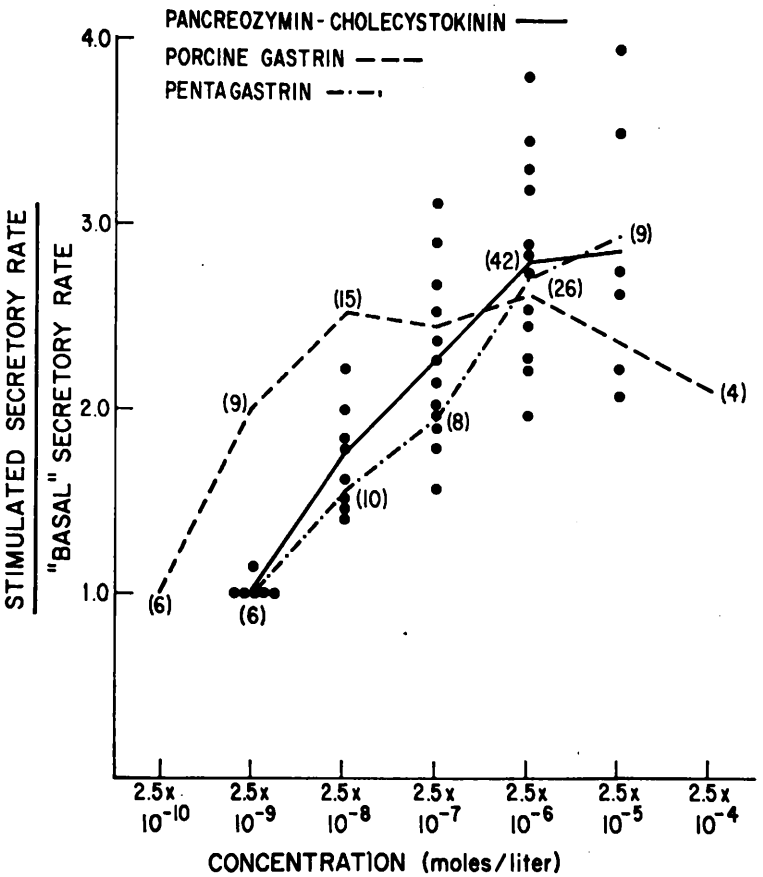

FIG. 2. Response of 88 mucosae to 84 doses of porcine gastrin, 34 doses of PZ-CCK and 75 doses of pentagastrin: Each point represents an individual response to PZ-CCK at the indicated dose level; (—), the mean responses at each dose level; nos. in parentheses indicate the no. of responses to porcine gastrin and pentagastrin at each dose level.

TABLE I. Structure of Porcine Gastrin, Pentagastrin, and Porcine PZ-CCK.*

Porcine gastrin

Glu–Gly–Pro–Trp–Met–Glu–Glu–Glu–Glu–Glu–Ala–Tyr–Gly–Trp–Met–Asp–Phe–NH_2

Pentagastrin

t-butyloxycarbonyl-β-Ala–Trp–Met–Asp–Phe–NH_2

Porcine pancreozymin-cholecystokinin

Lys–(Ala, Gly, Pro, Ser)–Arg–(Arg$_2$, Asp$_4$, Glu$_2$, His, Ile$_2$, Leu$_2$, Lys$_1$, Met$_2$, Pro, Ser$_3$, Tyr, Val)–*Gly–Trp–Met*–Asp–Phe–NH_2.

* The sequence of amino acids enclosed in parentheses has not been established. The position of the italicized tripeptide Gly–Trp–Met in PZ-CCK has been established only tentatively (5).

properties of the PZ-CCK preparation.

Discussion. It has been shown previously that PZ-CCK is a stimulant of acid secretion in humans (9) and in dogs with a denervated gastric pouch (10, 11) or gastric fistula (11). Additionally, the rapid intravenous administration of large doses of PZ-CCK inhibits acid secretion in dogs (10, 12). Gastrin and its synthetic analogues also possess this property of inhibiting gastric secretion when given intravenously in large doses (4). Recently caerulein, a decapeptide found in the skin of certain Australian and South American toads, has been isolated and characterized (13). It has been shown to possess gastrin, pancreozymin, and cholecystokinin activities in the intact animal and isolated gastric mucosa (14). Interestingly, this peptide shares the same C-terminal pentapeptide as gastrin and PZ-CCK (13). Our study indicates that PZ-CCK is a potent stimulant of acid secretion in the isolated gastric mucosa of the bullfrog. The similarity of action of gastrin and PZ-CCK is probably related to the identical amino acid sequence shared by both hormones.

Summary. Pancreozymin–cholecystokinin stimulates acid secretion by the isolated gastric mucosa of the bullfrog. On a molar basis it is 2–3 times as potent as pentagastrin; porcine gastrin is 25–35 times as potent as pancreozymin–cholecystokinin. The maximal secretory responses to porcine gastrin, pentagastrin, and pancreozymin–cholecystokinin were achieved at 2.5×10^{-8}, 2.5×10^{-6}, and 2.5×10^{-6} *M*, respectively.

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