

Stimulation of Gastric Inhibitory Polypeptide Release in *ob/ob* Mice by Oral Administration of Sugars and Their Analogues

PETER R. FLATT,† PIOTR KWASOWSKI AND CLIFFORD J. BAILEY*

Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, †Department of Biological and Biomedical Sciences, University of Ulster at Coleraine, Co. Londonderry, BT52 1SA and *Biology Division, Department of Pharmaceutical Sciences, Aston University, Birmingham, B4 7ET, United Kingdom

ABSTRACT The effect of oral administration of sugars and their analogues (glucose, galactose, fructose, mannose, sucrose, *N*-acetylglucosamine, 2-deoxyglucose, 3-O-methylglucose and α -methyl-glucoside) on plasma gastric inhibitory polypeptide (GIP) concentration was examined in 18-h fasted *ob/ob* mice. Administration of sucrose (5.52 mol/kg body wt), or the monosaccharides (11.04 mol/kg body wt) glucose, galactose or fructose, elicited prompt GIP responses that peaked at 30 min. Similar effects were induced by 3-O-methylglucose or α -methyl-glucoside, but the stimulatory action of 2-deoxyglucose was delayed. In contrast to the other sugars, *N*-acetylglucosamine decreased plasma GIP concentration, while mannose exerted no effect. The results suggest that sugars using the Na⁺-glucose cotransporter at the luminal brush border stimulate GIP release without the necessity of being metabolized or removed from the cell by the glucose transporter at the basolateral membrane. The ability of fructose to stimulate GIP release in *ob/ob* mice suggests that the Na⁺-glucose cotransporter does not represent an exclusive trigger for sugar-induced GIP secretion. *J. Nutr.* 119: 1300–1303, 1989.

INDEXING KEY WORDS:

- enteroinsular axis • gastric inhibitory polypeptide (GIP) • *ob/ob* mice • mannose
- fructose • sucrose • glucose • galactose
- sugar analogues

Studies in humans and experimental animals have shown that obesity-diabetes syndromes are often associated with increased activity of the enteroinsular axis, as exemplified by an enhanced secretion or insulinotropic action of gastric inhibitory polypeptide (GIP) (1–3). This abnormality is particularly marked in adult obese hyperglycemic (*ob/ob*) mice that serve as a useful model to investigate the causes and consequences of

GIP hypersecretion. Previous studies have indicated that hyperplasia of GIP-secreting K-cells, elevated intestinal GIP content and increased plasma GIP concentration of *ob/ob* mice are determined by the quantity and nutrient composition of the diet (4, 5).

Orally administered glucose, a mixture of amino acids and a fat emulsion each evoked a prominent increase in plasma GIP in adult *ob/ob* mice (6). Furthermore, neutral and basic amino acids were equipotent, whereas fatty acids produced particularly marked, but variable, GIP responses (7; Flatt, P. R., Kwasowski, P. & Bailey, C. J., unpublished data). Long-chain fatty acids were more potent stimulators of GIP release than fatty acids that are not esterified into triglycerides following absorption (7). To further evaluate the specificity and mechanism of nutrient-stimulated GIP release, the present study examined plasma GIP responses of *ob/ob* mice after oral administration of natural sugars and glucose analogues with well-defined intestinal transport characteristics.

MATERIALS AND METHODS

Animals. Groups of obese hyperglycemic (*ob/ob*) mice on the Aston background were used at 12–17 wk of age. The origin and characteristics of these mice have been described elsewhere (8–10). Briefly, heterozygous C57BL/6J *ob/+* breeding pairs from the Jackson Laboratory, Bar Harbor, ME were obtained in 1957 and outcrossed to two noninbred strains selected for high litter size and increased growth rate. Heterozygous breeding pairs from this stock were transferred to the University of Aston in 1966, where they were used to establish a closed noninbred colony. The severity of the diabetes in the Aston stock is intermediate between that of C57BL/6J and C57BL/KsJ *ob/ob* mice (9, 10).

Mice were housed in an air-conditioned room at $22 \pm 2^\circ\text{C}$ with a 12-h light/dark cycle. A standard pelleted nonpurified diet (mouse breeding diet, Heygate & Sons, Northampton, England) and tap water were supplied ad libitum. The diet consisted of 2.5% fat, 17.6% protein and 46.8% carbohydrate (digestible energy 15.3 MJ/kg diet) with added fiber, vitamins and minerals as described elsewhere (11).

Experimental procedure. Sugars (Sigma Chemical, Poole, England) were administered intragastrically to conscious 18-h fasted *ob/ob* mice. The sugars (all D-stereoisomers) tested were glucose, galactose, fructose, mannose, sucrose, N-acetylglucosamine, 2-deoxyglucose, 3-O-methylglucose and α -methyl-glucoside. All sugars except sucrose were administered at a dose of 11.04 mmol/(8 ml · kg body wt) corresponding to a dose of 2 g glucose/kg body wt that is commonly employed in glucose tolerance tests (6, 12). Sucrose, which yields equimolar amounts of glucose and fructose on hydrolysis, was given at half of this dose [5.52 mmol/(8 ml · kg body wt)]. Blood samples (60 μl) were taken from the tail tip of the mice immediately before and at 30, 60 and 120 min after administration of the sugars.

Analyses. Plasma immunoreactive GIP was measured by double-antibody radioimmunoassay (13) using donkey anti-rabbit gamma globulin antiserum to separate bound and free antigen. Immunoabsorbed GIP-

free plasma was used to minimize nonspecific interference, and parallelism was demonstrated between the standard curve and serially diluted *ob/ob* mouse plasma. Natural porcine GIP was used to prepare [^{125}I]GIP and as a standard. The GIP antiserum (RIC34/III), raised in rabbit against a porcine GIP-glutaraldehyde-ovalbumin conjugate, exhibits negligible cross-reactivity with other enteropancreatic hormones.

Statistics. Data were assessed a priori by means of two-way (sugar \times time) analysis of variance. Examination of the data in Figure 1 indicated heteroscedasticity (Hartley's $F_{\text{max}} = 105$). These data were therefore analyzed following \log_{10} transformation (Hartley's $F_{\text{max}} = 49$, indicating homoscedasticity). Differences between individual data points were assessed by one-way analysis of variance, followed by Duncan's multiple range analysis. Differences were considered to be significant if $P < 0.05$.

RESULTS

Plasma GIP responses of *ob/ob* mice to orally administered sugars are shown in Figure 1. Of the sugars tested, only mannose lacked a significant effect on plasma GIP concentration. Administration of sucrose, glucose, galactose or fructose elicited prompt GIP re-

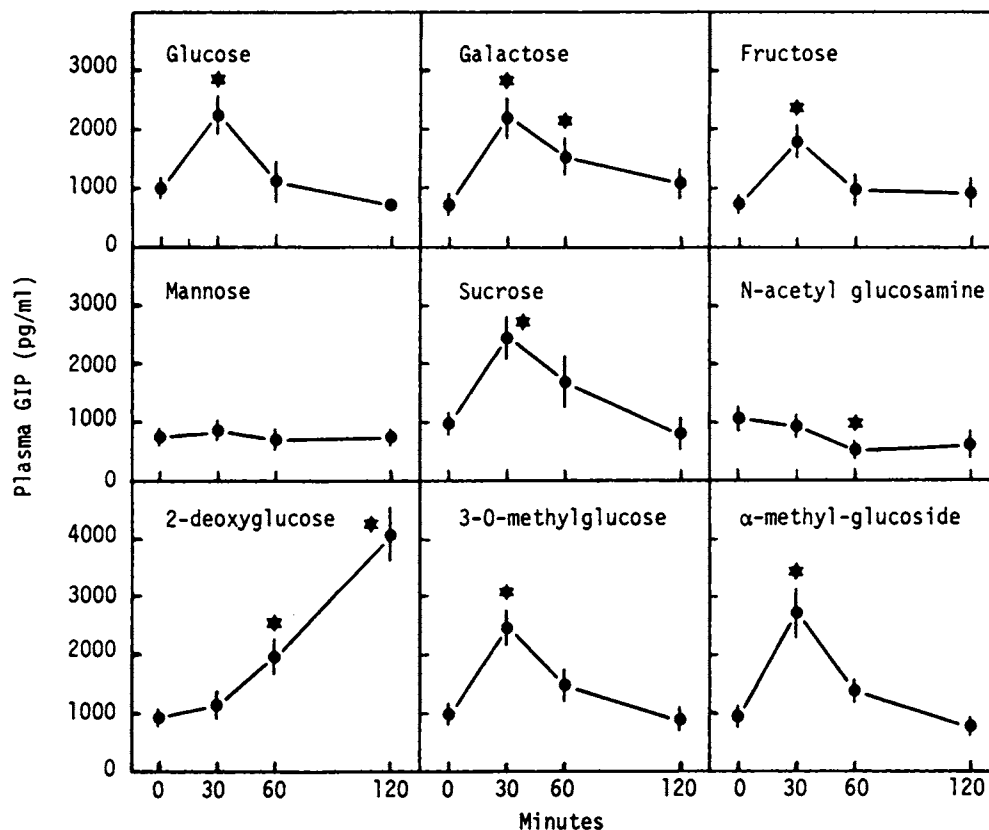


FIGURE 1 Plasma gastric inhibitory polypeptide (GIP) responses to sugars in 18-h fasted *ob/ob* mice. Values are means \pm SEM of groups of five mice. * $P < 0.05$ compared with time 0. ANOVA confirmed that plasma GIP concentration was significantly affected by time and by administration of different sugars ($P < 0.0001$). There was also a significant interaction between these two variables ($P < 0.0001$).

TABLE 1

Effects of sugars on plasma gastric inhibitory polypeptide (GIP) concentration in 18-h fasted *ob/ob* mice¹

Sugar	Incremental GIP response (0–30 min)	Integrated GIP response (0–120 min)
	pg/ml	pg/(ml·h)
Glucose	1215 ± 384 ^{ab}	313 ± 456 ^{ab}
Galactose	1467 ± 366 ^{ab}	1491 ± 175 ^b
Fructose	864 ± 353	861 ± 316 ^{ab}
Mannose	79 ± 341	-144 ± 393 ^a
Sucrose	1412 ± 315 ^{ab}	893 ± 470 ^{ab}
N-Acetylglucosamine	-148 ± 163	-994 ± 233 ^a
2-Deoxyglucose	113 ± 243	2367 ± 623 ^b
3-O-Methylglucose	1496 ± 440 ^{ab}	1030 ± 520 ^{ab}
α-Methyl-glucoside	1806 ± 515 ^{ab}	1112 ± 366 ^{ab}

¹Values are means ± SEM of groups of five mice. Superscript letters indicate $P < 0.05$ compared with ^a2-deoxyglucose and ^bN-acetylglucosamine, as assessed by Duncan's multiple range analysis. The incremental GIP responses and the integrated GIP responses to glucose, galactose, fructose, sucrose, 3-O-methylglucose and α-methyl-glucoside were not significantly different, as assessed by Duncan's multiple range analysis.

sponses that peaked at 30 min. Similarly rapid effects were observed in response to the analogues 3-O-methylglucose or α-methyl-glucoside. However, the stimulatory action of 2-deoxyglucose was considerably delayed, with GIP concentration continuing to increase at 120 min. In contrast to the other sugars, N-acetylglucosamine decreased plasma GIP concentration by 60 min.

Table 1 summarizes the incremental GIP response between 0 and 30 min, as well as the overall integrated response. The latter was calculated as: $\text{pg}/(\text{ml} \cdot \text{h}) = [\text{GIP at } (30 + 60 + 120 \text{ min}) - (3 \cdot \text{basal GIP})]/2$. No significant differences were noted in the incremental response of those sugars that exerted prompt stimulatory effects on GIP release, namely, sucrose, glucose, galactose, fructose, 3-O-methylglucose and α-methyl-glucoside. The overall integrated GIP response to these sugars was also similar. Compared with the other sugars, the overall integrated GIP response to 2-deoxyglucose was significantly enhanced (except compared with galactose), and the response to N-acetylglucosamine was significantly reduced (except compared with mannose).

DISCUSSION

Hyperinsulinemia is an early pathogenic feature in obese hyperglycemic (*ob/ob*) mice which makes an important contribution to the severity of insulin resistance and glucose intolerance (10). Short-term feeding of *ob/ob* mice with isoenergetic diets of different nutrient composition has shown that carbohydrate represents the major stimulus to the hyperinsulinemia (11, 14). Since intraperitoneal injection of glucose fails to elicit an insulin response in nonfasted *ob/ob* mice (12),

attention has focused on the involvement of the overactive enteroinsular axis and hypersecretion of GIP in the hyperinsulinemia of these mice (1). The present study has shown that a range of naturally occurring sugars, including glucose, galactose, fructose and sucrose, elicit rapid and prominent GIP responses in *ob/ob* mice. This observation, together with the earlier demonstration (6) that GIP provides a physiological stimulus to insulin secretion in *ob/ob* mice, offers a mechanistic link between the ingestion of carbohydrate and the hyperinsulinemia.

The present observation that sugars that serve as a substrate for the Na⁺-glucose cotransporter in the luminal brush border (15, 16) elicit GIP response is consistent with results obtained by single time-point sampling of the hepatic portal vein of anesthetized rats (17). Thus, glucose, galactose, α-methyl-glucoside and 3-O-methylglucose were equally effective in raising the plasma GIP concentration of *ob/ob* mice. Although these sugars utilize the same transport carrier at the brush border, α-methyl-glucoside is not a substrate for the glucose transporter at the basolateral membrane, and 3-O-methylglucose is not metabolized within the intestinal cells (16). This indicates that the GIP-releasing action of glucose and galactose requires neither their metabolism nor transport out of the cell. Nevertheless, the observation that fructose increased plasma GIP in *ob/ob* mice raises doubt as to whether the Na⁺-glucose cotransporter represents the sole trigger to GIP secretion. Although not confirmed in mice, fructose absorption is generally considered to involve a brush border transporter independent of both Na⁺ and the glucose transport system (15, 16).

The GIP-releasing action of fructose in *ob/ob* mice contrasts with the reported lack of effect of this sugar on circulating GIP concentration in normal human subjects and rats (17, 18). This lack of effect in the rat may reflect the use of a single blood sample to assess GIP release 30 min after luminal perfusion of the sugar (17). The divergence from humans represents either a pathological feature associated with obesity-diabetes or a peculiarity to mice. In this respect it is notable that sucrose, which is hydrolyzed to glucose and fructose by brush border enzymes, elicited a GIP response comparable to twice the molar dose of glucose or fructose alone. Since the effect of glucose on GIP release is dose-dependent in all species studied (18–20), a smaller GIP response to sucrose would be predicted if fructose were not stimulatory. Consistent with this observation, an equimolar concentration of glucose and sucrose elicited comparable GIP responses in the rat (17). Moreover, it is of interest that incorporation of fructose, instead of glucose, into the diet of *db/db* mice did not ameliorate the diabetes-obesity syndrome (21).

Absorption of mannose, 2-deoxyglucose and N-acetylglucosamine involves neither the glucose nor fructose luminal transport system (15, 16). Mannose did not elicit a GIP response, whereas 2-deoxyglucose pro-

duced a delayed, but substantial, elevation of plasma GIP concentration. The latter effect suggests an action that is independent of intestinal transport, such as activation of the sympathetic nervous system (22) and the release of somatostatin (23). The mechanism underlying the inhibitory effect of *N*-acetylglucosamine on circulating GIP concentrations is also unclear. Although this sugar stimulates insulin release in the rat (24), suppression of plasma GIP does not involve insulin-feedback inhibition of intestinal K-cells, which is defective in *ob/ob* mice (6).

ACKNOWLEDGMENTS

The authors wish to thank R. J. Howland and V. Marks for constructive comments.

LITERATURE CITED

1. BAILEY, C. J. & FLATT, P. R. (1988) The enteroinsular axis in models of hyperinsulinaemic and hypoinsulinaemic diabetes. In: *Frontiers in Diabetes Research: Lessons from Animals Diabetes*, Vol. 2 (Shafir, E. & Renold, A. E., eds.), pp. 217–224, John Libbey, London.
2. MORGAN, L. M., FLATT, P. R. & MARKS, V. (1988) Nutrient regulation of the enteroinsular axis and insulin secretion. *Nutr. Res. Rev.* 1: 79–97.
3. KRARUP, T. (1988) Immunoreactive gastric inhibitory polypeptide. *Endocr. Rev.* 9: 122–134.
4. FLATT, P. R., BAILEY, C. J., KWASOWSKI, P., SWANSTON-FLATT, S. K. & MARKS, V. (1985) Glucoregulatory effects of cafeteria feeding and diet restriction in genetically obese hyperglycaemic (*ob/ob*) mice. *Nutr. Rep. Int.* 32: 847–854.
5. BAILEY, C. J., FLATT, P. R., KWASOWSKI, P., POWELL, C. J. & MARKS, V. (1986) Immunoreactive gastric inhibitory polypeptide and K-cell hyperplasia in obese hyperglycaemic (*ob/ob*) mice fed high fat and high carbohydrate cafeteria diets. *Acta Endocrinol.* (Copenhagen) 112: 224–229.
6. FLATT, P. R., BAILEY, C. J., KWASOWSKI, P., PAGE, T. & MARKS, V. (1984) Plasma immunoreactive gastric inhibitory polypeptide in obese hyperglycaemic (*ob/ob*) mice. *J. Endocrinol.* 101: 249–256.
7. KWASOWSKI, P., FLATT, P. R., BAILEY, C. J. & MARKS, V. (1985) Effects of fatty acid chain length and saturation on gastric inhibitory polypeptide release in obese hyperglycaemic (*ob/ob*) mice. *Biosci. Rep.* 5: 701–705.
8. FLATT, P. R. & BAILEY, C. J. (1981) Abnormal plasma glucose and insulin responses in heterozygous lean (*ob/+*) mice. *Diabetologia* 20: 573–577.
9. BAILEY, C. J., FLATT, P. R. & ATKINS, T. W. (1982) Influence of genetic background and age on the expression of the obese hyperglycaemic syndrome in Aston *ob/ob* mice. *Int. J. Obes.* 6: 11–21.
10. BAILEY, C. J. & FLATT, P. R. (1986) Animal models of diabetes. In: *Recent Advances in Diabetes*, Vol. 2 (Nattrass, M., ed.), pp. 71–89, Churchill Livingstone, Edinburgh.
11. FLATT, P. R. & BAILEY, C. J. (1982) Role of dietary factors in the hyperinsulinemia of genetically obese hyperglycaemic (*ob/ob*) mice. *J. Nutr.* 112: 2212–2216.
12. FLATT, P. R. & BAILEY, C. J. (1981) Development of glucose intolerance and impaired plasma insulin response to glucose in obese hyperglycaemic (*ob/ob*) mice. *Horm. Metabol. Res.* 13: 556–560.
13. MORGAN, L. M., MORRIS, B. & MARKS, V. (1978) Radioimmunoassay of gastric inhibitory polypeptide. *Ann. Clin. Biochem.* 15: 172–177.
14. FLATT, P. R. & BAILEY, C. J. (1984) Dietary components and plasma insulin responses to fasting and refeeding in genetically obese hyperglycaemic (*ob/ob*) mice. *Br. J. Nutr.* 51: 403–413.
15. KIMMICH, G. A. (1981) Intestinal absorption of sugar. In: *Physiology of the Gastrointestinal Tract*, 1st ed. (Johnson, L. R., ed.), pp. 1035–1061, Raven Press, New York.
16. HOPFER, U. (1987) Membrane transport mechanisms for hexoses and amino acids in the small intestine. In: *Physiology of the Gastrointestinal Tract*, 2nd ed. (Johnson, L. R., ed.), pp. 1499–1526, Raven Press, New York.
17. SYKES, S., MORGAN, L. M., ENGLISH, J. & MARKS, V. (1980) Evidence for preferential stimulation of gastric inhibitory polypeptide secretion in the rat by actively transported carbohydrates and their analogues. *J. Endocrinol.* 85: 201–207.
18. MORGAN, L. M. (1979) Immunoassayable gastric inhibitory polypeptide: Investigations into its role in carbohydrate metabolism. *Ann. Clin. Biochem.* 16: 6–14.
19. PEDERSON, R. A., SCHUBERT, H. E. & BROWN, J. C. (1975) Gastric inhibitory polypeptide. Its physiological release and insulinotropic action in the dog. *Diabetes* 24: 1050–1056.
20. MARTIN, E. W., SIRINEK, K. R., CROCKETT, S. E., O'DORISIO, T. M., MAZZAFERRI, E. L., THOMFORD, N. R. & CATALAND, S. (1975) Release of gastric inhibitory polypeptide: Comparison of hyperosmolar carbohydrate solutions as stimuli. *Surg. Forum* 26: 381–382.
21. LEITER, E. H., COLEMAN, D. L., INGRAM, D. K. & REYNOLDS, M. A. (1983) Influence of dietary carbohydrate on the induction of diabetes in C57BL/KsJ-*db/db* diabetes mice. *J. Nutr.* 113: 184–195.
22. FISHER, D. A. & BROWN, M. R. (1980) Somatostatin analogue: Plasma catecholamine suppression mediated by the central nervous system. *Endocrinology* 107: 714–718.
23. BODEN, G., MASTER, R. W., SATTTLER, M. A., MARTIN, J. S., TANSY, M. F. & OWEN, O. E. (1982) Adrenergic control of somatostatin release. *Endocrinology* 111: 1166–1172.
24. ASHCROFT, S. J. H. & CROSSLEY, J. R. (1975) The effect of glucose, *N*-acetylglucosamine, glyceraldehyde and other sugars on insulin release in vivo. *Diabetologia* 11: 279–284.