Effects of Gastric Inhibitory Polypeptide in the Response to Prolonged Parenteral or Enteral Alimentation in Rats

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SUMMARY

To examine the effects of long-term elevation of plasma gastric inhibitory polypeptide (GIP), the responses to parenteral (PA) or enteral (EA) alimentation were studied in conscious rats with duodenal and venous cannulae. A weight-maintaining liquid diet (84% as glucose, 16% as amino acids) was infused at a constant rate for 6 days by either route, and daily blood samples were taken. A subset of animals receiving PA also received porcine GIP with the infusate (PA plus GIP; plateau plasma immunoreactive GIP, IRGIP, 610 ± 120 pg/ml). With PA, plasma IRGIP did not change from basal levels, whereas with EA IRGIP rose to virtual plateau levels (mean 530 ± 110 pg/ml). In the steady state, plasma immunoreactive insulin (IRI) was significantly lower with EA (mean, 153 ± 5 μU/ml) than with PA (mean, 226 ± 15 μU/ml), which in turn was lower than with PA plus GIP (mean, 375 ± 23 μU/ml, $P < 0.001$ by ANOVA). A similar ranking of plasma glucose levels occurred in the steady state, with means of 113 ± 7 (EA), 126 ± 3 (PA), and 184 ± 9 (PA plus GIP) mg/dl ($P < 0.001$ by ANOVA). To assess the response to transient hyperglycemia in the steady state, an intravenous glucose bolus was given to each group on the fifth day. Peak plasma IRI levels did not differ among the three groups; however, the glucose disappearance rate was significantly slower with PA plus GIP compared with either EA or PA. Assuming that porcine GIP did not stimulate glucose production, this peptide appeared to induce hyperinsulinemia with insulin resistance during parenteral alimentation. The contrasting features of relatively low glucose and insulin levels during enteral alimentation associated with high levels of endogenous IRGIP in the blood suggest either (1) that the findings depend on variations of GIP or its actions in the different species, or (2) that mechanisms originating in the intestine act to preserve insulin sensitivity during absorption of nutrients from the gut under physiologic conditions. **DIABETES 1985; 34:1108–12.**

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In man, the concentration of immunoreactive gastric inhibitory polypeptide (IRGIP) in the plasma is elevated after ingestion of a meal and does not return to the postabsorptive (basal) level for several hours.[1] Thus, with normal feeding patterns, plasma levels of IRGIP may be elevated above basal postabsorptive values for most of diurnal period. It has been shown that parenteral administration of GIP can augment the secretion of insulin in response to a rise of blood glucose in animals, and it has been suggested that this peptide acts as a hormonal mediator of the enteroinsular axis.[2,3] The demonstration of high blood levels of IRGIP in obese subjects with or without abnormal glucose tolerance has also led to the suggestion that GIP may be partly responsible for hyperinsulinemia in such subjects, and thus may be involved in the development of insulin resistance in these states.[4]

Earlier investigations have generally been restricted to studies of acute responses to administration of nutrients. In the present study, we have examined the effects of sustained elevation of plasma concentrations of IRGIP derived from either endogenous or exogenous (porcine) sources in normal rats. The results show that under pharmacologic conditions, with parenteral delivery of nutrients together with porcine GIP, long-term elevation of plasma GIP can result in glucose intolerance and hyperinsulinemia. In contrast, during intestinal delivery of nutrients, relatively low plasma levels of glucose and insulin were observed, again associated with maintained high blood levels of endogenous IRGIP. The results suggest either (1) that the findings depend on variations of the nature of GIP or of its actions in the different species, or (2) that sustained high levels of GIP in the blood can cause insulin resistance, and that hitherto unrecognized physiologic mechanisms serve to preserve or enhance insulin sensitivity during absorption of nutrients from the gut.

MATERIALS AND METHODS

Preparation of animals. Male Sprague-Dawley rats (initial body weight 250–350 g) were prepared under anesthesia by placement of a blood-sampling cannula in the right jugular vein, and of a feeding cannula in the proximal duodenum or the external iliac vein. The cannulae were tunneled subcutaneously (s.c.) and exteriorized in the midscapular region; during the infusions they were attached to polyethylene tubing protected by wire coils that exited at the top of the cage and allowed the animals to move about freely. No additional water other than the infusate was supplied, and, to discourage coprophagy, open-mesh cages were used. The urine was drained through funnels to collecting vessels.

Experimental protocol. Infusion studies were initiated 4 days after implantation of the cannulae, when the animals had recovered their preoperative weights while feeding on regular chow. The nutrient solution delivered 84% of the calories as glucose and 16% as amino acids, and was close in composition to infusates used in other studies of parenteral nutrition in rats.[5] The calorie load was approximately 280 kcal/kg/day, and was designed to maintain the mean body weight in the enterally fed group through the 6-day infusion period. The animals were infused with the nutrient solution via the central vein or duodenal cannula at a constant rate through 6 days. A subset of the animals received the intravenous (i.v.) infusion together with highly purified porcine GIP 1.6 μg/kg, a dose designed to raise the plasma level of IRGIP into the same range as that observed during duodenal infusions of the nutrient mixture. Blood samples were taken after a 10-h fast before beginning the infusions, and at 24, 48, 72, 96, 120, and 144 h. The sampling procedure was successful in the majority of animals over the full time course of the experiment, but was interrupted in some. Intravenous glucose tolerance tests were carried out on the fifth day; the continuous infusion was not interrupted, and a bolus dose of glucose was given by i.v. injection (1.1 g/kg body wt): blood samples were taken at 2, 8, 20, and 50 min thereafter for determination of plasma glucose and IRI.

Assays. Plasma was separated and frozen for later determination of glucose (using a Beckman glucose analyzer, Beckman Instruments, Fullerton, California), and for radioimmunoassays for immunoreactive insulin (IRI), immunoreactive glucagon (IRG, antiserum 30K provided by Dr. R. H. Unger, Dallas, Texas), and immunoreactive GIP (antiserum GP24 provided by Dr. J. C. Brown, Vancouver, British Columbia) by methods previously described.[2,3] With this assay, endogenous rat IRGIP showed similar dilution characteristics to porcine standard GIP.

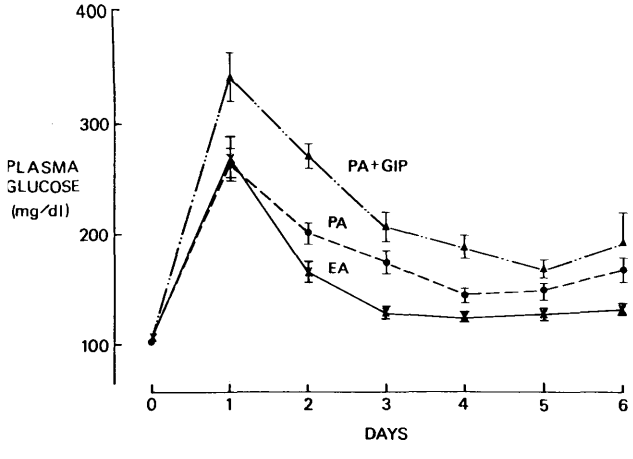

FIGURE 1. Mean venous plasma glucose concentrations in the three groups of animals (± SEM). EA indicates enteral alimentation, PA represents parenteral alimentation, and PA + GIP represents parenteral alimentation with addition of GIP, as described in the text.

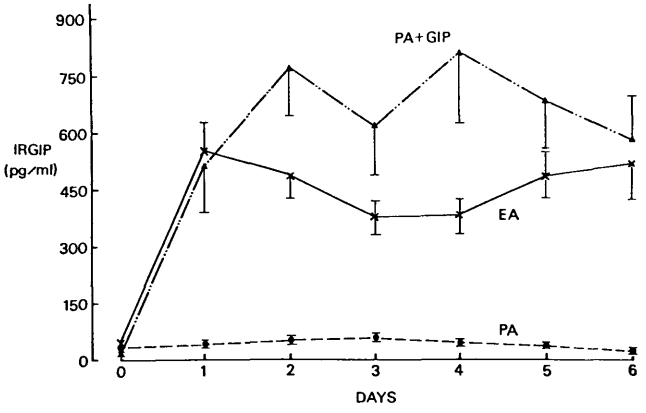

FIGURE 2. Mean plasma immunoreactive GIP concentrations in the three groups of animals (± SEM). EA indicates enteral alimentation, PA represents parenteral alimentation, and PA + GIP represents parenteral alimentation with addition of GIP, as described in the text.

Statistics. Comparisons between the means of the several observations at given times were analyzed using Student's *t*-test. To determine the significance of differences among the study groups when comparing sets of sequential observations, a nested-design analysis of variance (ANOVA) was employed.[6]

RESULTS

Plasma hormone and glucose concentrations. Mean plasma glucose concentrations determined over the 6-day study period are shown in Figure 1. At 24 h, the mean plasma glucose concentrations in the enterally and parenterally fed (without GIP) groups were not significantly different. The means then diverged, those in the enterally fed animals falling significantly lower at each subsequent sampling time ($P < 0.05$–0.005), with an overall significance of $P < 0.001$ as determined by ANOVA. In the rats infused i.v. with the same nutrient solution together with GIP, the mean plasma glucose concentration was significantly higher than in those receiving i.v. infusions without GIP at 24, 48, and 96 h, with an overall significance of $P < 0.001$.

The mean plasma levels of IRGIP in the three groups of rats are shown in Figure 2. There was no significant change of plasma IRGIP in the parenterally fed rats that did not receive GIP. During enteral alimentation, there was a highly significant rise of plasma IRGIP, which was maintained throughout the study. The addition of GIP to the parenteral infusate resulted in plasma IRGIP levels in a range overlapping values for endogenous rat IRGIP observed in the enterally fed animals, with mean values significantly greater at days 2 and 4, with an overall significance of $P < 0.001$.

The mean plasma concentrations of IRI in the three groups of rats are shown in Figure 3. These were not significantly different at 24 h; subsequently they diverged, the mean concentration falling to the lowest level in the enterally fed ani-

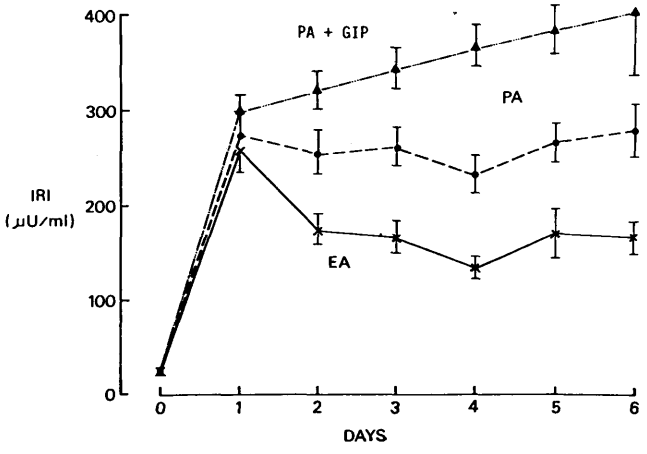

FIGURE 3. Mean plasma immunoreactive insulin levels in the three groups of animals (± SEM). EA indicates enteral alimentation, PA represents parenteral alimentation, and PA + GIP represents parenteral alimentation with addition of GIP, as described in the text.

mals. The level achieved by 24 h in the parenterally fed rats that did not receive GIP was maintained through the study. In the rats receiving parenteral alimentation with addition of GIP, the mean plasma levels of IRI rose progressively through the 6-day period. The mean values in the three sets were significantly different at each time point after 48 h, and the overall differences among them were significant ($P < 0.001$). Mean plasma concentrations of total IRG (Figure 4) did not change significantly from the baseline in any group.

Intravenous glucose tolerance tests. The results of the i.v. glucose tolerance tests are shown in Figure 5 and Table 1. Glucose disappearance rates ($t_{1/2}$, time taken for the plasma glucose to fall to one-half of any given value) were calculated using linear regression analysis after logarithmic transformation of the data. The $t_{1/2}$ was least in the enterally alimented group, followed by the group receiving parenteral alimentation alone. The greatest $t_{1/2}$ was observed in the group receiving parenteral alimentation with GIP. The associated insulin responses were similar at the peaks in all three groups (Figure 6), and there were no significant differences thereafter between enterally or parenterally fed groups as IRI levels returned to the prebolus baseline concentrations. However, the rats that received exogenous GIP showed a sustained elevation of plasma IRI levels.

Body weights at initiation and termination of the nutrient infusions. Table 2 shows the mean body weights at commencement and termination of the infusions. The mean weight of the enterally fed rats did not change significantly from the initial value. A modest but significant increase in mean body weight was observed in both groups of parenterally fed rats.

DISCUSSION

In acute studies of the response to nutrients delivered i.v. or into the gastrointestinal tract in normal rats, enhancement of plasma insulin levels associated with absorption of glucose from the gut, with relative lowering of blood glucose levels after the intestinal delivery of glucose, have been observed.[7] Intravenous administration of porcine GIP enhances the acute insulin response to parenteral administration of glucose in postabsorptive rats and man, and lowers the resulting plasma glucose levels in both species.[2,3] Accordingly, the rat appears to provide a valid model for studies of the so-called enteroinsular axis, as it was described in man.

In the present study of prolonged alimentation, approximate steady-state conditions were attained through the second half of the experiment, when the state of each group was distinct from the others. During enteral alimentation, the nutrient load was assimilated with relatively small increments of the blood glucose and insulin levels compared with those resulting from parenteral delivery of the same nutrient load. This adaptation occurred between 48 and 72 h of alimentation. Assuming that the very low levels of initial (hepatic portal) extraction of glucose demonstrated in dogs during portal i.v. infusion of glucose,[8] and in man during absorption of glucose from the gut,[9] also occur in rats, the present findings are unlikely to be accounted for by changes in first-pass

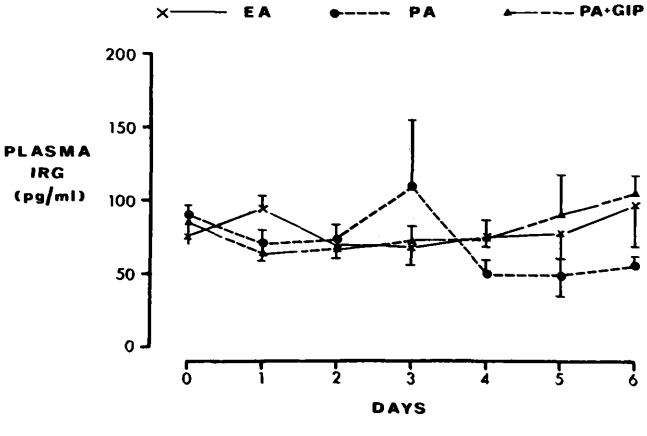

FIGURE 4. Mean plasma immunoreactive glucagon levels in the three groups of animals (± SEM). EA indicates enteral alimentation, PA represents parenteral alimentation, and PA + GIP represents parenteral alimentation with addition of GIP, as described in the text.

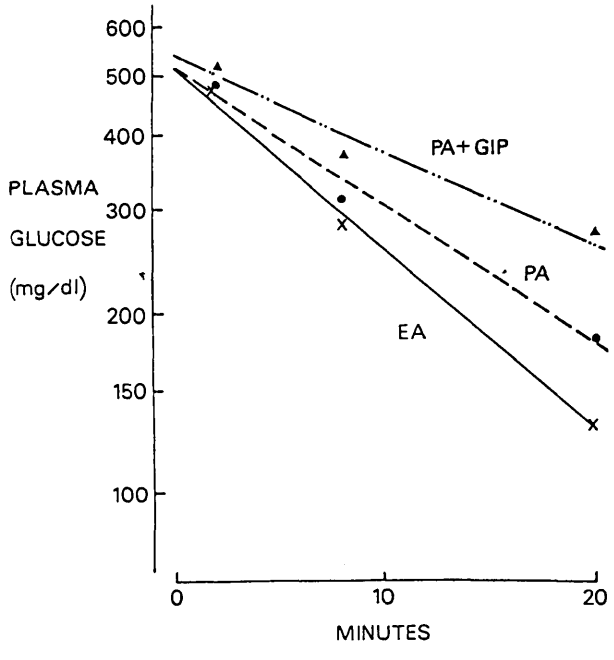

FIGURE 5. Mean plasma glucose concentrations after the bolus injection of glucose during the study (± SEM), as described in the text.

TABLE 1
Intravenous glucose tolerance test on day 5 of infusion (Figure 5)

Study group	$T_{\cdot 2}$ (min)
EA	9.93 ± 0.52
PA	14.47 ± 1.72
PA + GIP	19.78 ± 1.25

Glucose disappearance rates after an i.v. glucose bolus on day 5 given to rats receiving enteral (EA), parenteral (PA), or GIP-supplemented parenteral alimentation (PA + GIP). The differences between the mean values for $t_{\cdot 2}$ in the three sets were significant by ANOVA ($P < 0.05$).

hepatic extraction of glucose. They are also unlikely to be accounted for by higher rates of production of glucose in the parenterally alimented groups, given the relative hyperinsulinemia of the rats that did not receive infusions of GIP, and the lack of change of plasma IRG in either group.

When porcine GIP was added to the parenteral infusions, the elevation of blood glucose was greater than with administration of parenteral nutrient alone, and this was associated with yet higher plasma insulin levels. Since there was no increase of plasma immunoreactive glucagon levels in the rats that received GIP by infusion, and since infusion of GIP in dogs does not stimulate endogenous production of glucose,[10] it is unlikely that the different elevations of blood glucose in the rats fed parenterally with and without infusion of GIP depended on higher rates of glucose production in the animals that received GIP. The relative hyperglycemia in the GIP-infused rats, which developed in spite of their high plasma insulin levels, is therefore most readily attributable to impaired disposal of glucose. The findings thus suggest that long-term administration of porcine GIP induced a state of insulin resistance in the rats. It may be noted that decreased clearance of insulin from the blood has been suggested as a factor in development of hyperinsulinemia in obesity in human subjects.[11] Whether GIP can retard the clearance of insulin under the conditions of the present experiment remains to be determined, but such an effect would also be consistent with the same interpretation regarding insulin resistance.

The results of the i.v. glucose tolerance tests are likewise consistent with induction of insulin resistance as a result of administration of porcine GIP. Although the mean incremental response of IRI to the parenteral infusion with GIP added was smaller than that to the parenteral infusion alone, the mean peak levels of IRI attained in the three sets of animals were similar. Addition of GIP to the infusate resulted in slowing of the glucose disappearance rate associated with maintained elevation of plasma IRI levels. By contrast, plasma glucose fell most rapidly in the enterally alimented rats, although the plasma insulin concentrations in these animals were not higher than those in the parenterally alimented group that did not receive GIP, suggesting that insulin sensitivity was highest in the enterally alimented animals.

In the present study, the enterally administered nutrient load was sufficient to maintain or slightly increase the body weights of the rats through the 6-day period. Significant mean weight gains were achieved in the parenterally fed groups, but not in the enterally fed group. Relatively greater gains in weight are to be expected during parenteral alimentation, which results in retention of sodium and water through mechanisms that are not fully understood, but which may be related to the relatively high plasma insulin levels that occur when nutrients are administered this way.[12] It was of interest, therefore, that the mean weights in the three groups at termination of the infusions were ranked in order of the mean plasma levels of IRI. The enterally fed animals showed no diarrhea, and there were no detectable differences in the urine outputs among the three groups. On the basis of these observations it is not possible to assess the efficiency of absorption and metabolism of the nutrients in the three groups. Since some of the enterally fed rats did not gain weight, and still had mean plasma glucose and IRI levels lower than those of the parenterally fed groups, the findings are unlikely to be due to differences in absorption of the nutrient load.

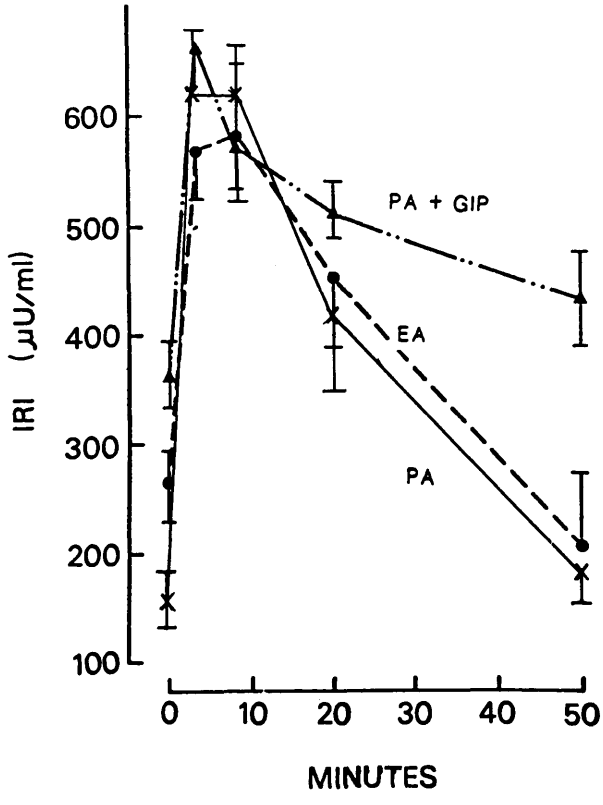

FIGURE 6. Mean plasma immunoreactive insulin levels after the bolus injection of glucose during the study (± SEM), as described in the text.

Although the mechanisms of the different responses to parenteral and enteral nutrients and of the effects of the prolonged i.v. infusion of GIP remain to be clarified, some speculations can be entertained. The addition of apparently physiologic doses of porcine GIP to the parenterally administered nutrients failed to elicit a state simulating that observed with

TABLE 2
Mean initial and final body weights (±SEM) in rats receiving enteral alimentation (EA), parenteral alimentation (PA), and parenteral alimentation with addition of GIP i.v. (PA + GIP)

Body wt (g)	EA	PA	PA + GIP
Initial	338 ± 5.6	341 ± 7.1	321 ± 4.8
Final	338 ± 5.3	354 ± 6.5	355 ± 7.9

enteral alimentation. Instead, the infusion of GIP exacerbated the hyperglycemia and hyperinsulinemia by comparison with the effects of parenteral alimentation. The condition thus brought about resembles that observed in obese normal and diabetic (type II diabetes) humans who exhibit exaggerated increments of plasma IRGIP after ingestion of glucose or of mixed meals.[4] Such findings in man have been interpreted as manifestations of insulin resistance, and have led to the suggestion that endogenous GIP can contribute to the generation of this condition. Although in man it has not been possible to test the possible causal role of GIP in the development of this state, it is notable that both the hyperglycemia and the elevated plasma levels of IRGIP can be corrected by food restriction.[4] The present studies with administration of porcine GIP in rats demonstrate that this peptide is capable of modifying the response to parenteral alimentation with glucose and amino acids in a manner consistent with the suggestion that it can elicit insulin resistance. This interpretation of the pathophysiologic states in man, and of the pharmacologically induced condition in the rat, depends on the indirect evidence cited suggesting that the hyperglycemia is not dependent on stimulation of glucose production. It is also apparent from the nature of the steady state developed during long-term enteral alimentation in the rats either that the actions of exogenous porcine GIP and endogenous rat GIP are different, or that other and perhaps physiologic mechanisms initiated in the gastrointestinal tract lead to reduction of plasma glucose concentrations by means that do not depend in a simple fashion on the levels of insulin or GIP in the blood. The latter inference suggests the possibility that failure of such mechanisms could lead to development of hyperglycemia and hyperinsulinemia. In either case, the background evidence and the results of the present study indicate that further examination of long-term effects of GIP on insulin secretion, on sensitivity to insulin, and on glucose production should be undertaken to elucidate the mechanisms of the effects here demonstrated in the rat, and their relevance to the pathophysiology of obesity and non-insulin-dependent diabetes mellitus.

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