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Gastric Inhibitory Polypeptide and Insulin Secretion after Infusion of Acetylcholine, Catecholamines, and Gut Hormones[1] (40719)

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In some patients with mild adult-onset diabetes oral glucose produces hypernormal increments in plasma immunoreactive insulin (1) or a delayed but prolonged rise of insulin (2), which sometimes causes reactive hypoglycemia. These and other observations emphasize the importance of characterizing factors that influence insulin release and the mechanisms involved. The nervous system, gut and pancreatic islets are key participants in modulating insulin secretion (3–9), and their functions are coordinated by hormonal and neuronal actions. Some of the messages are sent long distances via nerves (neuroendocrine) or the blood circulation (endocrine), whereas others act locally, exerting neurocrine or paracrine actions (4, 7–9). The autonomic nervous system transmits many afferent and efferent impulses through cholinergic, adrenergic, and peptidergic nerves (3–5). Pancreatic islets and the gut are richly innervated by these nerve fibers, which have terminal synapses closely associated with the endocrine cells of the gastroenteropancreatic system and with intramural nerves in the gut (5, 7–9).

Since insulin secretion is strongly stimulated by gastric inhibitory polypeptide (GIP) (6) we have investigated factors that might affect GIP secretion. Extensive studies have shown that oral glucose, triglyceride, amino acids, and a mixed meal increase GIP secretion (6, 10–13), but only a small number of hormones have been found to influence it. Because of many actions of acetylcholine in the gut and because atropine inhibits glucose-induced GIP rise (14), we have investigated in dogs the effect of intravenous infusion of acetylcholine, given alone or with oral glucose or corn oil. Also, since adrenergens exert potent actions on the islets and gut, and have many coordinated interactions with cholinergic functions (3–5), we investigated epinephrine, with and without adrenergic blocking compounds. We included serotonin and vasoactive inhibitory polypeptide (VIP), because of their large concentration in the gut and pancreas and their effect on insulin secretion (15–18). Although pancreatic polypeptide (PP) has not been reported to affect insulin secretion, its plasma concentration increases rapidly with food ingestion, and PP tends to counteract gastrin, cholecystokinin, and secretin actions (19). Therefore, we investigated the possibility that it might affect GIP secretion. Because GIP inhibits gastrin-stimulated acid secretion (6) and because of the frequent reciprocal interactions of gut hormones, we included gastrin in our studies, using a different experimental design from one showing that gastrin augments glucose-induced increase in plasma GIP (20).

In summary, we determined the effect of infusion of acetylcholine, epinephrine, serotonin, PP, gastrin, and VIP upon plasma levels of GIP, insulin, glucose, and glucagon. All of these hormones have many common characteristics. Each is secreted by cells of ectoblastic origin (21)—APUD (Amine Precursor Uptake Decarboxylation) cells. Each is in nerve terminals and/or specific endocrine cells in the gut and/or pancreas (3–5, 7–9, 17–19, 21, 22), and most of them are released by food (3, 4, 6, 15, 17, 19, 23–25). We also tested iso-

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proterenol because of its beta adrenergic action.

Materials and methods. Supplies. We were kindly supplied glucagon antibody (30K) by Dr. Rober Unger, Veterans Hospital, Dallas; GIP antibody (RO2) by Drs. John Brown, University of British Columbia, and Werner Creutzfeldt, University of Gottingen; GIP by Dr. John Brown; synthetic porcine secretin and hepadecapeptide gastrin (G-17-1) by Dr. Morton Grossman, Veterans Hospital, Los Angeles; bovine PP by Dr. Ronald Chance, Lilly Laboratories, Indianapolis; VIP by Dr. Sami Said, Veterans Hospital, Dallas. Benzamidine was obtained from Aldrich Chemical Company, Milwaukee, Wisc. The other compounds were high-grade, readily available commercial preparations.

Dogs. Purebred female foxhounds were obtained from the same breeding supplier and were kept in good health (examined weekly by veterinarian). Five dogs weighing 17 to 21 kg were used in each experiment, but each was given a rest for at least 3 days between experiments.

Collection of blood specimens. After a 16-hr fast the dogs were kept in a Pavlov jacket for 20 min before receiving a test compound. They were in a relatively relaxed state. Blood specimens were drawn through a 20-gauge plastic cannula inserted into the cephalic vein of the leg opposite the one for infusion of test compounds. The cannula was kept patent by continuous slow infusion of 0.9% saline. Two baseline specimens were drawn 5 min apart and then, following initiation of the infusion of the test compound, every 5–15 min for the first hour, and 5–30 min thereafter. Experiments with corn oil were for 150 min, but others were shorter. Aliquots for assays for GIP, insulin, and glucose were placed in EDTA, and those for glucagon were put in heparin and 0.05 *M* benzamidine. All tubes were submerged in ice immediately. Plasma for glucagon assays were removed within an hour, but for other assays as much as 2 hr elapsed before removal of plasma. All plasma was stored at −70°C until assayed.

Administration of test compounds. All glucose, 1 g/kg, or corn oil, 1 ml/kg, had water added to a total volume of 200 ml and was put into the stomach by gastric tube; in control experiments water, 200 ml, was given likewise. These solutions were given within 2 min. In most experiments with glucose administration [^{14}C]glucose (5 μCi) was included with the glucose, in order to gain a coarse index of the net time required for gastric emptying, intestinal transit, and absorption of glucose. Each of the hormone infusions was given at a constant rate with a Harvard pump through an indwelling plastic cannula in the cephalic vein. Propranolol and phentolamine were infused in a similar manner. Details regarding the dosages of the hormones, timing of infusion, and other aspects are presented in the legends to the Figures; those for VIP and serotonin, given without glucose, are described under Results.

Assays. Glucose was assayed by the glucose oxidase method, using the Beckman analyzer. GIP, insulin, and glucagon were measured by radioimmunoassay, using the respective methods of Kuzio *et al.* (26), Morgan and Lazarow (27), and Ensinck *et al.* (28). With the GIP antibodies used there was no significant cross-reactivity by secretin, PP, neurotensin, substance P, motilin, VIP, growth hormone or gastrin, using 10 ng/ml. Insulin, glucagon and GIP reacted significantly only with its respective antibody. All assays were run in duplicate. The lower limit of sensitivity for the GIP assay was 125 pg/ml. All of the specimens assayed had values significantly higher than this. Intraassay variation was 9% and interassay variation was 20%.

Presentation of data. In the figures are presented the mean absolute values (±SEM) for glucose, but for GIP the mean of the fasting values for each dog is calculated and the mean difference (±SEM) from this zero point is plotted for each time point. The data for insulin and glucagon are presented likewise. This was done to reconcile the interanimal variations in baseline levels of hormones. Mean baseline levels (±SEM) in 10 experiments were GIP 455 ± 41 (pg/ml):, insulin 7 ± 1, and glucagon 40 ± 5. The statistical significance of the responses was determined, by a paired Student's *t* test, on the natural logarithm between baseline and response levels in

each dog. Logarithmic transformation was performed to approximate better the Gaussian distribution required for this parametric statistical analysis. Confidence intervals for the difference between treatments, calculated by the technique of spherical exchangeability, were used to determine significant changes in GIP levels (29). Except for differences that are obviously large, symbols are used in the Figs to signify differences with $P < 0.05$, or less.

Results. Acetylcholine alone and with glucose or corn oil. Acetylcholine (Ach) did not alter the baseline level of GIP, glucose, or glucagon, but caused a slight transient rise in plasma insulin (Fig. 1). It inhibited the glucose-stimulated increase in GIP, but did not alter significantly the glucose effect on insulin, glucagon, or plasma glucose, except for slightly prolonging the elevation of glucose. The plasma ^{14}C (after oral [^{14}C]glucose) rose equally rapidly whether or not Ach was given with glucose. Ach slightly inhibited corn oil-stimulated rise in GIP, but given with corn oil plasma glucose or insulin levels were the same as with corn oil alone. However, Ach plus corn oil caused an immediate slight increment in

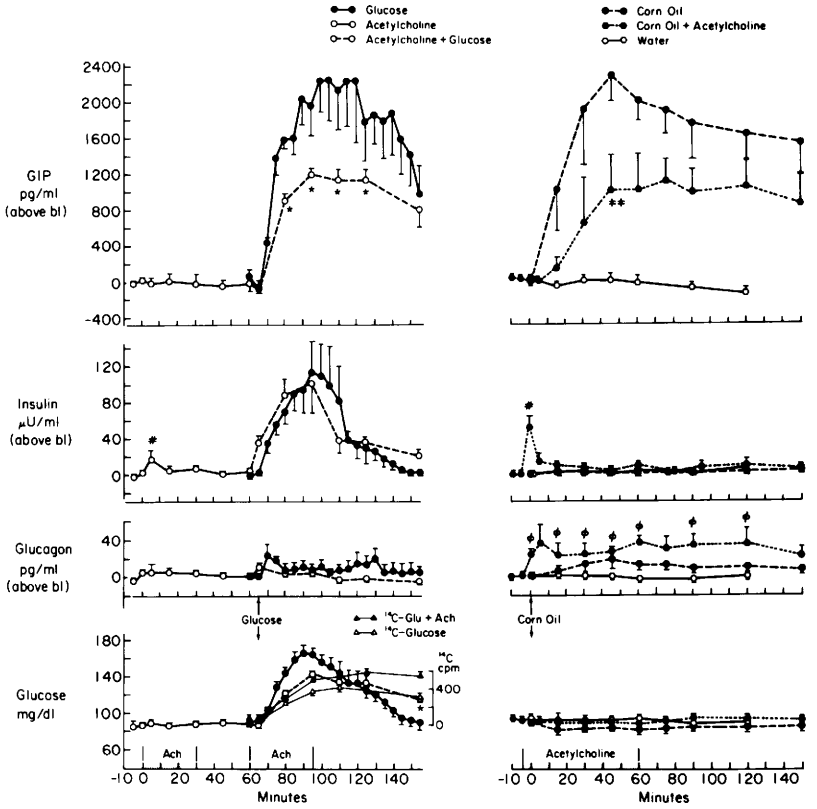

FIG. 1. After obtaining baseline blood specimens 5 min apart, test compounds were given in the following five experimental patterns: (a) glucose plus [^{14}C]glucose, (b) corn oil, (c) water, (d) acetylcholine, 8.6 μg/kg/min, infused for 30 min, stopped for 30 min, and then infused for 35 min. Glucose and [^{14}C]glucose were given 5 min after the second infusion was started. (e) Acetylcholine, 8.6 μg/kg/min, was infused for 65 min. Five minutes after starting this infusion, corn oil was given. All glucose, 1 g/kg, and [^{14}C]glucose, 5 mCi, were given at 65 min. Corn oil, 1 ml/kg, or water was given at time 0. The mean absolute values for glucose are shown, but for GIP, insulin, and glucagon the fasting values are designated as zero and subsequent results as mean deviations from this point. Significant differences ($P < 0.05$): vs pretreatment baseline No.; glucose vs glucose plus Ach (*); crn oil vs corn oil plus Ach (‡); corn oil plus Ach vs water (Φ).

plasma glucagon, which persisted throughout most of the experiment.

Isoproterenol alone or with glucose or corn oil. Whereas isoproterenol alone did not alter GIP levels, it inhibited glucose-induced GIP rise at 15, 30, and 45 min ($P < 0.05$) (Fig. 2); it plus glucose caused a much larger rise in insulin and glucose than was produced by either compound alone. When [^{14}C]glucose was given with glucose plus isoproterenol the plasma levels of ^{14}C were similar to those obtained when it was given with glucose without isoproterenol (Fig. 2). Isoproterenol did not alter significantly the GIP increase produced by corn oil. With both given together there was less rise in plasma glucose than with isoproterenol alone ($P < 0.05$). The apogee for insulin rise was the same for isoproterenol alone as with it plus corn oil. Plasma glucagon was

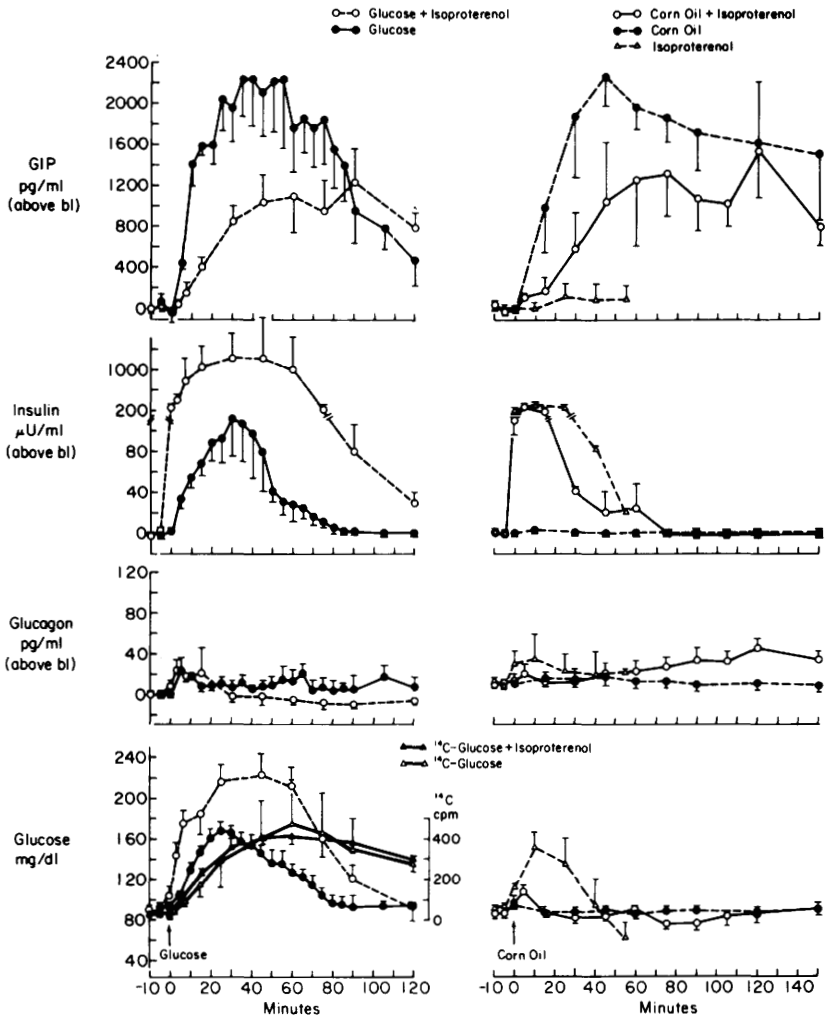

FIG. 2. After obtaining two baseline blood samples, 5 min apart, compounds were tested using five experimental patterns: (a) glucose, (b) corn oil, (c) isoproterenol, 0.1/μg/kg/min for 30 min, (d) isoproterenol for 65 min with glucose given 5 min after starting infusion, and (e) isoproterenol for 65 min, with corn oil given 5 min after starting the infusion. Each infusion of isoproterenol was started at −5 min. Each dose of glucose, 1 g/kg, and of corn oil, 1 ml/kg, was given by gastric tube at 0 time; [^{14}C]glucose (5 μCi) was given with the glucose.

increased slightly at 120 and 150 min ($P < 0.05$) when isoproterenol was combined with corn oil.

Epinephrine alone and epinephrine plus glucose, with or without the administration of propranolol or phentolamine. Epinephrine did not affect the basal GIP levels, but it inhibited glucose-induced increments in GIP ($P < 0.05$, paired t test). When epinephrine was given with either phentolamine or propranolol, glucose-induced GIP response was also inhibited (Fig. 3). Although epinephrine plus glucose caused much more hyperglycemia than did either compound alone, epinephrine markedly inhibited glucose-stimulated insulin secretion, with or without the addition of propranolol. However, when phentolamine was combined with epinephrine and glucose, most of the inhibitory effect of epinephrine was removed. In experiments with epinephrine plus glucose, the addition of propranolol or

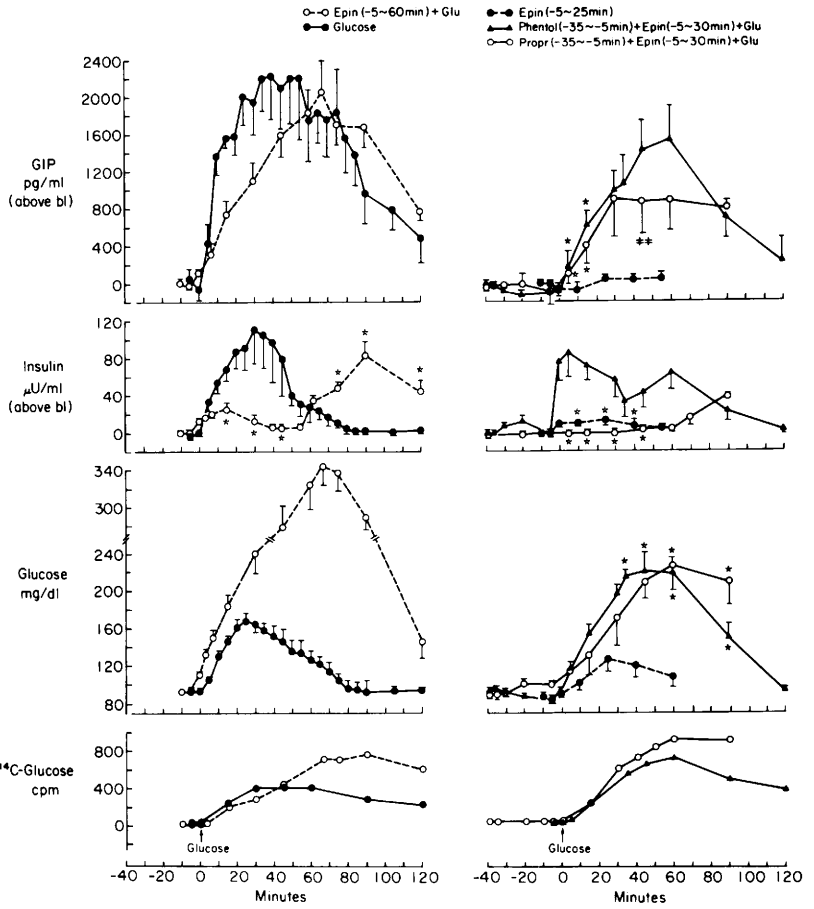

FIG. 3. After obtaining two baseline blood specimens 5 min apart, a series of four experiments was conducted testing the effect of epinephrine, infused at the rate of 0.3 μg/kg/min: (a) epinephrine alone for 30 min, (b) epinephrine for 65 min, with glucose given 5 min after starting epinephrine, (c) propranolol, 25 μg/kg/min for 30 min, and then epinephrine plus glucose as in (b), (d) same pattern as (c), with propranolol replaced by phentolamine, infusing 100 μg/kg as a bolus and then 10 μg/kg/min for 30 min. A fifth series consisted of glucose. All dogs given glucose received 1 g/kg by gastric tube at 0 time. [^{14}C]Glucose, 5 μCi, was included with glucose in the majority of the experiments. An asterisk signifies differences ($P < 0.05$) between results after glucose alone vs those after other compounds or combinations. ‡‡, Differences ($P < 0.05$) in GIP response to glucose plus epinephrine vs this combination plus propranolol.

phentolamine decreased the amount of hyperglycemia, but there was more of it than was found with glucose alone. The rate of rise of ^{14}C, after [^{14}C]glucose, was as fast with glucose plus epinephrine (with or without its blockers) as with glucose alone.

Glucose alone vs glucose plus either PP, gastrin, or VIP. With infusion of PP, gastrin, or VIP into dogs given oral glucose, changes in plasma GIP, insulin, glucose, and glucagon were comparable with those produced by glucose alone (Fig. 4), except VIP caused a very slight decrease in glucagon at 25 min ($P < 0.05$).

VIP, serotonin. In these experiments no glucose was given. After obtaining baseline blood specimens the following intravenous infusions were started at the times indicated and completed within 1 min: VIP, 1.25 μg/kg, at time 0; serotonin, 25 μg/kg, at 30

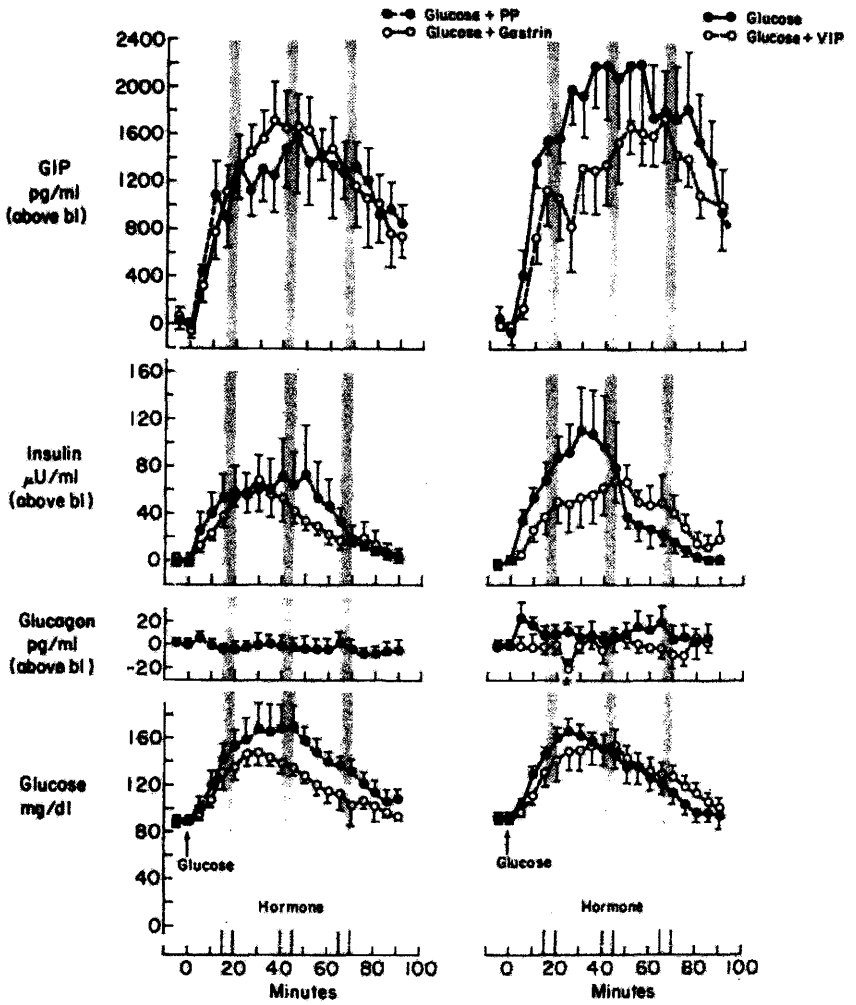

FIG. 4. The pattern for this four series of experiments was the same. After two baseline blood specimens were obtained, 5 min apart, glucose (1 g/kg) was given by gastric tube (time 0). One series consisted of only glucose administration while the other three series included intravenous infusion of the same hormone for each of three intervals: 15–20, 40–45, and 65–70 min. The amount of each hormone/kg/min was: gastrin, 600 ng; PP, 25 ng; and VIP, 25 ng. The mean ± SE for each time is plotted. An asterisk signifies different ($P < 0.05$) from effects of glucose alone. (Glucagon assays were not conducted in the gastrin experiments.)

min; and 250 μg/kg at 45 min. Blood specimens were obtained every 5 min for 80 min. Data are not presented because no new positive information was found. VIP alone caused an immediate brief increase in insulin, but no change in the levels of GIP, glucose, or glucagon. Neither dose of serotonin increased GIP, but the larger dose caused a small increase in glucagon and glucose.

Discussion. Except for PP, each hormone that we studied has been reported to modify the secretion of insulin in intact subjects (3, 4, 15–19, 30); most of these hormones also affect insulin secretion *in vitro* (3, 4, 16, 31–33). Part of their *in vivo* action conceivably could result from their effect on GIP secretion, but we found that none of them, when infused alone, changed fasting levels of GIP. However, glucose-induced GIP rise was inhibited by acetylcholine, isoproterenol and epinephrine alone or plus either propranolol or phentolamine, but not influenced by gastrin, VIP, or PP.

While catecholamines affect gastrointestinal secretion, mobility and blood flow (5), our observations of the rate of absorption of [^{14}C]glucose suggest that these factors may not have impaired significantly the net rate of glucose transport through the upper gut and into the plasma. Moreover, isoproterenol did not alter corn oil-induced GIP rise. The failure of relatively large doses of catecholamines alone to influence GIP secretion is in keeping with impressions that they do not affect directly the release of very many gastrointestinal hormones (5). However, they increase gastrin and somatostatin release (34, 35).

Conversely, acetylcholine and/or vagal stimulation increases the secretion of many gastroenteropancreatic hormones, including insulin, glucagon, gastrin, PP, VIP, substance P, catecholamine, and serotonin, but decreases somatostatin (3, 4, 19, 23, 24, 32, 33, 35–39). Whereas atropine blocks many of these effects, it does not block the vagal release of gastrin, VIP, or substance P (38). This apparently is because these hormones occur in peptidergic fibers (noncholinergic and nonadrenergic) which do not act through muscarinic receptors (5). Atropine decreases the insulin-releasing effect of oral glucose, but not intravenous glucose (40) suggesting that atropine might decrease the release of an insulin-stimulating gut hormone. Supporting this thesis are the studies of Larrimer *et al.* (14), who found that atropine decreases the GIP rise with glucose introduced into the duodenum. Sirinek *et al.* (20) reported that gastrin alone did not alter GIP levels, but augmented the GIP response to glucose. Their experimental design differed from our in ways that cause major differences in reactions; they gave glucose intraduodenally, and the gastrin infusion was started 30 min before glucose administration, and continued for 120 min thereafter.

Since acetylcholine, catecholamine, VIP, serotonin, gastrin, and PP are found in gut nerves, and gastrin, VIP, serotonin, and PP in gut endocrine cells (21, 22, 38), there is a possibility that each one, with their endogenous release, exerts chiefly a neurocrine and/or paracrine action (5, 7–9, 15–19, 21, 23–25, 30, 33–35, 37, 38, 41), thereby increasing the specificity and intensity of their reactions. Therefore, it is conceivable that significant effects on GIP secretion could result from changes in their concentration in the gut. However, our systemic administration of the hormones gives them an endocrine rather than a paracrine or neurocrine role, and this could possibly account for our not showing more effect on GIP secretion. Likewise, this possibly could explain why we have not observed changes in plasma GIP after systemic infusion of secretin, neurotensin, substance P, and bombesin, or cholecystokinin (unpublished). Similarly, food substances when given orally can affect markedly GIP secretion, but not when given intravenously, suggesting a need for a high concentration near the GIP-secreting cells.

Summary. In studies pertinent to pathophysiologic changes in diabetes and reactive hypoglycemia, we have investigated, in dogs, the effect of gastroenteropancreatic hormones upon gastric inhibitory polypeptide (GIP), since it is a potent insulin-releasing hormone. Intravenous infusion of pharmacologic doses of vasoactive inhibitory polypeptide (VIP), acetyl-

choline, isoproterenol, epinephrine (with or without phentolamine or propranolol), serotonin, or pancreatic polypeptide (PP) did not change fasting plasma GIP levels, despite the fact that several of them increased plasma insulin. However, glucose-induced increase in GIP was inhibited by acetylcholine, iosproterenol, and epinephrine (alone or plus phentolamine or plus propranolol), but was not affected by gastrin, VIP, or PP. Corn oil-stimulated GIP rise was inhibited by acetylcholine but not by isoproterenol. By infusing the hormones systemically we were testing an endocrine effect, but their endogenous release in the gut would conceivably promote neurocrinic and /or paracrinic actions. Their release in close proximity to the K cells (GIP-producing) would presumably provide higher concentrations and stronger actions on these cells.

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