PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 379–385 (1983)

Gastric Inhibitory Polypeptide (GIP) Release by Actively Transported, Structurally Similar Carbohydrates (41660)

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Abstract. Six awake adult dogs prepared with a duodenocutaneous fistula were infused intraduodenally with one of the following solutions: 3% saline, 10% glucose, 20% glucose, 20% galactose, 20% fructose, 20% mannose, 20% sorbitol, 20% maltose, 20% lactose, or 20% sucrose. Both 10 and 20% glucose stimulated GIP release, and the response appeared to be dose related. Actively transported galactose (C-4 epimer) stimulated GIP release, but less than glucose. Fructose (C-2 keto sugar) which is absorbed by facilitated transport did not stimulate GIP release. Mannose (C-2 epimer) which is passively absorbed by diffusion did not release GIP. Sorbitol (reduced alcohol of glucose) which is not absorbed did not release GIP. Of the disaccharides tested, only maltose stimulated the release of GIP. The results suggest that structural integrity of the glucose molecule from the C-1 to C-4 carbon atoms, a free aldehyde group on the C-1 carbon atom, and a cyclic structure are all necessary for both the active transport of glucose and the release of endogenous GIP. It would appear that structurally similar receptors exist for both the active transport of glucose and for the release of GIP.

Beaumont, in his historical studies of Alexis St. Martin in the early 1830s, was the first of many to observe that gastric emptying time is determined by the composition of the food ingested (1). Subsequent studies have shown intraduodenal fat (2, 3) hydrochloric acid (4), glucose (5, 6), and hypertonic solutions (5, 7) to be potent inhibitors of both gastric acid secretion and gastric emptying. The studies of Leconte, and Day and Komarov suggested that glucose and other polysaccharides inhibited gastric acid secretion and delayed gastric emptying by stimulating a common osmoreceptor mechanism sensitive to duodenal contents with osmolarity above a certain threshold (5, 6).

Gastric inhibitory polypeptide (GIP) is an intestinal peptide capable of inhibiting both gastric motility and gastric acid secretion (8). Intraduodenal glucose is a potent stimulus for GIP release in both normal humans and in dogs prepared with Mann–Bollman fistulas (9, 10). This release of GIP by enteral glucose, coupled with the capacity of GIP to augment insulin release in the presence of hyperglycemia, has prompted several investigators to postulate that GIP is the incretin factor of the enteroinsular axis (11, 12). Recognition of the role of GIP in carbohydrate metabolism has prompted a suggested name change of GIP to Glucose-dependent Insulinotropic Peptide.

The present study was designed to investigate the mechanism by which glucose causes GIP secretion. Therefore carbohydrate compounds with structural similarity to the glucose molecule but with absorptive mechanisms different from glucose were used in an attempt to stimulate the release of endogenous GIP in the awake dog. These included both monosaccharides (galactose, fructose, mannose, sorbitol) and disaccharides (maltose, lactose, sucrose). The results of these stimulatory studies were then used to formulate a possible mechanism of GIP secretion.

Materials and Methods. Six adult conditioned dogs weighing 20 to 25 kg each were surgically prepared with a Mann–Bollman duodenocutaneous fistula (13). The fistula was constructed by placement of a 15-cm antiperistaltic segment of isolated ileum between the abdominal wall (matured stoma) and the second portion of the duodenum (duodenoenterostomy). Animals were allowed to recover for a minimum of 6 weeks after operation. Following this recovery period, each dog

0037-9727/83 $1.50

underwent 20 separate daily infusions, one test per week for 20 weeks.

Prior to testing, each animal was fasted for 18 hr, but was allowed water *ad libitum*. During the study period, animals were placed in loose restraints on a Pavlov stand. A soft rubber catheter was advanced into the duodenum through the Mann–Bollman fistula to a depth of 7 cm and fixed in place. An indwelling, heparinized, 19-gauge needle was placed in a peripheral vein to facilitate serial blood sampling. The animal was then allowed to stabilize for 30 min before testing. Care was taken at all times to limit external stimuli.

Following stabilization, 50 ml of one of the following solutions (3% saline, 10% glucose, 20% glucose, 20% galactose, 20% fructose, 20% mannose, 20% sorbitol, 20% β-maltose, 20% lactose, or 20% sucrose) was infused into the duodenum over a 10-min period through the rubber catheter in the Mann–Bollman fistula. Each animal underwent two separate tests with each of the 10 experimental solutions, for a total of 20 separate studies per dog over a 20-week period. All solutions were buffered to pH 7.5 using sodium bicarbonate.

Venous blood samples were obtained from the indwelling catheter at 0 (fasting), 5, 15, 30, 45, 60, 120, and 180 min with the 0-min sample coinciding with the start of the intraduodenal infusion. Blood samples for GIP determinations were immediately centrifuged at room temperature, and the serum was frozen and stored at −20°C. Serum immunoreactive GIP (IR-GIP) was measured by the method of Kuzio *et al.* (14) with some modifications (4). Porcine GIP was used as a standard. The lower sensitivity of the GIP assay was between 25 and 50 pg/ml of incubate. This allowed serum concentrations as low as 125 pg/ml to be measured. The coefficient of variation was 8% for intraassay and 15% for interassay reproducibility.

The experimental results were expressed as the mean ± the standard error of the mean ($\bar{X} \pm$ SEM). Both dependent and independent means were compared using the Scheffé modification of the two-way analysis of variance. A P value of 0.05 or less was considered significant. The integrated incremental area for the IR-GIP response (picograms per minute per milliliter) was calculated using the trapezoidal rule (15).

Results. All animals remained calm during the period of restraint required for each experimental study. The perfusates caused no salivation, lacrimation, vomiting, diarrhea, micturition, or tachycardia. The onset, magnitude, and duration of response for IR-GIP were consistent for the animls within each experimental group.

Intraduodenal saline. Compared to fasting concentrations, significant changes in serum IR-GIP did not occur following infusion of 3% saline into the duodenum (Table I).

Intraduodenal glucose. Following infusion of 50 ml of 10% glucose into the duodenum, serum IR-GIP increased significantly to a peak mean response of 659 ± 93 pg/ml at 15 min (Table I). IR-GIP remained significantly increased at 30 and 45 min. The mean serum IR-GIP concentrations at 60, 120, and 180 min were not significantly different from fasting concentrations. Following infusion of 50 ml of 20% glucose into the duodenum, IR-GIP increased significantly to a peak mean response of 1026 ± 186 pg/ml at 15 min (Table I). The IR-GIP remained significantly increased at 30, 45, and 60 min. The mean serum IR-GIP concentrations at 120 and 180 min were not significantly different from fasting concentrations. Compared to the response to 10% glucose, the IR-GIP response to 20% glucose was significantly greater at 15, 30, 45, and 60 min. Also the mean integrated incremental area for IR-GIP (Table II) was significantly greater following 20% glucose than after 10% glucose infusion (31,334 ± 7591 vs 14,293 ± 3953 $pg \cdot ml \cdot min^{-1}$).

Intraduodenal galactose. Following infusion of 50 ml of 20% galactose into the duodenum, IR-GIP increased significantly to a peak mean response of 630 ± 117 pg/ml at 15 min (Table I) and remained increased at 30 min (457 ± 40 pg/ml). The mean serum IR-GIP concentrations at 45, 60, 120, and 180 min were not significantly different from fasting concentrations. The mean integrated incremental area for IR-GIP was significantly less following 20% galactose than after either 10 or 20% glucose infusion (Table II).

Intraduodenal fructose, mannose, and sorbitol. Serum concentrations of IR-GIP did not change compared to fasting levels following intraduodenal infusions of either 20% fructose or 20% mannose (Table I). Infusions of

TABLE I. THE IR-GIP RESPONSE ($\bar{X}$ ± SEM) IN PICOGRAMS PER MILLILITER FOLLOWING INFUSION OF 50 ml OF SOLUTION INTO THE DUODENUM OVER A 10-min PERIOD IN AWAKE DOGS

Intraduodenal perfusate	Time 0 (fasting)	Minutes postinfusion						
		5	15	30	45	60	120	180
3% Saline	293 ± 45	348 ± 62	377 ± 65	321 ± 61	296 ± 52	309 ± 61	275 ± 48	302 ± 49
10% Glucose	286 ± 30	325 ± 36	659 ± 93*	531 ± 49*	452 ± 67*	350 ± 35	282 ± 21	305 ± 54
20% Glucose	275 ± 54	322 ± 19	1026 ± 186*	920 ± 135*	663 ± 95*	438 ± 55*	280 ± 5	271 ± 12
20% Galactose	317 ± 29	489 ± 44*	630 ± 117*	457 ± 40*	445 ± 30	396 ± 4	317 ± 31	320 ± 12
20% Fructose	235 ± 0	235	235	235	235	235	235	235
20% Mannose	220 ± 0	220	220	220	220	220	220	220
20% Sorbitol	333 ± 58	370 ± 55	412 ± 80	380 ± 82	338 ± 62	361 ± 61	269 ± 51	277 ± 60
20% Maltose	266 ± 16	306 ± 16	638 ± 111*	696 ± 117*	482 ± 72*	306 ± 16	270 ± 40	281 ± 52
20% Lactose	230 ± 30	230 ± 30	243 ± 27	259 ± 43	258 ± 43	250 ± 50	231 ± 31	272 ± 22
20% Sucrose	275 ± 0	275	275	275	275	275	275	275

* $P < 0.05$ or less.

20% sorbitol resulted in nonsignificant changes in IR-GIP and an integrated incremental IR-GIP area not different from that of saline (Table II).

Intraduodenal β-maltose. Following infusion of 50 ml of 20% maltose into the duodenum, IR-GIP increased significantly to a peak mean response of 696 ± 117 pg/ml at 30 min. The mean serum IR-GIP concentrations at 60, 120, and 180 min were not significantly different from fasting concentrations. The IR-GIP response to 20% maltose at 15, 30, and 45 min was of a magnitude similar to that seen with 10% glucose infusion (Table I). Compared to the IR-GIP response to 20% glucose, the IR-GIP response to 20% maltose was significantly less following 20% maltose (31,334 ± 7591 vs 15,292 ± 4464 $pg \cdot ml \cdot min^{-1}$).

Intraduodenal lactose and sucrose. Compared to fasting concentrations and to 3% saline, significant changes in serum IR-GIP did not occur following intraduodenal infusions of either 20% lactose or 20% sucrose (Tables I and II).

Discussion. Almost 70 years after Beaumont's initial observations, the detailed studies of Marbaix in 1898 confirmed the regulatory role of gastric contents on gastric emptying (1, 16). More importantly, he introduced the concept of duodenal regulation. Subsequent studies have shown that intraduodenal introduction of fat (2, 3), hydrochloric acid (4), glucose (5, 6), and hypertonic solutions (5, 7) caused marked inhibition of both gastric acid secretion and gastric emptying. The mechanism of acid inhibition and delay in gastric emptying following either intraduodenal fat or acid appears to be humoral, acting via a hormone released from the small intestine; that is, an enterogastrone (17). The physiology of gastric acid inhibition by hyperosmolar carbohydrate solutions is poorly defined and remains a controversial issue.

In 1900, Leconte demonstrated the ability of intraduodenal hypertonic glucose to inhibit gastric acid secretion (6). Early reports suggested that this inhibition occurred secondary to hyperglycemia (18). However, subsequent studies demonstrated that gastric acid output did not change during epinephrine-induced hyperglycemia or with the intravenous infusion of glucose (19, 20). In 1939, Day and

TABLE II. THE INTEGRATED INCREMENTAL IR-GIP RESPONSE ($\bar{X} \pm$ SEM) FOLLOWING INFUSION OF VARIOUS CARBOHYDRATES INTO THE DUODENUM OVER A 10-min PERIOD

Intraduodenal perfusate	Integrated incremental IR-GIP ($pg \cdot ml \cdot min^{-1}$)
3% Saline	3519 ± 1221
10% Glucose	14,293 ± 3953
20% Glucose	31,334 ± 7591
20% Galactose	5155 ± 2246
20% Fructose	0
20% Mannose	0
20% Sorbitol	3804 ± 1193
20% Maltose	15,292 ± 4464
20% Lactose	3930 ± 3268
20% Sucrose	0

Komarov found that the duodenal instillation of glucose solutions of 20% or greater inhibited the gastric secretory response to sham feeding (5). In addition, they were able to demonstrate that an intravenous infusion of glucose produced only a slight inhibition when compared to that caused by duodenal infusion of an equivalent glucose load. Further studies showing similar inhibition with other sugars, polysaccharides, and saline suggested a common osmoreceptor mechanism sensitive to duodenal contents above a certain threshold of osmolarity (5).

Gastric inhibitory polypeptide (GIP) is an intestinal polypeptide isolated from crude extracts of cholecystokinin by Brown *et al.* in 1969 (21). Detailed studies have demonstrated its capacity to inhibit gastric motility and to inhibit gastric acid secretion in response to histamine, pentagastrin, and insulin hypoglycemia (8). Both oral glucose and fat have been shown to be potent stimuli for the release of GIP in normal humans and in dogs prepared with Mann–Bollman fistulas (9, 10, 22). Prior studies from our laboratory have shown that infusion of hyperosmolar solutions of either mannitol or saline into the duodenum of awake dogs results in gastric acid inhibition in the absence of any change in GIP levels (23). Hunt, in 1956, and Sircus, in 1958, demonstrated inhibition of gastric acid secretion by carbohydrates other than glucose (24, 25). The present experiment was designed to determine if carbohydrates other than glucose were capable of releasing endogenous GIP, thereby providing a plausible mechanism for the above phenomenon. In addition, the structural configuration and method of gastrointestinal absorption of these other carbohydrates were compared to those of glucose in order to provide a theoretical mechanism by which glucose causes GIP secretion.

In this study, both 10 and 20% intraduodenal glucose were potent stimuli for the release of endogenous GIP. However, there were notable differences in the magnitude and duration of the GIP responses (Table I). Compared to 10% glucose, the GIP response to 20% glucose peaked at a significantly higher level (1026 ± 186 vs 659 ± 93 pg/ml) and remained significantly elevated at 60 min (438 ± 55 vs 350 ± 35 pg/ml). The integrated incremental IR-GIP response was greater ($P < 0.05$) following 20% glucose than with 10% glucose (Table II). Thus, the GIP response to intraduodenal glucose appears to be dose related.

Infusion of a 20% galactose solution into the duodenum was also associated with endogenous GIP release. However, the GIP response was markedly reduced compared to the amount released by an osmotically identical 20% glucose solution (Table I). Other investigators have found similar results for galactose-induced GIP release in normal humans (26, 27). This difference in capacity to release gastric inhibitory polypeptide by these two carbohydrates coincides with the observation of Elias *et al.*, in 1968, that galactose was less effective per osmole in slowing gastric emptying than was glucose (28). Since an infusion of a hypertonic saline solution failed to stimulate GIP release and equimolar solutions of each carbohydrate were used, it would seem that these different responses for glucose and galactose occurred via some mechanism other than by stimulation of duodenal osmoreceptors. Although glucose and galactose are absorped by an identical active transport mechanism, there is preferential translocation of glucose over galactose by the membrane carrier (29). The structural difference of the galactose molecule (C-4 epimer) compared to glucose (Fig. 1) might account for this difference in transport between these two monosaccharides. The diminished GIP response to galactose may have occurred secondary to either decreased carrier transport

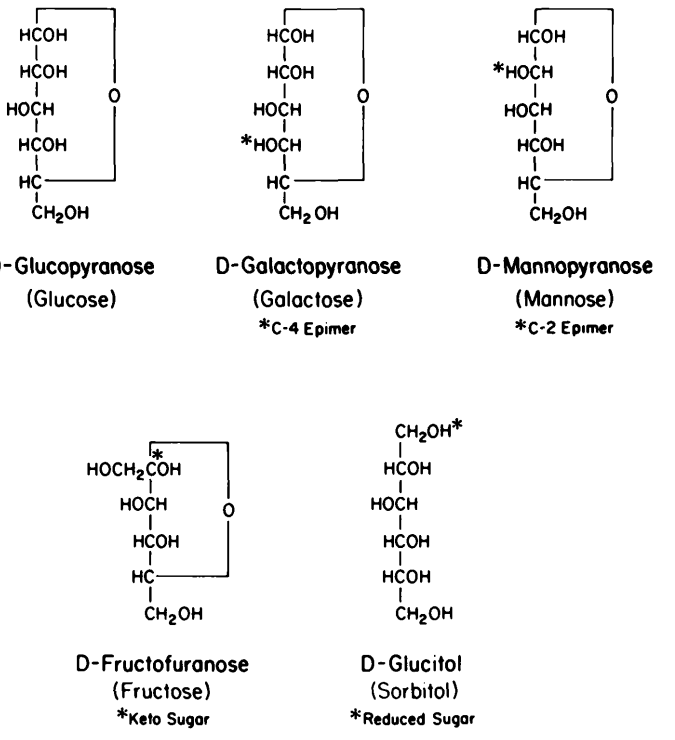


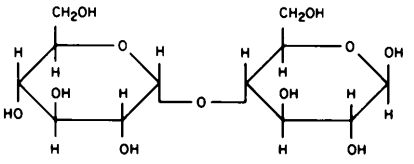
FIG. 1. Structural configuration of glucose and its related monosaccharides.

or to configurational differences of the galactose molecule compared to that of glucose.

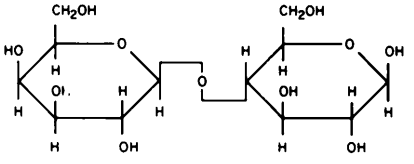
Intraduodenal infusion of either fructose, mannose, or sorbitol did not stimulate GIP release (Table I). These three molecules are not actively transported by the intestinal mucosa. There is facilitated transport of fructose, passive diffusion of mannose, and no appreciable absorption of sorbitol. Structural modification of the glucose molecule, as occurs in fructose (C-2 keto sugar), mannose (C-2 epimer), and sorbitol (reduced alcohol of glucose), results in a loss of both active transport of the molecule and the capacity to release GIP (Fig. 1). These results suggest that, in addition to maintaining structural integrity of the glucose molecule from C-1 to C-4 carbon atoms, the carbohydrate molecule must maintain a six-membered ring (pyranose) conformation and an aldehyde group at C-1 to preserve both active transport and GIP release.

Of the disaccharides tested, only β-maltose stimulated the release of endogenous GIP. Unexpectedly, the magnitude of the GIP response to 20% maltose paralleled the GIP response to 10% glucose instead of 20% glucose (Table I). Complete hydrolysis of maltose should have yielded an identical number of glucose molecules as in 20% glucose (Fig. 2). However, it has been shown that glucose derived from disaccharides is translocated across the intestinal mucosal cell by an active transport mechanism different from that utilized by glucose itself (29). The diminished GIP response following maltose suggests that either hydrolysis may have been incomplete or that a delay in hydrolysis affected both active transport of glucose by this other carrier mechanism and subsequently GIP release. The fact that the peak mean GIP response following maltose occurred 15 min later than the peak for glucose suggests that there indeed was a delay in hydrolysis of maltose.

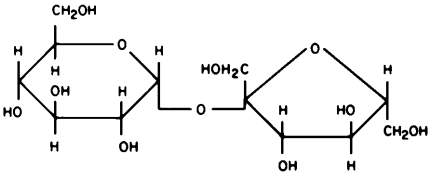
Intraduodenal 20% lactose did not stimulate GIP release (Table I). The adult dog is deficient in lactase activity. Lack of hydrolysis to glucose and galactose and failure to release GIP demonstrates that the isolated glucose molecule is necessary for both active trans-



4-O-α-D-Glucopyranosyl-D-Glucopyranose
(β-Maltose)



4-O-β-D-Galactopyranosyl-D-Glucopyranose
(β-Lactose)



α-D-Glucopyranosyl-β-D-Fructofuranoside
(Sucrose)

FIG. 2. Structural configuration of the three disaccharides: maltose, lactose, and sucrose.

port and GIP release. Intraduodenal 20% sucrose failed to stimulate GIP release in this animal model (Table I). However, other investigators have found a release of endogenous GIP following ingestion of sucrose (30). This discrepancy may be secondary to species variation. Further studies are necessary to solve this problem.

The results of this study suggest that structurally similar receptors exist for both the active transport of glucose and for the release of endogenous GIP. This further suggests that GIP release is indeed coupled to the active transport of glucose. However, the mechanism of action to explain how this transmembrane carbohydrate movement in one type of cell affects the release of GIP from an adjacent neuroendocrine cell remains to be elucidated in further studies.

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Received February 23, 1983. P.S.E.B.M. 1983, Vol. 173.