EVIDENCE FOR PREFERENTIAL STIMULATION OF GASTRIC
INHIBITORY POLYPEPTIDE SECRETION IN THE RAT BY
ACTIVELY TRANSPORTED CARBOHYDRATES
AND THEIR ANALOGUES

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SUMMARY

A rat intestinal perfusion technique has been used to assess the ability of a number of
monosaccharides, monosaccharide analogues and disaccharides to stimulate intestinal
release of immunoreactive gastric inhibitory polypeptide (GIP).

Perfusates containing glucose, sucrose, galactose, maltose, 3-O-methylglucose or α- or β-
methylglucoside at concentrations of 100 mmol/l in Krebs–Ringer phosphate buffer (KRP)
produced significant stimulation of GIP release compared with the control perfusions with
KRP alone (P < 0.02). Mannose, D-deoxygalactose, D-deoxyglucose, myoinositol, fructose or
lactose (100 mmol/l of each) did not stimulate GIP release compared with controls. There
was no significant difference in the ability of sucrose, maltose or β-methylglucoside
(100 mmol/l of each) to release GIP compared with 100 mmol glucose/l, but galactose, 3-O-
methylglucose and α-methylglucoside (100 mmol/l of each) produced significantly lower
GIP responses than did glucose (P < 0.02). Addition of 5 mmol phloridzin/l to a perfusate
containing 50 mmol glucose/l prevented intestinal absorption of glucose and abolished the
GIP response.

The molecular configuration of monosaccharides which have the ability to stimulate GIP
release agreed well with the structural requirements for active transport by the sodium-
dependent hexose pathway.

INTRODUCTION

Gastric inhibitory polypeptide (GIP) is a polypeptide released from the neuroendocrine K
cells of the duodenum and the upper small intestine (Buffa, Polak, Pearse, Solcia, Grimelius
& Capella, 1975) after the ingestion of a mixed meal (Kuzio, Dryburgh, Malloy & Brown,
1970). Its most important physiological role is now thought to be as an insulinotropic agent
in the presence of hyperglycaemia when it plays a major role in the intestinal regulation of
insulin secretion (Dupré, Ross, Watson & Brown, 1973; Marks & Turner, 1977; Brown &
Otté, 1978). The most important stimulants of GIP release are oral fat and glucose
(Cataland, Crockett, Brown & Mazaferri, 1974; Brown, Dryburgh, Ross & Dupré, 1975). In
rat and man, galactose and sucrose, but not fructose, also stimulate GIP release (Morgan,
1979; Sykes, Morgan, Hampton & Marks, 1979). Glucose and galactose are actively
absorbed from the small intestine by the same sodium-dependent transport mechanism
situated at the mucosal cell brush-border (Crane, 1965, 1968; Gary, 1975). Although it is not

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known how the mucosal cell effects GIP release from its neighbouring neuroendocrine cell, it appears that active transport by this pathway is necessary. In the present study using a rat intestinal perfusion technique in vivo some monosaccharides and their analogues and a number of disaccharides have been assessed for their ability to stimulate immunoreactive GIP release. Correlation of the plasma immunoreactive GIP response to the various carbohydrate stimuli with the molecular structure of the carbohydrates has provided information on the minimal structural requirements for the release of immunoreactive GIP and has allowed deductions to be made about the nature of the active transport mechanisms involved.

MATERIALS AND METHODS

β-D-Glucose, β-D-galactose, β-D-fructose, lactose, sucrose, maltose and D-mannose of Analar grade were purchased from British Drug Houses Ltd, Poole, Dorset. Phloridzin, 2-deoxyglucose, α-methylglucoside, β-methylglucoside, 3-O-methyl-α-D-glucose, 6-deoxygalactose and myoinositol were purchased from Sigma Chemical Co. Ltd, London.

Male Wistar albino rats weighing between 200 and 220 g were starved for 16 h before operation and were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (Nembutal, 0·1 ml/100 g). The gut loop perfusion technique was a modification of one previously described (English, Chakraborty & Marks, 1976). The lumen of the small intestine was perfused from a point just below the pyloric sphincter to the ileocaecal junction, the blood system remaining essentially intact. Krebs–Ringer phosphate buffer (pH 7·4; KRP) maintained at 37 °C was pumped at a rate of 2 ml/min through a plastic cannula (internal diameter 4 mm) into the upper end of the small intestine and allowed to drain through another plastic cannula from the ileal end. Except for experiments comparing the effects of 5 mmol phloridzin/l on the absorption of 50 mmol glucose/l, all perfusions were carried out with concentrations of 100 mmol of the various carbohydrates/l KRP.

Blood was collected from the hepatic portal vein into lithium heparinized tubes 30 min after commencing the perfusion and the rats were then killed under anaesthesia. A sample of whole blood was immediately deproteinized with 0·33 m-perchloric acid and the supernatant fraction stored at −20 °C for glucose estimation. Aprotinin (Novo Research Institute, Copenhagen, Denmark; 1000 units/ml blood) was added to the remaining blood for estimation of plasma immunoreactive GIP. The blood was separated immediately by centrifugation at 1500 g for 5 min and stored at −20 °C until assay.

Preliminary experiments under these conditions established that a constant concentration of the intestinal effluent, as assessed by measurement of tritiated glucose in the intestinal effluent, was consistently achieved within 12 min of the start of the infusion, and that levels of portal vein GIP and glucose reached a plateau after 30 min with a wide range of glucose concentrations (Watts, Morgan & Marks, 1979).

Immunoreactive GIP was measured by double-antibody radioimmunoassay (Morgan, Morris & Marks, 1978). Sensitivity was approximately 110 ng/l and the antiserum exhibited <1% cross-reactivity with cholecystokinin, insulin, pancreatic polypeptide, pancreatic glucagon, porcine gut glucagon-like immunoreactivity, secretin or vasoactive intestinal polypeptide. Preliminary work in man suggested that the antiserum recognizes big GIP (apparent mol. wt 8000) as well as standard GIP (mol. wt 5105). Parallelism of rat GIP with porcine GIP standard was verified. Glucose levels in whole blood were measured by a hexokinase NADP–NADPH linked method (Boehringer Corporation Ltd, Lewes, Sussex).

Control animals, perfused with KRP alone or with 100 mmol glucose/l, were used in each experiment.

The GIP responses to the various carbohydrates were compared with those produced by perfusions in control animals using the two-tailed Student's t-test for unpaired observations (Table 1).
RESULTS

Glucose (100 mmol/l in KRP) produced highly significant \( (P < 0.01) \) increases in concentrations of GIP and glucose in portal vein blood which were approximately 60% of those observed when the glucose perfusate concentration was 200 mmol/l (Fig. 1). There was a

![Graph showing concentrations of immunoreactive gastric inhibitory polypeptide (GIP) and glucose in portal vein blood of rats after intestinal perfusion with various concentrations of glucose for 30 min. Vertical lines indicate \( \pm \) S.E.M.]

Fig. 1. Concentrations of immunoreactive gastric inhibitory polypeptide (GIP) (■) and glucose (▲) in portal vein blood of rats after intestinal perfusion with various concentrations of glucose for 30 min. Vertical lines indicate \( \pm \) S.E.M.
Table 1. Levels of immunoreactive gastric inhibitory polypeptide (GIP) in the plasma from the portal vein of rats in response to intestinal perfusion for 30 min with various carbohydrate solutions (100 mmol/l). Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>Plasma GIP (pmol/l)</th>
<th>P value compared with control perfusion</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control†</td>
<td>61.3 ± 4.5</td>
<td>—</td>
<td>28</td>
</tr>
<tr>
<td>Mannose</td>
<td>34.9 ± 7.0</td>
<td>NS</td>
<td>6</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>40.5 ± 6.5</td>
<td>NS</td>
<td>4</td>
</tr>
<tr>
<td>6-Deoxygalactose</td>
<td>42.3 ± 3.3</td>
<td>NS</td>
<td>5</td>
</tr>
<tr>
<td>Myoinositol</td>
<td>50.5 ± 15.9</td>
<td>NS</td>
<td>5</td>
</tr>
<tr>
<td>Fructose</td>
<td>55.2 ± 7.1</td>
<td>NS</td>
<td>4</td>
</tr>
<tr>
<td>Lactose</td>
<td>56.4 ± 8.6</td>
<td>NS</td>
<td>5</td>
</tr>
<tr>
<td>3-O-Methylglucose</td>
<td>107 ± 10</td>
<td>&lt; 0.02</td>
<td>5</td>
</tr>
<tr>
<td>α-Methylglucoside</td>
<td>118 ± 24</td>
<td>&lt; 0.02</td>
<td>5</td>
</tr>
<tr>
<td>Galactose</td>
<td>139 ± 19</td>
<td>&lt; 0.005</td>
<td>5</td>
</tr>
<tr>
<td>β-Methylglucoside</td>
<td>163 ± 35</td>
<td>&lt; 0.01</td>
<td>5</td>
</tr>
<tr>
<td>Maltose</td>
<td>177 ± 21</td>
<td>&lt; 0.001</td>
<td>5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>226 ± 29</td>
<td>&lt; 0.001</td>
<td>5</td>
</tr>
<tr>
<td>Glucose</td>
<td>302 ± 32</td>
<td>&lt; 0.001</td>
<td>9</td>
</tr>
<tr>
<td>Glucose* (50 mmol/l)</td>
<td>210 ± 20</td>
<td>&lt; 0.001</td>
<td>12</td>
</tr>
<tr>
<td>Glucose* (200 mmol/l)</td>
<td>383 ± 51</td>
<td>&lt; 0.001</td>
<td>4</td>
</tr>
</tbody>
</table>

NS, not significant.
* Glucose was also perfused at concentrations of 50 and 200 mmol/l.
† Control perfusion was Krebs–Ringer phosphate buffer only.
significant ($r = 0.58$, $P < 0.01$) correlation between the amount of glucose absorbed and the size of the GIP response, as reflected in their respective plasma levels.

Solutions containing sucrose, maltose, $\beta$-methylglucoside, galactose, $\alpha$-methylglucoside or 3-0-methylglucose all produced significant stimulation of GIP when compared with control rats infused with KRP (Fig. 2 and Table 1), but there was no significant difference compared with control rats in portal vein GIP levels after perfusion with solutions of mannose, 2-deoxyglucose, 6-deoxygalactose, myoinositol, fructose or lactose (Table 1).

The stimulatory properties of glucose, sucrose, maltose or $\beta$-methylglucoside (100 mmol/l of each) were not significantly different. The stimulatory properties of 3-0-methylglucoside and $\alpha$-methylglucoside on the other hand were significantly less than those of glucose ($P < 0.025$).

Addition of 5 mmol phloridzin/l to a perfusate containing 50 mmol glucose/l prevented intestinal absorption of glucose and abolished the GIP response to it (Table 2).

Table 2. Levels of immunoreactive gastric inhibitory polypeptide (GIP) and glucose in the portal vein blood of rats after the addition of 5 mmol phloridzin/l to a perfusate of 50 mmol glucose/l. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Perusate</th>
<th>Glucose (mmol/l)</th>
<th>GIP (pmol/l)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krebs–Ringer</td>
<td>4.4 ± 0.5</td>
<td>58.8 ± 22.9</td>
<td>4</td>
</tr>
<tr>
<td>phosphate buffer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mm-glucose</td>
<td>11.2 ± 0.4**</td>
<td>184 ± 19*</td>
<td>5</td>
</tr>
<tr>
<td>50 mm-glucose+ phloridzin</td>
<td>3.7 ± 0.3</td>
<td>50.9 ± 10.8</td>
<td>5</td>
</tr>
</tbody>
</table>

* $P < 0.005$.  ** $P < 0.001$: when compared with control rats perfused with Krebs–Ringer phosphate buffer only (Student’s $t$-test).

**DISCUSSION**

Crane (1965, 1968) has postulated that glucose and galactose are actively transported across the mucosal cell brush-border in rat and man by a common sodium-dependent carrier protein which can be inhibited by phloridzin and which possesses a lower $K_m$ for glucose than for galactose. Studies have shown that hexoses with a minimal structural requirement of an intact pyranose ring and an hydroxyl group in the $\alpha$ position on the second carbon atom (C-2), are necessary for translocation across the brush-border membrane using the same transport system. The monosaccharide, fructose, which is not transported by this system, probably utilizes a separate membrane-bound carrier. Hydrolysis of disaccharides to constituent monosaccharides is carried out by specific enzymes situated at, or very close to, the luminal side of the brush-border. For sucrose and maltose at least, hydrolysis by their respective enzymes, sucrase and maltase, is not rate-limiting and does not restrict the supply of monosaccharides for active transport (Gary, 1975). It has been demonstrated in the hamster (Malathi, Ramaswami, Caspary & Crane, 1973; Ramaswami, Malathi, Caspary & Crane, 1974) that intestinal translocation of glucose derived from a variety of di- and oligosaccharides is different from that of glucose itself, suggesting the existence of separate, active-transport mechanisms for glucose in close spatial contact with their specific hydrolases which are distinct from the sodium-dependent transport system available for free glucose and galactose. Sirinek, Thomford, Pace, O’Dorisio, Crockett, Mazzaferrri & Cataland (1979) have measured plasma GIP responses in dogs after duodenal infusions of glucose, galactose, fructose, mannose, sorbitol, maltose, lactose or sucrose. Apart from 20% sucrose, which, in their experiments, did not stimulate GIP, our results are in broad agreement. Sirinek et al. (1979) concluded that structural integrity of the 6-membered ring conformation and the presence of an aldehyde group on C-1 is necessary to preserve active transport and GIP release. Examination of the molecular structures of the perfused carbohydrates and analogues in our study showed that complete loss of ability to stimulate GIP resulted from loss of the hydroxyl group at C-6 (6-deoxygalactose, fructose), loss or rotation to the $\beta$ form of the $\alpha$-hydroxyl group at C-2 (2-deoxyglucose, fructose, D-mannose)
or loss of the pyranose ring conformation (myoinositol). All the monosaccharides that stimulated release of GIP possessed a \(-\text{CH}_2\text{OH}\) group at C-5, a C-2 \(\alpha\)-hydroxyl group and an intact pyranose ring. Manipulation of the glucose molecule at the C-1 hydroxyl group, by substitution with a methoxy group in either the \(\alpha\) or the \(\beta\) orientation, was consonant with definite GIP release although in the case of \(\alpha\)-methylglucoside, this was significantly less than for an equimolar concentration of glucose. Substitution of the C-3 \(\beta\)-hydroxyl group by a \(\beta\)-methoxy group (3-\(O\)-methylglucose) was also consonant with definite but reduced release of GIP. The diminished response of GIP to galactose compared with glucose is in agreement with the known differences in affinity of the two molecules for the hexose transport system. The data presented define a basic molecular configuration which agrees with the requirements for active transport by the hexose pathway as postulated by Crane (1968) and establishes a link between release of GIP and this system. This link was further substantiated by the observation that in the presence of phloridzin the GIP response to glucose was abolished.

Of the various disaccharides tested, sucrose and maltose but not lactose stimulated GIP release. The failure of lactose to stimulate release of GIP is almost certainly due to low levels of lactose in the intestinal mucosa of the mature rat (Rubino, Zimbalatti & Auricchio, 1964).

Portal vein plasma GIP levels did not differ significantly after perfusion with equimolar solutions of glucose, sucrose or maltose (100 mmol/l of each). On hydrolysis, 100 mmol maltose/l should release twice the molar amount of glucose and this was reflected in portal vein glucose concentrations of 18.0±2.0 mmol/l following perfusion with 100 mmol maltose/l. These values were not significantly different from those obtained after perfusion with 200 mmol glucose/l. If all the glucose released by hydrolysis of maltose was available for transport by the sodium-dependent hexose transport system, the rise in plasma GIP levels provoked by 100 mmol maltose/l might have been expected to match those provoked by 200 mmol glucose/l (Table 1). This was not so, however, for 200 mmol glucose/l provoked significantly more \((P<0.05)\) release of GIP than 100 mmol maltose/l, thereby providing some additional evidence for the existence of the separate hydrolase-related transport system for glucose derived from maltose as postulated by Ramaswami et al. (1974).

Although it was not possible in the present study on rats to demonstrate evidence of hydrolase-related active transport for glucose derived from sucrose, our recent work on fasting human volunteers has shown differences in the time-courses of GIP release after oral sucrose and glucose that are consistent with this hypothesis (Sykes et al. 1979).

The results of experiments both on man and rats have suggested that glucose derived from disaccharide hydrolysis within the brush-borders of the intestinal mucosal cells is capable of stimulating GIP release and that the mechanism for stimulating GIP secretion, in addition to being linked to active transport of free glucose, is also linked to each of the specific hydrolase-related active transport systems. The question as to how the monosaccharides produced in the mucosal cell brush-border initiate release of GIP from the K cells, where it is produced, remains unsolved. Tight junctions between the two types of cells have been proposed as a possible channel of communication between them. If this is indeed so then changes in flux across the tight junctions of sodium ions generated in the mucosal cells by interaction between the stimulatory monosaccharides and their transport proteins might offer a possible explanation for their ability to stimulate release of GIP (J. D. Sheridan, personal communication). Variations in release of GIP in response to stimulation by sugars are probably functions of the relative affinities of the different monosaccharides for active transport proteins and the nature of the transport proteins themselves.

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GIP stimulation by actively transported sugars

REFERENCES


