GASTROINTESTINAL FUNCTION

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REGULATION AND DISTURBANCES
VOLUME 7

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Editors:

Fusahiro Nagao
Department of Surgery
The Jikei University School of Medicine, Tokyo

Yutaka Matsuo
Department of Internal Medicine
Nihon University School of Medicine, Tokyo

Yutaka Kasuya
Department of Pharmacology
Hoshi University, Tokyo

Masaharu Tsuchiya
Department of Internal Medicine
School of Medicine, Keio University, Tokyo

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Preface

The symposia on the Regulation and Disturbances of Gastrointestinal Function form a series of study meetings at which the functions of the digestive tract are examined in four areas: secretion, motility, blood flow, and inflammation. There have been some remarkable developments in research in this field through clinical investigations of the physiological significance of different regions of the digestive tract and of manifestations of the various diseases affecting it. For this, the 7th symposium, experts in each of the 4 fields were present, so that the topics discussed were extremely interesting, and the overall content was most valuable. Among the presentations, several results that could almost be termed sensational were reported, and as a result, the question-and-answer sessions were very lively.

Whenever one of the symposia in this series is held, well-known researchers from overseas are invited to lecture on aspects of their current work. On this occasion, Professor John C. Brown, of the University of British Columbia, Vancouver, a distinguished expert in the field of GIP and motilin, gave the Special Lecture. The audience heard a detailed account of the mechanisms of secretion of insulin through the mediation of the digestive tract hormones, and the vigorous discussion that it stimulated was extremely impressive.

It appears that since research on gastrointestinal regulatory factors is necessarily very complex and difficult, the number of investigators working in this field is proportionately small. However, because of the difficulties involved, the significance and value of this symposium in its contribution to progress in this area are great.

Finally, clarification of the subtleties and complexities of the physiology and pathophysiology of the digestive organs through future symposia in this field is eagerly awaited, so that this newfound knowledge may be applied to the clinical sphere for the diagnosis and treatment of all types of digestive tract disease.

F. Nagao, M.D.
Y. Matsuo, M.D.
Y. Kasuya, Ph.D.
M. Tsuchiya, M.D.

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I. Special lecture

The regulation of insulin secretion by gastrointestinal hormones

John C. Brown

Medical Research Council of Canada, Group on Regulatory Peptides, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada

INTRODUCTION

The observed effects of highly purified preparations of the gastrointestinal hormones, secretin and cholecystokinin-pancreozymin (CCK), and a crude extract of the pyloric antrum, containing gastrin, on secretion of pancreatic islet hormones led to the suggestion of the existence of an "enteroinsular axis"(1). These studies were performed in anesthetized dogs and the preparations were introduced into the mesenteric vein as a rapid single injection. All preparations produced a significant increase in insulin concentration in the pancreaticoduodenal venous effluent, which peaked within 1 min and declined to preinjection levels almost immediately. In addition to the insulinotropic action, CCK caused a dramatic rise in glucagon concentration which peaked at 3 min and was accompanied by hyperglycemia. It was concluded that "the demonstration that these three hormones possess insulin-releasing activity suggests that there is in the gastrointestinal tract a chain of ß-cytotropic hormones from antrum to ileum that is capable of augmenting insulin secretion as required for disposal of substrate loads" (1).

The possibility that an insulinotropic response might be induced by a hormone from the gastrointestinal tract had been proposed earlier (2), when it was shown that a humoral factor which increased the endocrine secretion of the pancreas could be isolated from the gastrointestinal tract. It did not demonstrate secretin activity (3) and the name "incretin" (4) was introduced to describe this hypoglycemic factor. Even earlier studies (5) had indicated that the duodenum "supplies a chemical excitant for the internal secretion of the pancreas" and the hypothesis was proposed that diabetes might be caused by "the absence of an intestinal excitant of the internal secretion of the pancreas." A duodenal factor, duodenin, that improved postprandial hyperglycemia has also been described

(5). It was rather surprising when the above studies and others (6,7) demonstrating the existence of insulinotropic factors in mucosal extracts of the duodenum were later discredited (8), when hypoglycemic effects could not be isolated from the duodenum and hydrochloric acid introduced into the duodenum did not change circulating glucose levels in either fasting or hyperglycemic animals. Comparisons of several extracts from the mucosa of the small intestine described by others to have hypoglycemic activity led to conclusions that: "i) carefully prepared pancreatic tissue-free extracts of intestinal mucosa obtained by a number of methods which have been reported to yield a hypoglycemic substance have consistently been found to be without effect on the blood sugar level of fasted unanesthetized dogs; ii) this and previous studies have failed to provide evidence in favour of the theory that the duodenum exerts a hormonal control over carbohydrate metabolism"(9).

Confirmation of the existence of an insulinotropic factor (incretin) in mucosal extracts of the gastrointestinal tract quickly followed the development of a radioimmunoassay for insulin. A significantly greater rise in plasma immunoreactive insulin (IRI) followed the oral, as compared to the intravenous administration of glucose. A comparison of the effect of intravenous glucose with that of glucose introduced directly into the jejunum on IRI release confirmed the greater insulin response, even though glucose levels achieved via the intravenous route of administration were higher (10). This study identified the small intestine as the probable site of the release of the insulinotropic factor. The importance of the role of the intestinal mucosa in the regulation of insulin release was confirmed when it was estimated that 50% of the insulin released following an oral glucose load was by mechanisms of gastrointestinal origin (11).

GASTROINTESTINAL HORMONES

Unequivocal evidence supporting a role for gastrointestinal hormones in insulin release has been obtained with difficulty. Since the earlier observations that a factor of gastrointestinal origin was involved in insulin release, secretin, CCK, and gastrin have been considered at one time or another to be the candidate hormone. The studies that led to this conclusion were inappropriate in that the preparations used were impure, administered as a single bolus injection achieving nonphysiological blood levels, and used in experiments in which the circulating glucose levels were either basal, or not controlled (12).

Secretin

Studies in humans suggested that secretin induced insulin release, and that a degree of hyperglycemia produced a greater effect (13–15). The secretin preparations used were impure, and the subsequent studies demonstrated

that doses of secretin which gave excellent pancreatic exocrine responses did not increase circulating levels of insulin (16). Single injections of secretin revealed a more profound effect on insulin release from a readily releasable insulin pool and little or no effect on insulin synthesis (17). Further evidence against a role for secretin in insulin release included the demonstration that intraduodenal glucose did not stimulate the exocrine secretion of the pancreas (18), and the inability of glucose (19,20) or a mixed meal (21,22) to elevate serum immunoreactive secretin levels.

It is generally agreed that secretin is not involved in the enteroinsular axis.

Gastrin

Insulin release in response to injected gastrin has been shown to be transitory, monophasic, and uninfluenced by the prevailing state of glycemia (15,23,24). However, conflicting reports on the effect of administration of exogenous gastrin have appeared. Both stimulation (1,15,25) of insulin release and no effect on release (26) have been described. The absence of any correlation between the increase in circulating gastrin levels by appropriate stimuli and increases in plasma insulin levels confirms that gastrin is not important in the regulation of insulin release (27).

Cholecystokinin-pancreozymin

The physiological role of CCK has been described from studies in which bioassays have been extensively used. The development of a specific radioimmunoassay for CCK has proven to be difficult, making interpretation of results equivocal. Bioassay results have demonstrated that CCK release can be induced by the duodenal administration of protein hydrolysates, fats, and to a lesser extent, carbohydrates (18). Amino acids, particularly L-tryptophan and L-phenylalanine, have been demonstrated to be the most consistent secretagogues (18,28–30). Radioimmunoassay studies have confirmed these findings and also indicated that there was an increase in circulating IR-CCK following ingestion of glucose (31). It has been concluded that CCK exerts an insulinotropic effect in the presence of hyperaminoacidemia, is capable of potentiating glucose-stimulated insulin release (32), but is unlikely to contribute significantly to insulin release following oral glucose (27) (Fig 1).

Glucose-dependent insulinotropic polypeptide: gastric inhibitory polypeptide

Glucose-dependent insulinotropic polypeptide: gastric inhibitory polypeptide (GIP) was isolated from a highly purified preparation of CCK, when it was observed that this preparation probably contained an inhibitor of acid secretion, other than CCK itself (33). Figure 2 illustrates this

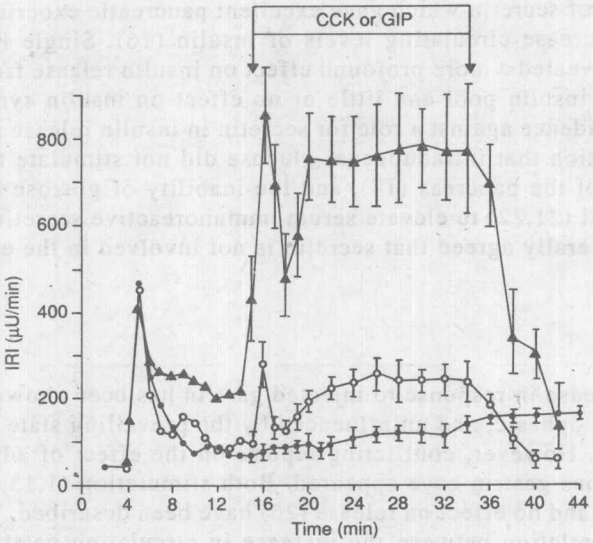

Fig 1 Effect of a 20-min infusion of 770 pg CCK/mL (99% pure) and 1.0 ng GIP/mL (equimolar concentrations) on insulin release from the isolated perfused rat in the presence of 160 mg glucose/dL. ● Control (n = 9); ○ CCK 770 pg/mL (n = 4); ▲ GIP 1 ng/mL (n = 7).

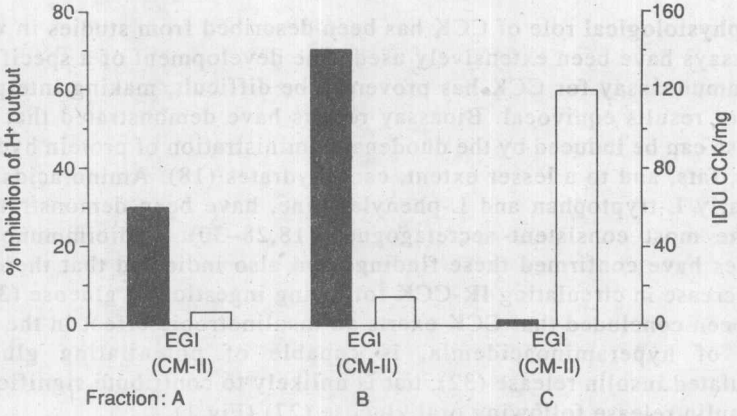

Fig 2 Bioassay results from fractionation of GIP starting material on carboxymethyl cellulose (CM 11). CCK activity was bioassayed using an in vitro guinea pig gallbladder assay and inhibitory activity for gastric acid secretion was assayed in dog with a Bickel pouch. CCK-like activity is expressed as Ivy Dog Units (IDU) and acid inhibitory activity is expressed as a percentage of the inhibition of pentagastrin-stimulated acid secretion produced by 1.0 $\mu g.kg^{-1}h^{-1}$ on each fraction. EGI refers to starting material in the purification process. ■ % Inhibition; □ IDU CCK/mg.

point, in that gallbladder contracting activity (CCK) and acid inhibitory activity (GIP) could be separated from the starting material following chromatography using a cation-exchange cellulose. Purification was achieved using a bioassay for measuring the inhibition of gastric acid secretion (34–36). An amino acid sequence for GIP was reported (37) and later corrected (38). Amino acid sequences have now been reported for porcine, human, and bovine GIP (Fig 3) and a high degree of conservation is seen.

HOG

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Ser-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-

HUMAN

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln

BOVINE

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Ser-Asp-Trp-Ile-His-Asn-Ile-Thr-Gln

Fig 3 Amino acid sequences of porcine, bovine, and human GIP. Note the high degree of conservation.

A highly purified preparation of GIP was infused into normal human volunteers at a dose of 1.0 μg/min for 60 min and in the presence of elevated serum glucose (0.5 g/min iv for 60 min), a marked elevation of plasma IRI levels above control was observed (Fig 4) (39). The glucose-dependent nature of the insulinotropic effect of GIP was confirmed in both the rat (40) and man (41), and in addition it was demonstrated that there was a threshold concentration of glucose below which GIP was not insulinotropic. This has been confirmed to be 4.4 mM. The potentiating effect of GIP at a concentration of 5 ng/mL was shown to be maximum in the rat at 16 mM glucose which corresponded to the maximum response observed with glucose alone. However, in the presence of GIP, the maximum insulin output was several-fold higher than with glucose alone.

Immunoreactive GIP

Serum immunoreactive GIP (IR-GIP) levels increase following the passage of glucose into the duodenum (42). It is the only gastrointestinal

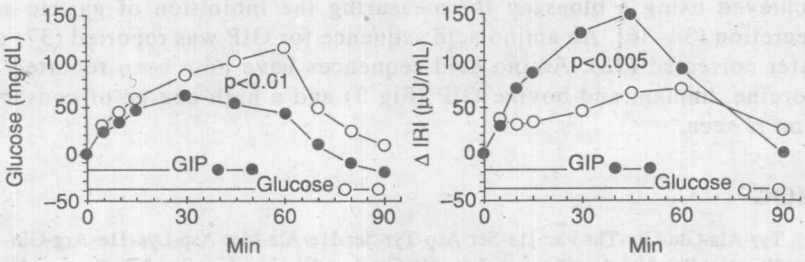

Fig 4 Comparison of the insulinotropic effect of an iv infusion of glucose in the absence (○) and presence (●) of an iv infusion of porcine GIP (1.0 μg/min) in paired studies in 6 normal human volunteers.

hormone that is significantly increased following glucose ingestion. Although controversy exists as to the absolute plasma values in both basal and stimulated situations, most studies have indicated that IR-GIP levels increase approximately 4-fold following glucose ingestion (42). IR-GIP levels in plasma have been shown to become elevated following ingestion of a mixed meal (43,44), amino acids (45), and fatty acids (46,47), as well as carbohydrates. Fasting and postprandial IR-GIP levels have recently been studied using 7 different antisera to GIP (48). Fasting GIP levels varied from 12 to 92 pmol/L and postprandially from 35 to 235 pmol/L, depending on which antiserum was used. Fractionation of the IR-GIP in serum samples, and subsequent assay with the antisera revealed that all of the antisera recognized 3 circulating forms of IR-GIP, the largest component eluting at a volume indicating a molecular size of 5 kDa. A similar study used 5 antisera raised to porcine GIP to compare measurements of IR-GIP in porcine and human gastrointestinal tissue (49). All of the antisera gave comparable values for the porcine tissue, however the results from the human tissue extract varied considerably. Most of the antisera used recognized an epitope within the GIP sequence 15–42 in which amino acid substitutions have been shown to occur. It has been reported that within the human GIP amino acid sequence histidine replaces arginine at 18 and asparagine replaces serine at 34 (50). It is possible that antisera directed to this part of the porcine molecule may not accurately measure human GIP, contributing to the equivocal values which are given for plasma IR-GIP in man (12).

CELLS OF ORIGIN

Immunoreactive GIP cells have been shown, in man and dog, to be con-

fined to the upper small intestine, distributed diffusely among the epithelial cells (51). The distribution of GIP cells overlaps with several other peptide-containing cells, including the structurally related peptides, secretin and gut glucagon, as well as somatostatin, neurotensin, and CCK. No evidence of colocalization of peptides in endocrine type cells has been reported. Ultrastructural localization of GIP within the human small intestine has confirmed that the cell previously identified as the K cell was the cell of origin for GIP (52). It is defined by the characteristic appearance of the intracellular secretory granules which have a small electron-dense core, surrounded by a concentric electron-lucent halo. The cell type identified as the GIP-secreting cell in the dog small intestine differs from the human and is more characteristic of the I cell of the endocrine cell classification.

Immunocytochemical studies using rabbit antisera suggested that IR-GIP may be present in the pancreatic islet A cell of mammals (53). These studies, however, were considered controversial when it was demonstrated that the rabbit antiserum used could be blocked by preincubation with both glicentin (54) and pancreatic glucagon (52). GIP-like immunoreactivity of the glucagon cells of the rat pancreas has also been described (55). Infusion of GIP and resection of the upper small intestine did not change the effect, indicating that the glucagon cells produced this GIP-like peptide rather than it accumulating there from the circulation.

GIP RECEPTORS

Physiological and pharmacological concentrations of porcine GIP have been shown to evoke responses from a variety of tissues, including pancreas, stomach, liver, fat cells, smooth muscle, and cells of the anterior pituitary. The identification of receptors through which the tissue response would be mediated has met with limited success. The suggestion has been made that GIP may interact with glucagon receptors in some tissues. The peptide has been shown to block the binding of ^{125}I-labeled glucagon to adipocytes (56) and to exert a strong antagonistic effect to the actions of glucagon (56, 57).

Preparation of ^{125}I-GIP suitable for receptor studies has recently been achieved (58). This superior preparation and the use of tissue with a relatively high density of binding sites, the hamster transplantable insulinoma, has led to some characterization studies (58–60). An insulin-secreting B-cell line (In 111) has also been used (61, 62). Two types of binding sites have been identified, a small population of high-affinity and a larger population of low-affinity binding sites. Binding to either receptor was unaffected by the related peptides glucagon, secretin, and vasoactive intestinal peptide (VIP) (58,59,61,62). The presence of a 59-kDa membrane protein which specifically bound ^{125}I-GIP has been described (59). However, it has not been possible to demonstrate receptors on normal tissue,

which probably reflects the relatively small number of high-affinity binding sites and loss of receptor during cell and membrane preparation.

PATHOPHYSIOLOGY

Animal models

The possible involvement of GIP in the hyperinsulemia observed in animal models of obesity and diabetes has been investigated by several laboratories. In the obese Zucker rat (fa/fa), which is characterized by hyperphagia, hyperinsulinemia, hyperlipemia, and an increased deposition of fat into adipocytes, glucose and GIP responses to an oral glucose challenge were similar to normal controls (63). However, the response of the isolated perfused pancreas of the obese animal to a 300-mg/dL glucose challenge was significantly elevated and a gradient of GIP, 0–1.0 ng/dL, increased insulin output in both lean and obese rats 3-fold. At basal glucose concentrations (80 mg/dL), GIP stimulated insulin release in the obese animal, whereas in the lean control, no insulinotropic effect was observed (Fig 5). Immunocytochemical studies indicated that the obese rats had greatly enlarged islets due to an increase in B cells, whereas the other cellular components appeared normal. Similar numbers of GIP cells were identified in the gastrointestinal tract. The primary cause of the altered response of the B cell to GIP in the obese animal, an insulinotropic response at basal glucose concentrations, is not known, but is indicative of a defect within the islet and probably at the level of the receptor.

The obese hyperglycemic mouse (ob/ob) is characterized by hyperphagia, obesity, moderate hyperglycemia, and severe hyperinsulinemia (64). Hyperplasia of IR-GIP-containing cells in the mucosa of the gastrointestinal tract had been reported earlier (65) and plasma IR-GIP concentrations were reported to be 15-fold higher in the obese animal compared to lean littermates (66). Plasma IRI and IR-GIP were elevated between 10–20 weeks in the ob/ob mouse and a significant degree of hyperinsulinemia was observed preceding the hypersecretion of GIP (67). Both IRI and IR-GIP plasma levels were suppressed by fasting and restored by refeeding. Regulation of IR-GIP release was apparently normal in these animals, responding to fat, glucose, and amino acids, and only the latter two induced an increase in insulin.

Man

Since the development of a radioimmunoassay for GIP, numerous studies investigating a possible role for the hormone in pathophysiological states have been reported. Most of these studies have concentrated on the measurement of circulating levels of IR-GIP in response to a wide variety of provocative challenges varying from a 25-g oral glucose challenge to a