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Effect of Phenformin on the Response of Plasma Intestinal Glucagon-like Immunoreactivity (GLI) to Oral Glucose in Gastrectomized Subjects (39009)

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(Introduced by Piero P. Foà)

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It has been established that the secretion of intestinal glucagon-like immunoreactivity (GLI) is stimulated by the oral or intraduodenal administration of various sugars (1–5), salts (6) and fat (7). In a previous paper concerning the effectiveness of different sugars in stimulating GLI secretion in gastrectomized subjects, we have reported evidence that this GLI response is the result of chemical, mechanical or osmotic stimuli (8). However, the possible role of the intestinal absorption of sugar upon GLI release remains unknown. In order to clarify this point, we have studied the plasma GLI response to the oral or intravenous administration of glucose in gastrectomized subjects before and after treatment with phenformin, a drug believed to impair the intestinal absorption of glucose (9–11). Gastrectomized subjects were used because of their exaggerated GLI response to ingested sugars.

Materials and methods. The observations were carried out in 24 male and 2 female, 22–60-year-old non-obese patients who had been gastrectomized for various benign gastric or duodenal diseases 1–19 years previously except for three cases for gastric carcinoma (Billroth I, 16 cases; Billroth II, 10 cases). It could not be explored whether or not vagotomy had been included. All patients had a nondiabetic glucose tolerance. The following glucose loading tests were performed: 22 subjects were given the equivalent of 100 g of glucose in the form of Toleran G[1] orally after an overnight fast; 4 patients received 25 g of glucose dissolved in 100 ml of water, intravenously. Within 2 months, the tests were repeated 60 min after the ingestion of 150 mg of phenformin hydrochloride.

Heparinized venous blood was obtained before and after the glucose load, as indicated in Figs. 1 and 2. In addition, a blood sample was obtained immediately before the administration of phenformin. The specimens were placed in an ice bath and were centrifuged as soon as possible. The plasma was removed, Trasylol[2] was added (4000 KIU/ml of sample) to prevent the proteolytic destruction of glucagon, and the mixture was stored at −20° until analyzed. Glucagon was measured in 0.2 ml of plasma using a double-antibody radioimmunoassay previously described (12), with minor modifications (13). Two rabbit antiglucagon sera (AGS) were used: AGS 159[3] which binds both pancreatic and gut glucagon (14) and, hence, measures "total GLI," and AGS K47[4] which measures mostly pancreatic glucagon (IRG, 15). All specimens obtained from the same subject were assayed in duplicate and in the same run to eliminate errors due to interassay variation. Plasma glucose was measured using a modification of the Hoffman ferricyanide technique adapted to an AutoAnalyzer (16); immunoreactive insulin (IRI) was determined using an insulin assay kit.[5]

The statistical significance of the data was calculated using the paired *t* test.

Results. Sixty minutes after the administration of phenformin hydrochloride the

[1] Gift of Shimizu Pharmaceutical Co., Shimizu, Japan.

[2] Trasylol, Bayer, Leverkusen, Germany.

[3] Gift of Dr. P. P. Foà, Department of Research, Sinai Hospital of Detroit, Detroit, MI.

[4] Gift of Dr. L. Heding, Novo Research Institute, Copenhagen, Denmark.

[5] Phadebas, Pharmacia Laboratories, Inc., Sweden.

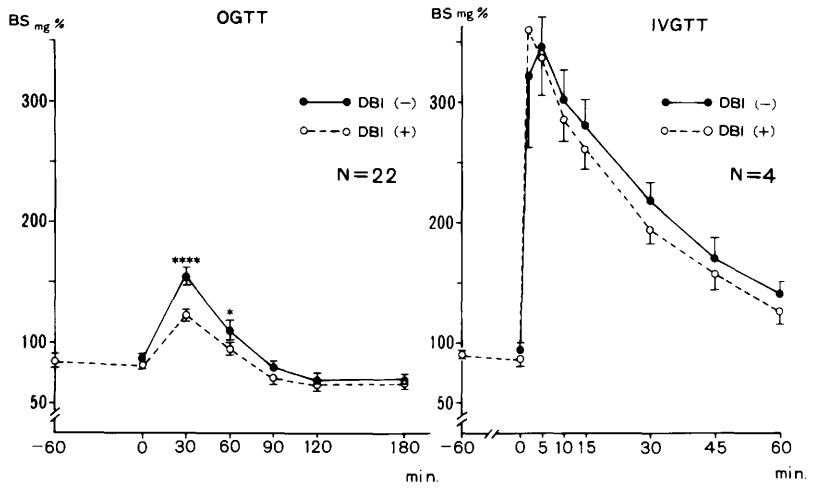

FIG. 1. Effect of phenformin (DBI) on the mean blood sugar responses to oral and intravenous glucose loading. Broken lines, with DBI; solid lines, without DBI; ****, $P < 0.005$; *, $P < 0.05$.

mean fasting blood sugar level of 22 gastrectomized subjects remained unchanged (84 ± 1.8 vs 83.1 ± 1.6 mg). However, the drug suppressed the average glucose response to an oral load. Thus, the glucose tolerance curve reached a peak of 124.5 ± 4.3 mg/100 ml in the drug-treated patients but rose to 154.0 ± 7.4 mg/100 ml in the untreated patients ($P < 0.005$). A significant difference was noted also at the 60-min point ($P < 0.05$). In contrast, the rate of glucose disappearance (K value) after intravenous loading was not affected by the previous administration of phenformin (control, K = 3.11 ± 0.66; after DBI, K = 3.22 ± 0.13). The changes in plasma GLI and pancreatic IRG levels after the oral and the intravenous administration of glucose are shown in Table I. The drug did not alter either the basal or the peak level of plasma GLI attained 30 min following the ingestion of glucose. However, 2 and/or 3 hr thereafter, all but one of the subjects exhibited higher GLI concentrations after phenformin treatment than before. On the other hand, the plasma total GLI response to the intravenous injection of glucose was not modified by pretreatment with the drug. Nor did the drug have any effect on the plasma pancreatic IRG response to oral glucose. There was no significant difference in plasma GLI and pancreatic IRG responses between the types of gastrectomy. The effect of phenformin on the plasma IRI response to the oral and intravenous administration of glucose is seen in Fig. 2. Phenformin pretreatment did not alter fasting plasma IRI levels but decreased the response to oral glucose. The difference was significant at the 30-min point ($P < 0.05$). In contrast, the mean IRI curve obtained after rapid intravenous glucose injection was unaltered by phenformin treatment.

Discussion. Although the factors affecting the secretion of intestinal GLI have been studied repeatedly (3, 6, 7), little is known about the mechanism by which intestinal GLI is released. Gastrectomized subjects receiving an intestinal bolus of relatively concentrated glucose solution showed a plasma GLI response greater than that noted in normal subjects (1, 8, 17). This response increased with the amount of glucose solution ingested, suggesting that mechanical or osmotic stimuli to the gut may have contributed to the release of GLI (8). However, the possibility that the intestinal absorption of glucose, per se, may be a factor, could not be excluded because the hyperglucagonemia observed was always associated with hyperglycemia.

The present study demonstrates that the plasma GLI response to oral glucose is not reduced but, rather, augmented in patients pretreated with phenformin, even though the drug decreased the accompanying hyper-

TABLE I. EFFECT OF PHENFORMIN (DBI) ON MEAN PLASMA IRG RESPONSE TO ORAL AND INTRAVENOUS GLUCOSE LOADING.

	Time after oral administration of glucose (min)						
	−60	0	30	60	90	120	180
Oral glucose loading							
Total GLI (22)[a] (ng/ml)							
Without DBI		0.73 ±0.08[b]	1.55 ±0.17	1.36 ±0.15	1.34 ±0.16	1.21 ±0.18	1.07 ±0.15
With DBI	0.77 ±0.08	0.73 ±0.07	1.67 ±0.18	1.60 ±0.16	1.59 ±0.18	1.57 ±0.14	1.47 ±0.18
P						<0.005	<0.005
Pancreatic IRG (16)[a] (ng/ml)							
Without DBI		0.28 ±0.05	0.34 ±0.06	0.32 ±0.05	0.33 ±0.05	0.32 ±0.06	0.34 ±0.06
With DBI	0.31 ±0.06	0.29 ±0.05	0.39 ±0.06	0.31 ±0.05	0.39 ±0.06	0.34 ±0.05	0.34 ±0.04

	Time after intravenous injection of glucose (min)							
	−60	0	2	5	15	30	45	60
Intravenous glucose loading								
Total GLI (4)[a] (ng/ml)								
Without DBI		0.77 ±0.18	0.63 ±0.19	0.58 ±0.13	0.67 ±0.14	0.63 ±0.14	0.62 ±0.11	0.59 ±0.13	0.70 ±0.18
With DBI	0.80 ±0.15	0.68 ±0.12	0.57 ±0.12	0.62 ±0.11	0.77 ±0.15	0.80 ±0.15	0.66 ±0.13	0.77 ±0.19	0.72 ±0.11

[a] Number of cases measured in parentheses.
[b] Mean ± SEM.

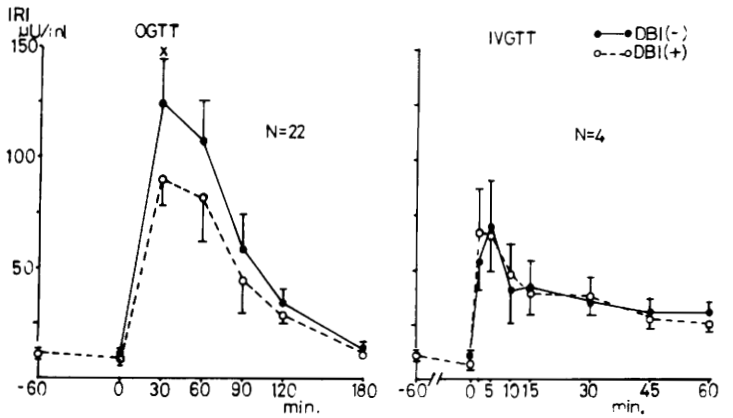

FIG. 2. Effect of phenformin (DBI) on the mean plasma IRI responses to oral and intravenous glucose loading. Broken lines, with DBI; solid lines, without DBI; *, $P < 0.05$.

glycemia, perhaps by reducing the intestinal absorption of glucose (9–11). Since no significant changes in plasma pancreatic IRG levels could be demonstrated during the tests, the observed rise in plasma GLI probably represents an increase in the intestinal release of GLI. Thus, as other investigators have suggested (18), the intestinal absorption of glucose does not appear to be a significant factor in the GLI response to oral glucose. The higher concentration of plasma GLI observed 2 and 3 hr after the ingestion of glucose in patients pretreated with phenformin could be attributed to a persistent stimulation by glucose retained in the gut as a result of an impaired intestinal absorption. Butylbiguanide, closely related to phenformin, was shown to diminish glucose uptake from *in vivo* perfused canine intestine (9), and phenformin inhibited glu-

cose uptake in everted sacs of rat intestine (11). Additional evidence for this inhibitory action was obtained by Wingate *et al.* (19) who demonstrated that phenformin significantly diminished mucosal glucose uptake by isolated sheets of human ileum. Taking into consideration these facts and the finding that phenformin pretreatment flattened the blood sugar response to oral glucose without altering the response to intravenous glucose in our subjects, it is reasonably presumed that intestinal glucose absorption had been indeed impaired in our subjects, though we have no data concerning the extent to which the drug impaired glucose absorption. Of course, the possibility that phenformin may have an effect on the intestinal GLI response to glucose has not been excluded, even though the drug did not affect either the facting plasma GLI levels nor their response to intravenously administered glucose.

It has been reported that the sulfonylureas suppress the secretion of pancreatic glucagon (20, 21); however, the effect of biguanide derivatives has scarcely been studied (22): Our data suggest that phenformin, in doses capable of reducing the hyperglycemic response to an oral glucose load, does not alter plasma pancreatic IRG levels. The normally observed suppression of pancreatic IRG after the ingestion of glucose (15) was not observed in our gastrectomized subjects. This unexpected result could be accounted for by the even-if-minimal cross-reactivity of AGS K47, which could have picked up a significant amount of intestinal GLI in view of its markedly increased concentration after oral glucose loading. It should be noted that the levels of plasma pancreatic IRG reported herein are higher than those obtained by other investigators (23) but are similar to the levels reported by Heding *et al.* (15) whose antibody we employed in our assay.

The suppression of insulin response to oral but not to intravenous glucose after previous administration of phenformin argues against a direct inhibitory effect of the drug on the B cells of the pancreatic islets.

Summary and conclusion. The effect of phenformin (DBI) on the plasma intestinal glucagon-like immunoreactivity (GLI) and pancreatic glucagon (IRG) responses to oral and intravenous glucose loads were studied in 26 gastrectomized subjects, using a cross-reacting and an IRG-specific antiserum. The drug produced no significant changes in fasting GLI and IRG levels. Thirty minutes after oral glucose alone, the total GLI level rose to a peak of 1.55 ± 0.17 ng/ml in the untreated subjects and to a maximum level of 1.67 ± 0.18 ng/ml in the DBI-pretreated subjects. However, the mean GLI levels obtained 120 and 180 min after oral glucose were significantly higher after treatment with DBI. The blood sugar and IRI responses to oral glucose were lowered significantly by DBI pretreatment. DBI did not alter the glucose, IRI, IRG and GLI response to intravenous glucose. These results suggest that the release of intestinal GLI is not related to the intestinal absorption of glucose.

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