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Gastric Inhibitory Polypeptide, Insulin, and Glucose Changes Produced by Growth Hormone, Prednisolone, Glucagon, Insulin, Fasting, or Diazoxide (40637)[1]

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Since gastric inhibitory polypeptide (GIP) augments glucose-stimulated insulin secretion (1–5), and since the amount of plasma insulin is important in diabetes, hypoglycemia, and other clinical disorders, we have investigated effects on plasma GIP levels of a series of compounds and metabolic states that have been found to affect insulin release. We tested the effects of growth hormone, glucosteroid (prednisolone), and glucagon; the effects of epinephrine are presented in a separate report (unpublished). Each of these hormones can influence the secretion and net actions of insulin and are involved significantly in preventing hypoglycemia, whether associated with a high absolute level of insulin (injection, insulinoma) or relative increase (6, 7). We included studies of prolonged fasting and of diazoxide; the latter was studied because of its inhibitory action on insulin secretion (6). We sought correlations of changes in plasma GIP, insulin, and glucose.

Materials and methods. Dogs. Five or more healthy purebred female foxhounds weighing between 17 and 21 kg were used for each experiment. Three days or more elapsed between tests. After a 16-hr fast, they were maintained in a calm and relaxed state in Pavlov jackets. A 20-gauge plastic cannula was inserted into the cephalic vein and was kept patent by a slow drip of normal saline. Before each test compound was given, two or more baseline blood specimens were obtained, 5 min apart. Growth hormone, glucagon, insulin, or diazoxide was infused at a constant rate (Harvard pump) into the cephalic vein of the opposite leg. Glucose and prednisolone were given by gastric tube (prednisolone also was injected intramuscularly). All compounds given orally were in a total volume of 200 ml of warm water. In control experiments, this amount of water alone was used. Each oral dose of glucose was 1 g/kg. The dosages of the other test substances and additional details of the experiments are presented in the legends with the figures.

Blood aliquots for GIP, insulin, and glucose assays were placed in EDTA; those for glucagon were put in tubes with heparin and 0.05 ml benzamidine (1 *M*). All tubes were submerged immediately in ice. Plasma for glucagon was separated within 1 hr and for the other tests within 2 or 3 hr. All specimens were stored at −70° until assayed.

Supplies. The antibody for glucagon (30 K) was kindly supplied by Dr. Roger Unger (Veterans Hospital, Dallas) and that for GIP by Drs. John Brown (University of British Columbia) and Werner Creutzfeldt (University of Göttingen). GIP was obtained from Dr. John Brown, growth hormone (bovine) from the National Institutes of Health, insulin and glucagon from the Eli Lilly Company, benzamidine from the Aldrich Chemical Company, and diazoxide from the Schering Corporation.

Assays. Glucose was assayed by the glucose oxidase method, using the Beckman Analyzer. GIP, insulin, and glucagon were measured by radioimmunoassay, using the respective methods of Kuzio *et al.* (8), Morgan and Lazarow (9), and Ensinck *et al.* (10). With the antibodies used there was no significant cross-reactivity by secretin, cholecystokinin, neurotensin, substance P, vasoactive intestinal polypeptide, bombesin, growth hormone, or gastrin. Insulin, glucagon, and GIP reacted significantly only with its respective antibody. All assays were run in duplicate. The lower limit of sensitivity for the GIP

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assay was 125 pg/ml. All of the specimens assayed had values significantly higher than this. Intraassay variation was 9% and interassay variation was 20%.

Presentation of data. In the figures are presented the mean absolute values (±SEM) for glucose, but for GIP the mean of the fasting values for each dog is calculated and the mean difference (±SEM) from this zero point is plotted for each time point. The data for insulin and glucagon are presented likewise. This was done to reconcile the interanimal variations in baseline levels of hormones. Typical mean baseline levels (±SEM) were: GIP, 455 ± 41 pg/ml; insulin, 7 ± 1 μU/ml; and glucagon, 40 ± 5 pg/ml. The statistical significance of the responses was determined by a paired Student's *t* test on the natural logarithm between baseline and response levels in each dog. Logarithmic transformation was performed to approximate better the Gaussian distribution required for this parametric statistical analysis. As needed, an asterisk or other symbol is used in the figures to indicate significant differences ($P < 0.05$). In the text all reports of observed changes are statistically significant to this extent, or more.

Results. Effect of growth hormone or prednisolone. Growth hormone alone increased plasma insulin and slightly decreased GIP, but produced no change in glucose (Fig. 1). It decreased the early increments in plasma GIP and glucose produced by glucose ($P < 0.05$), but did not affect significantly the increase in insulin.

Prednisolone alone did not change the levels of GIP, insulin, or glucose, and did not affect glucose-induced increments in plasma GIP or insulin (Fig. 1), but it slightly decreased the early rise in plasma glucose.

Effect of glucagon. Infusion of glucagon (5 ng/kg/min) for 15 min increased plasma insulin and markedly increased plasma glucagon, but did not change GIP or glucose levels (Fig. 2). Continuation of this infusion for 60 min after oral glucose did not affect significantly glucose-induced rises in plasma GIP, insulin, or glucose. Infusion of a large dose of glucagon, 50 ng/kg/min for 60 min, beginning 15 min after oral glucose, caused no significant inhibition of glucose-stimulated rises in plasma GIP. There were increments in insulin >400 μU/ml. and in glucose >100 mg/dl.

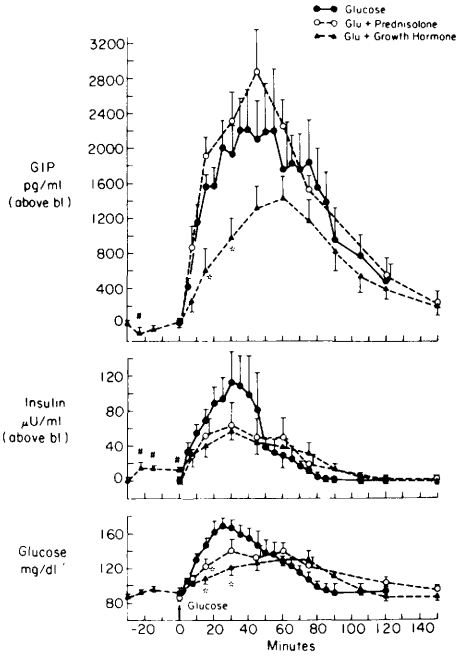

FIG. 1. There were three series of experiments, in all of which each dog was given glucose, 1 g/kg, by gastric tube at time 0. In one series, growth hormone (0.66 μg/kg/min) was infused for 1 hr, and glucose was given 30 min after starting the infusion. Blood specimens were acquired periodically during a period of 3 hr. Another series of experiments tested the effects of prednisolone alone, followed by oral glucose. Fasting blood specimens were obtained, at 8:00 and 8:05 AM, on the day preceding the main test. At 4:00 and 11:00 PM on that day and at 6:00 AM on the following day prednisolone sodium phosphate, 20 mg, was injected intramuscularly. Thereafter, blood was drawn at 8:00 and 8:05 AM. Then glucose plus prednisolone, 20 mg, were given by gastric tube. In a third series of experiments dogs were given only glucose.

The mean and SEM for each time are plotted. # Values that differed ($P < 0.05$) from baseline values obtained before any infusion of growth hormone. * Deviation ($P < 0.05$) from results found with oral glucose alone. The pretreatment baseline values (for times −5 and 0) were averaged and shown as one point. The baseline values obtained before prednisolone treatment are not shown; they differed little from the results obtained from those of specimens drawn immediately before oral glucose.

Effect of insulin. Although intravenous infusion of insulin, 57 mU/kg/min for 35 min, increased plasma IRI to levels >300 μU/ml and reduced plasma glucose to levels <40 mg/dl, it did not change plasma GIP levels significantly (Fig. 3). Moreover, infusion of insulin, 30 mU/kg/min, starting 20 min be-

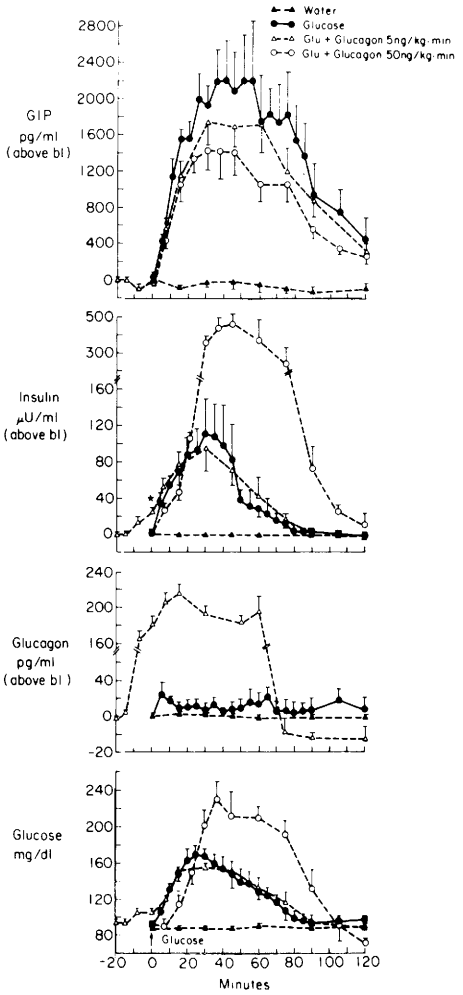

FIG. 2. Results from four series of experiments are shown. At time 0 one group was given only water by gastric tube. Three other series received either: (a) glucose alone, (b) glucose plus glucagon, 5 ng/kg/min (infused from −15 to 60 min), or (c) glucose plus glucagon, 50 ng/kg/min (infused from 15 to 75 min). All glucose (1 g/kg) was given by gastric tube. * Deviation from baseline values ($P < 0.05$).

fore oral glucose and continuing for 45 min after the glucose, did not alter the basal levels of GIP nor the GIP rises produced by glucose, despite increases in plasma insulin >100 μU/ml.

Effect of prolonged fasting or diazoxide. Fasting for 4 days plus the routine 16-hr fast did not change the plasma insulin or glucose levels, but decreased GIP levels ($P < 0.05$) (Fig. 4). Whereas glucose caused as much increase in GIP concentrations in dogs with the long fast as with the short fast, the actual levels attained were not as high during the early periods. After the long fast the mean basal glucagon level was 45.9 ± 4.2 pg/ml, which was comparable to that for the 16-hr fast (not plotted). Oral glucose given to long-fasted dogs caused a more prolonged rise in plasma glucose and insulin than it produced in dogs fasted for 16 hr. Diazoxide increased fasting plasma glucose (Fig. 4) but did not change plasma insulin or GIP. Compared with glucose alone, diazoxide plus glucose caused less insulin secretion, a slower rise in plasma GIP, and a more prolonged increase in glucose (Fig. 4). Diazoxide produced no significant changes in plasma glucagon levels

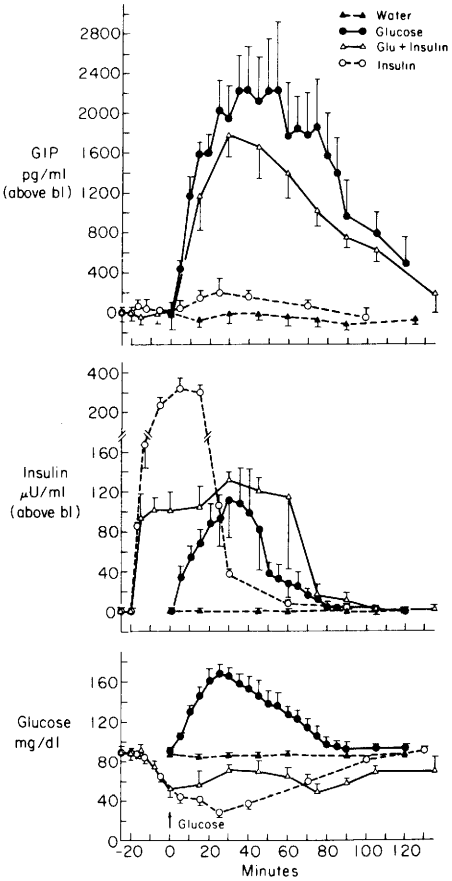

FIG. 3. Results from four series of experiments are shown. At time 0: one series was given water alone; another series was given glucose alone; a third series received glucose plus insulin, 30 mU/kg/min from −20 to 45 min. The fourth series received insulin alone, 57 mU/kg/min, from −20 to 15 min. The water and glucose, 1 g/kg, were given by gastric tube.

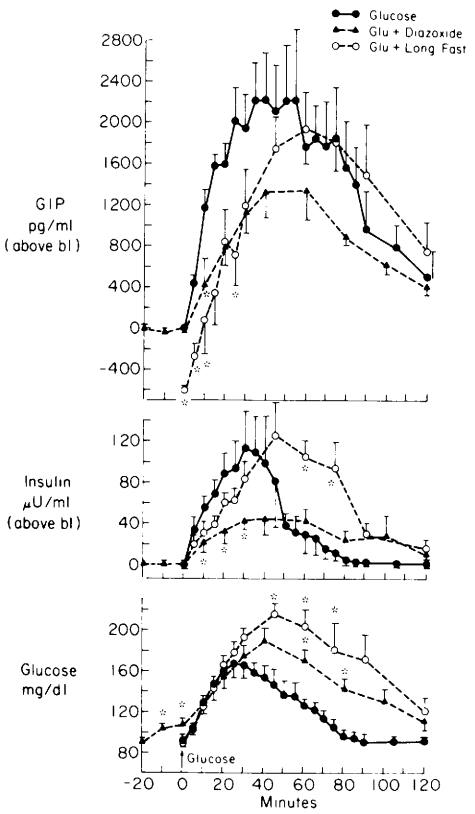

FIG. 4. In each of three series of experiments oral glucose, 1 g/kg, was given by gastric tube at time 0. In two series, glucose alone was given to dogs fasted for 16 hr or for 112 hr. The third series, fasted for 16 hr, were infused with diazoxide (5 mg/kg within 2 min), beginning 20 min before oral glucose. The mean decrease in GIP between a 16- and 112-hr fast was 600 pg/ml, and the baseline for the latter is shown at this level. * Differences ($P < 0.05$) in results obtained when glucose alone was given to dogs fasted 16 hr and those given glucose after a long fast or after diazoxide.

before or after glucose administration (not plotted).

Discussion. As indicated earlier, insulin secretion can be influenced by GIP and each of the factors that we studied (GH, prednisolone, glucagon, fasting, and diazoxide). In our studies we were interested most in correlating changes in plasma GIP, insulin, and glucose, observing especially how many of the factors influenced plasma GIP levels and evaluating the role of GIP in producing changes in insulin. Since the effects of GIP on plasma glucose result from its stimulation of the secretion of insulin and glucagon (3, 11), and these hormones have been considered to decrease GIP secretion (3, 12), we investigated these effects by infusing insulin and glucagon.

We found that infusion of GH alone increased plasma insulin yet decreased GIP. Since GH infusion decreased the early increase of plasma GIP, insulin, and glucose produced by oral glucose, we ask whether these changes were attributable to: (a) an increase in gastrointestinal transit time and/or decrease in intestinal absorption of glucose, (b) increased glucose utilization by peripheral tissues, (c) rapid increase in insulin release resulting from GH action on B cells, or (d) inhibition of GIP secretion by insulin, glucagon, or GH? Since GH promotes rapid release of insulin and glucagon from perfused pancreas (13), the GH-stimulated early rise in insulin presumably resulted from GH action on islet cells and was not mediated by GIP. GH can cause increased glucose uptake by muscle and adipose tissue by its action on these tissues (10). This action plus the early insulin increase may have accounted for the decreased early rise in plasma glucose; GH is known to decrease plasma glucose even without increase in plasma insulin (14). In view of the failure of large doses of insulin (Fig. 3) and glucagon (Fig. 2) to inhibit glucose-induced GIP rises, the slow increase in GIP that occurred with GH plus glucose does not seem to be related to effects of insulin or glucagon. Possibly GH caused some delay in gastrointestinal absorption of oral glucose. In two experiments in which we included [^{14}C]glucose with the oral glucose and followed ^{14}C levels in the plasma, the apogee was about 15 min later than with glucose alone (five tests); this possibly could be due partially to delayed absorption and/or GH-stimulated increase in glucose utilization.

Prednisolone did not affect baseline levels of GIP, insulin, or glucose, nor glucose-induced rises in GIP and insulin. However, it slightly decreased the early rise in plasma glucose. The basis for this is unknown; others have found that glucosteroid given for 3 days exaggerated plasma glucose and insulin rises after glucose administration (15).

Glucagon, 5 mg/kg/min, did not affect fasting GIP levels or its response to oral glucose. Moreover, when glucagon was infused in a pharmacologic dose, 50 mg/kg/min, starting 15 min after oral glucose, it did

not inhibit significantly the GIP rise. Ebert *et al.* (12) found that this large dose did not affect fasting GIP levels in man, but that when its infusion was begun before a large mixed meal it inhibited GIP increase. However, as they signified, this large dose is unphysiological and could inhibit gastric emptying. Even our smaller dose, 5 mg/kg/min, produced increments in plasma glucagon >200 pg/ml, which are higher than we have obtained with physiologic amounts of GIP. Therefore, it is questioned whether glucagon normally exerts a significant role in controlling GIP secretion.

In mild diabetics we have found normal GIP responses to glucose (13), but moderately severe diabetics had hypernormal GIP responses (3, 17, 18). Since insulin treatment decreases the responses (3), it has been suggested that insulin exerts feedback inhibition of GIP secretion. However, this inhibition may be indirect, because diabetes has been shown to increase glucose absorption from the intestine (19), and possibly in this manner increases GIP release.

Crockett *et al.* (20) found that insulin inhibited glucose-stimulated GIP rise, while Service *et al.* (21) did not observe this to occur unless hypoglycemia developed. However, we found no decrease in plasma GIP levels produced by insulin, regardless of whether insulin was given alone or with glucose, or whether hypoglycemia or euglycemia was present. As discussed earlier, hypoglycemia can increase the secretion of GH, glucagon, glucosteroid, and epinephrine. In testing these hormones, we found that only GH affected the GIP response to glucose.

Diazoxide decreased glucose-stimulated insulin secretion and increased plasma glucose. The increase in glucose results from decreased insulin secretion, increased glucose production, and inhibition of glucose uptake (18). Whether the inhibition of GIP is a direct or indirect action of diazoxide is unknown. Perfusion of pancreas with diazoxide decreased the secretion of glucagon, somatostatin, pancreatic polypeptide, and insulin (19).

We found that fasting for 112 hr significantly decreased plasma GIP, but did not change insulin or glucose. Whereas this fast increased the duration of glucose-induced rises in plasma insulin and glucose, it did not change the amount of GIP rise. Therefore, the persistence of the hyperinsulinemia did not inhibit the increase in GIP. Wilms *et al.* (24) found that fasting obese patients caused plasma GIP levels to increase and insulin to decrease within a few days. Despite the rise in baseline GIP, these fasted subjects experienced a decrease in glucose-induced GIP response. To what extent obesity and other factors accounted for the difference in results in obese patients and those in our nonobese dogs is unclear.

In conclusion, whereas GIP can stimulate insulin secretion, there are many factors that increase plasma insulin without increasing plasma GIP. Moreover, increase in plasma insulin may not suppress glucose-induced increase in plasma GIP.

Summary. Because of the strong augmentary action of gastric inhibitory polypeptide (GIP) on insulin secretion, we studied in foxhounds the effect on GIP secretion of several factors known to affect insulin secretion and plasma glucose. Growth hormone infusion decreased plasma GIP, yet increased insulin; no change in plasma glucose was found. Growth hormone inhibited oral glucose-induced increments in plasma GIP and glucose. Prednisolone did not affect levels of GIP or insulin, whether given alone or preceding oral glucose. Diazoxide decreased glucose-induced GIP and insulin secretion, but increased plasma glucose. Fasting for 112 hr decreased GIP but did not decrease the glucose-induced GIP rise. Infusion of glucagon or insulin in amounts sufficient to produce high physiologic or pharmacologic plasma levels did not change GIP concentration, nor did they alter GIP rises with glucose. In conclusion, GIP was not inhibited by insulin or glucagon but was decreased by growth hormone, fasting, or diazoxide. Whereas fasting increased glucose-stimulated insulin secretion, diazoxide decreased it. The insulin-releasing activity of a number of factors does not correlate with increases in plasma GIP, and increases in plasma insulin or glucagon may not suppress increases in plasma GIP. There was not a good correlation of the plasma levels of GIP, insulin, glucose, and glucagon.

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1. Dupre, J., Ross, S. A., Watson, D., and Brown, J. C., J. Clin. Endocrinol. Metabol. **37,** 826 (1973).
2. Cataland, S., Crockett, S. E., Brown, J. C., and Mazzaferri, E. L., J. Clin. Endocrinol. Metabol. **39,** 223 (1974).
3. Brown, J. C., Dryburgh, J. R., Ross, S. A., and Dupre, J., Recent Prog. Hormone Res. **31,** 487 (1975).
4. Pederson, R. A., Schubert, H. E., and Brown, J. C., Diabetes **24,** 1050 (1975).
5. Anderson, D. K., Elahi, D., Brown, J. C., Tobin, J. D., and Andres, R., J. Clin. Invest. **62,** 152 (1978).
6. Ensinck, J. W., and Williams, R. H., *in* "Textbook of Endocrinology" (R. H. Williams, ed.). Saunders, Philadelphia (1974).
7. Cahill, G. F., Jr., N. Engl. J. Med. **282,** 668 (1970).
8. Kuzio, M., Dryburgh, J. R., Malloy, K., and Brown, J. C., Gastroenterology **66,** 347 (1974).
9. Morgan, C. R., and Lazarow, A., Diabetes, **12,** 115 (1963).
10. Ensinck, J. W., Shepard, C., Dudl, R. J., and Williams, R. H., J. Clin. Endocrinol. Metabol. **35,** 463 (1972).
11. Kobric, M., and Dupre, J., Clin. Res. **24,** 681A (1976).
12. Ebert, R., Arnold, R., and Creutzfeldt, W., Gut **18,** 121 (1977).
13. Tai, T. Y., and Pek, S., Endocrinology **99,** 669 (1976).
14. Merimee, T. J., and Rabin, D., Metabolism **22,** 1235 (1973).
15. Kitabchi, A. E., Jones, G. M., and Duckworth, W. C., J. Clin. Endocrinol. Metabol. **37,** 79 (1973).
16. May, J. M., and Williams, R. H., Diabetes **27,** 849 (1978).
17. Crockett, S. E., Mazzaferri, E. L., and Cataland, S., Diabetes **25,** 931 (1976).
18. Ross, S. A., Brown, J. C., and Dupre, J., Diabetes **26,** 525 (1977).
19. Olsen, W. A., and Rosenberg, I. H., J. Clin. Invest. **29,** 96 (1970).
20. Crockett, S. E., Sirinek, K. R., O'Dorisio, T. M., Mazzaferri, E. L., Page, W. G., and Cataland, S., Clin. Res. **24,** 536A (1976).
21. Service, F. J., Go, V. L., Blix, P., and Nelson, R. L., Diabetes **26,** 374A (1977).
22. Altszuler, N., Hampshire, J., and Moraru, E., Diabetes **26,** 931 (1977).
23. Samols, E., Weir, G. C., Patel, Y. C., Fernandez-Durango, R., Arimura, A., and Loo, S. W., Diabetes **26,** 375 (1977).
24. Willms, B., Ebert, R., and Creutzfeldt, W., Diabetologia **14,** 379 (1978).

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