Lowering of fasting and food stimulated serum immunoreactive gastric inhibitory polypeptide (GIP) by glucagon

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SUMMARY The effect of intravenous glucagon infusion on serum levels of immunoreactive GIP (IR-GIP), insulin (IRI), gastrin (IRG), and on blood glucose has been investigated in six healthy volunteers in the fasting state and during ingestion of a mixed standard meal. Glucagon (500 ng/kg/min) lowered significantly serum levels of IR-GIP and IRG below the fasting values and increased the levels of IRI and blood glucose. Glucagon (50 ng/kg/min) infused 30 minutes before and continued 90 minutes after ingestion of a test meal abolished the IR-GIP response, suppressed significantly the IRG response, and left the IRI response unchanged. The same glucagon dose infused 60 minutes after ingestion of the test meal decreased significantly the raised levels of IR-GIP and IRG to fasting levels without changing IRI values. It is concluded that exogenous glucagon inhibits GIP release at the level of the GIP-producing cells.

Gastric inhibitory polypeptide (GIP) isolated from the small intestine is an inhibitor of basal and stimulated gastric secretion and potentiates the glucose induced insulin secretion (Brown *et al.*, 1975). GIP is released after ingestion of glucose (Cataland *et al.*, 1974; Brown *et al.*, 1975), fat (Brown *et al.*, 1975; Falko *et al.*, 1975) and amino acids (Thomas *et al.*, 1976). However, little is known about other factors influencing GIP release. The existence of such factors is of interest because an altered response to ingested glucose or a test meal has been described in different gastrointestinal and metabolic disorders (Bloom, 1975; Brown *et al.*, 1975; Crockett *et al.*, 1975; Botha *et al.*, 1976; Ebert *et al.*, 1976a, b). A feedback inhibition of GIP release by insulin has been suggested (Brown *et al.*, 1975; Cleator and Gourlay, 1975; Crockett *et al.*, 1976) and the rate of absorption as regulator of GIP release discussed (Brown *et al.*, 1975; Creutzfeldt *et al.*, 1976; Ebert *et al.*, 1976a).

Interactions of gastrointestinal hormones have been previously described. Secretin (Hansky *et al.*, 1971; Thompson *et al.*, 1972) and glucagon (Becker *et al.*, 1973; Hansky *et al.*, 1973) are able to lower fasting and food stimulated serum gastrin response

in healthy subjects and patients with duodenal ulcer. Therefore, the effect of glucagon infusions on the serum levels of immunoreactive GIP (IR-GIP), gastrin (IR-G) and insulin (IRI) was investigated in healthy subjects. It will be shown that glucagon infusion reduces basal IR-GIP levels and inhibits the IR-GIP response to a test meal.

Methods

SUBJECTS

The studies were performed in six healthy volunteers, two females and four males with a mean age of 25 years (range from 19-32 years). These subjects were within 10% of their ideal body weight (mean weight 68.6 kg) and had a normal glucose tolerance. Informed consent was obtained from all subjects.

TEST PROCEDURE

After an overnight fast and at least a 45 minute rest in the supine position an indwelling cannula was inserted into the antecubital vein of one arm for blood sampling and another cannula was inserted into the opposite arm for the infusion of glucagon. Both cannulas were kept patent by slow infusion of saline. The effect of glucagon on fasting and meal stimulated hormone levels was investigated. Three basal blood samples were collected, after which the intravenous infusion of glucagon was started. For

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30 minutes glucagon was administered at a concentration of 50 ng/kg/min, the concentration was then raised to 500 ng/kg/min during the next 30 minutes. In the other experiments, after taking basal samples, 550 ml of a mixed liquid test meal was ingested within 10 minutes. The test meal consisted of 400 ml Dextro^(R) OGT, containing 18 g glucose, 14 g maltose, 12 g maltotriose, 56 g oligosaccharides, corresponding to 100 g rapidly absorbable glucose (Pomps GmbH, Heilbronn), 100 ml cream (containing 30% fat), and 100 g Molico^(R) Instant (Nestle GmbH, Lindau), containing 36 g protein and 52 g lactose. This test meal contained 1031 calories. The osmolality was 1280 mosmol/l.

Blood samples were taken at 15 minute intervals one hour before and three hours after ingestion of the test meal. The samples were immediately placed on ice and centrifuged at 4°C. Five aliquots of each serum sample were frozen and stored at -20°C until assayed.

Glucagon (Glucagon, Eli Lilly Co, Gießen) for infusion was prepared immediately before use (approximately 10 µg/ml concentration) in physiological saline containing human serum albumin (Behringwerke AG, Marburg/Lahn) 2 g/100 ml, and a small amount of aprotinin (Trasylol, Bayer AG, Leverkusen) 50 KIU/ml, in order to prevent absorption and proteolytic breakdown of glucagon within the infusion apparatus. The glucagon infusion was started in one series of experiments 30 minutes before ingestion of the test meal and continued till 90 minutes after ingestion at an infusion rate of 50 ng/kg/min (protocol 1). In another series of experiments glucagon was infused from 60 minutes to 150 minutes after ingestion of the test meal at the same infusion rate (protocol 2). All subjects served as their own controls—that is, the test was repeated without glucagon within six to 14 days.

LABORATORY ANALYSES

Serum glucose was measured in duplicate on each sample by the glucose oxidase method after separating the serum. Serum calcium was determined complexometrically using the autoanalyser SMA 6/60.

IRI, IRG, and IR-GIP were determined in duplicate on each sample within four weeks after the test. Serum IRI was determined using human insulin as a standard (Melani *et al.*, 1965). Serum IRG was estimated as described previously (Mayer *et al.*, 1974) using antiserum 118/2/3. Serum IR-GIP was measured as described elsewhere (Creutzfeldt *et al.*, 1976) using the technique of Kuzio *et al.* (1974) with the antibody Van No. 8. Minor modifications were introduced improving the sensitivity of the assay. The amount of GIP for the labelling procedure was reduced from 6 µg to 0.5 µg per label. At the day of

use a batch of 0.5 g Dowex (Cl⁻, 50-100 mesh) was added to a portion of the label, mixed and centrifuged after five minutes. By this procedure free iodine was removed and thereby the non-specific binding reduced to about 4-6%. About 15% displacement of bound GIP was achieved by 6 pg, 50% displacement by 82 pg GIP (mean of 48 assays). The lower limit of sensitivity varied from assay to assay ranging from 3 pg to 10 pg. As final serum dilution in our assays is 1:10, the actual sensitivity for practical determination varies from 30 to 100 pg/ml of serum. Usually values of less than 50 pg/ml of serum were arbitrarily considered as 50 pg for statistical analysis. Non-specific binding was estimated in each assay for each subject and used in the calculation of the amount of immunologically active GIP in the unknown samples. The inter-assay variance was 12.6%, the intra-assay variance 9.1% (mean of 48 assays).

The GIP antiserum used did not cross-react with glucagon, secretin, CCK, and gastrin (Kuzio *et al.*, 1974). However, as high serum glucagon levels under the experimental conditions could be expected, it was reinvestigated to see if added glucagon interfered in the assay with the antibody against GIP. Glucagon in a concentration up to 2 ng/ml did not displace bound GIP. However, if 1 ng/ml glucagon was incubated together with fresh serum for one hour at room temperature a substance appeared in the serum which reacted with the antibody against GIP, possibly representing a breakdown product of glucagon. The formation of this substance was markedly reduced by the addition of Trasylol or by cooling the serum samples. Therefore, blood samples were immediately placed on ice and centrifuged at 4°C within 45 minutes and frozen. Addition of glucagon to GIP-containing solutions did not alter the measured IR-GIP levels. Results are expressed as the mean ± SEM. Student's *t* test for paired values was performed to determine the significance of differences between controls in the absence and presence of glucagon.

Results

EFFECT OF GLUCAGON ON BASAL CONCENTRATIONS OF SERUM IR-GIP, IRG, IRI AND GLUCOSE

The effect of intravenous glucagon infusion on fasting serum levels of glucose, gastrin, GIP, and insulin is shown in Fig. 1. The average basal value of GIP was 326 ± 61 pg/ml (n = 6); after infusion of glucagon at a concentration of 50 ng/kg/min the basal level dropped slightly but not significantly; whereas after increasing the glucagon infusion to 50 ng/kg/min a significant decrease of GIP levels was observed reaching a nadir of 109 pg ± 39 pg/ml after 20

Fig. 1 Effect of glucagon (50 ng/kg/min from 0-30 min and 500 ng/kg/min from 30-60 min) on serum levels of IR-GIP, IR-gastrin, IR-insulin, and blood glucose in six fasting healthy subjects. The vertical bars indicate SEM, asterisks significant differences from the pre-infusion values ($P < 0.05$).

minutes. GIP levels returned to basal values 10 minutes after the infusion was stopped.

Basal insulin levels were below the sensitivity of the assay ($< 6 \mu\text{U/ml}$ insulin) and increased during the glucagon infusion. Maximal insulin values were obtained 30 minutes after the infusion of 500 ng glucagon/kg/min had started. Glucose increased only slightly at low glucagon concentrations, significantly higher blood glucose levels occurred during the infusion of 500 ng/kg/min glucagon. The fall of serum gastrin in the presence of 50 ng/kg/min glucagon was not significant; however, significant reduction of gastrin was seen during the higher glucagon dose.

Serum calcium decreased from $9.3 \text{ mg/100 ml} \pm 0.4$ to $8.8 \text{ mg/100 ml} \pm 0.3$ 30 min after 50 ng/kg/min glucagon. A further, significant drop to $8.2 \pm 0.3 \text{ mg/100 ml}$ ($P < 0.05$) occurred 20 minutes after the start of the higher glucagon infusion.

EFFECT OF GLUCAGON ON RESPONSE OF IR-GIP, IRG, IRI, AND GLUCOSE TO A TEST MEAL

The reproducibility of the hormonal response to the standard test meal was investigated in four subjects. To each subject the meal was given three times within two weeks. The maximal variation between the tests was as follows: serum IR-GIP 24%, IR-insulin 27%, IR-gastrin 21%, and blood glucose 18%. The effect of the glucagon infusion (50 ng/kg/min) on the hormonal response to the test meal is demonstrated in Figs. 2 and 3. In protocol 1 the glucagon infusion was started 30 minutes before and continued until 90 minutes after ingestion of the test meal. The results are shown in Fig. 2. After onset of the glucagon infusion, basal levels of IR-GIP ($278 \pm 64 \text{ pg/ml}$) were slightly lowered; the sharp rise of serum levels of IR-GIP to $1051 \pm 306 \text{ pg/ml}$ which occurred in the absence of glucagon was completely suppressed over the total period of the glucagon

Fig. 2 Effect of glucagon (50 ng/kg/min) on the response of IR-GIP, IR-gastrin, IR-insulin, and blood glucose to a mixed test meal in six healthy subjects. The test meal started at time 0. The glucagon infusion started before the meal. $\circ$ — $\circ$ = test meal alone. $\bullet$ — $\bullet$ = test meal plus glucagon infusion. $\times$ = significant difference ($P < 0.05$) from control experiment.

infusion. After stopping the infusion IR-GIP increased sharply in a rebound pattern to 1688 ± 269 pg/ml.

The IR-gastrin response to the test meal was significantly lower during the glucagon infusion but not completely abolished as in the case of the GIP response. Serum IR-G levels increased immediately after the end of the glucagon infusion. The serum glucose concentration was significantly higher than the control values for the total period of glucagon application. IRI levels were significantly increased by glucagon only in the fasting period and 15 minutes after ingestion of the test meal. At later times there was no significant difference between IRI levels in the presence or in the absence of glucagon.

As glucagon inhibits gastric motility (Neches *et al.*, 1966), delayed gastric emptying could be responsible for the inhibition of IR-GIP release by glucagon. Therefore in protocol 2 the glucagon

infusion was started 60 minutes after ingestion of the test meal (Fig. 3). Again, IR-GIP levels dropped significantly after onset of the glucagon infusion and reaching the fasting values after 45 minutes. The IR-gastrin levels were also significantly reduced 30 minutes after the start of glucagon application. Serum glucose concentrations, on the other hand, increased during the infusion period significantly. The serum IRI values did not significantly differ from the control values during the glucagon application. They even tended to be lower than the controls at the time of the highest glucose levels.

Discussion

The data presented here demonstrate that exogenous glucagon lowers fasting IR-GIP levels (500 ng/kg/min) and suppresses completely the IR-GIP response to a mixed high caloric test meal

Fig. 3 Effect of glucagon (50 ng/kg/min) on meal stimulated IR-GIP, IR-gastrin, IR-insulin, and blood glucose in six healthy subjects. The test meal started at time 0. The glucagon infusion started 60 min after ingestion of the test meal. $\circ$ — $\circ$ = test meal alone. $\bullet$ — $\bullet$ = test meal plus glucagon infusion. $\times$ = significant difference from control experiment ($P < 0.05$).

(50 ng/kg/min) in man. However, the findings do not yet indicate that endogenous glucagon participates in the physiological regulation of GIP release. The lower glucagon dose used is probably still 30-50 times higher than the dose which is necessary to achieve glucagon plasma levels in the physiological range (Alford *et al.*, 1974; Gerich *et al.*, 1976).

The observed glucagon effect on IR-GIP levels closely resembles the suppressive effect of glucagon on fasting and meal stimulated serum gastrin levels (Becker *et al.*, 1973; Hansky *et al.*, 1973). The effect of glucagon on IR-gastrin has been confirmed in this study with even lower glucagon dosages. The significant suppression of fasting and meal stimulated serum IR-G levels occurred simultaneously with the suppression of IR-GIP values. Therefore, the glucagon effect could be mediated *via* gastrin if this hormone is normally involved in the release of GIP. However, this possibility can be ruled out because

it has been shown that patients with undetectable serum IR-gastrin levels after gastroduodeno-pancreatectomy (Whipple's procedure) have an exaggerated IR-GIP response to a test meal (Creutzfeldt *et al.*, 1976).

The following explanations for the lowering of IR-GIP levels by exogenous glucagon have to be discussed: (1) interference of high concentrations of glucagon with the radioimmunoassay for GIP, (2) promotion of destruction or excretion of GIP, (3) inhibition of GIP release.

The first possibility has been ruled out by *in vitro* addition of glucagon in concentrations up to 2 ng/ml. No interference with the estimation of IR-GIP could be detected. The second possibility (promotion of destruction or excretion of GIP) cannot be discussed at the moment because nothing is yet known about the site of destruction and excretion of GIP. However, the kinetics of the fall of

fasting and stimulated IR-GIP levels after the start of the glucagon infusion can be used as an argument against accelerated GIP excretion or destruction: glucagon induced a fall of the stimulated GIP levels of 50% within 30 minutes (Fig. 3). Also, in the fasting state the higher glucagon dose produced a fall of the serum IR-GIP level of 50% within 20 minutes (Fig. 1). This corresponds closely to the half-life time of exogenous GIP which has been reported to be 21 minutes (Brown *et al.*, 1975).

The rapid fall of fasting and stimulated IR-GIP after the onset of the glucagon infusion suggests inhibition of GIP release by glucagon. This could be a direct effect on the GIP-producing cells of the gut or an interaction with mechanisms regulating or modulating the GIP release, such as motility, assimilation of food, or blood levels of glucose or insulin. Each of these factors is influenced by exogenous application of high doses of glucagon and could theoretically interfere with the GIP release. However, some of these mechanisms cannot explain the lowering of fasting IR-GIP levels because they are relevant only for GIP response to food.

Gastric and jejunal motility can be markedly inhibited by exogenous glucagon (Neches *et al.*, 1966; Hicks and Turnberg, 1974). Therefore, the test meal may not reach the GIP-producing cells in the duodenal and jejunal mucosa. This possibility has been excluded by the second experimental protocol: the glucagon infusion was started 60 minutes after the ingestion of the test meal—that is, after it had entered the gut and thus was in contact with the GIP-producing cells. Also under these conditions glucagon produced a sharp fall of the IR-GIP levels.

It has been demonstrated that exogenous glucagon inhibits glucose absorption (Gottesbüren *et al.*, 1974). This may reduce the stimulus for GIP release because it has been suggested that the rate of absorption of nutrients is one of the facts which regulates GIP release (Brown *et al.*, 1975; Creutzfeldt *et al.*, 1976; Ebert *et al.*, 1976a). However, glucose absorption is not completely inhibited even with pharmacological glucagon doses. Furthermore, this would not explain the glucagon effect on the fasting IR-GIP levels.

The main effect of glucagon is the induction of hyperglycaemia. It has been found that the GIP response to fat ingestion is decreased by a simultaneous intravenous infusion of glucose suggesting a negative feedback control for GIP mediated by glucose (Cleator and Gourlay, 1975; Crockett *et al.*, 1976). As the blood glucose levels during the glucagon infusion were always higher than in the control period, these raised blood glucose levels could be the reason for the lowering effect of glucagon on serum IR-GIP. This argument is

invalidated by the observation of normal or exaggerated IR-GIP response to a test meal in patients with pathological glucose tolerance (Creutzfeldt *et al.*, 1976; Ebert *et al.*, 1976a, b)—that is, in the presence of blood glucose levels far in excess of the values found in this study during the glucagon infusion.

Exogenous glucagon releases insulin (Samols *et al.*, 1965). It has been suggested that insulin inhibits GIP release in the sense of a feedback control (Brown *et al.*, 1975; Cleator and Gourlay, 1975; Crockett *et al.*, 1976). Thus, high serum IRI levels might explain suppression of IR-GIP. However, the IRI increase during glucagon infusion was small only in the fasting state (Fig. 1) and not different from the control experiments after the test meal (Figs. 2 and 3). It is, therefore, not probable that endogenous insulin is involved in the suppressive effect of glucagon on serum IR-GIP. On the other hand, the similarity of the IRI response to the test meal with and without glucagon infusion demonstrates that pharmacological doses of glucagon (directly and/or *via* hyperglycaemia) can compensate for the abolished insulin-releasing potency of GIP.

The most probable mode of action of glucagon on GIP release seems to be a direct inhibition of secretion on the cellular level. As nothing is known about the stimulus secretion coupling of the GIP-producing cells the exact mechanism cannot yet be understood. As in the case of glucagon induced inhibition of gastrin release, glucagon induced changes of calcium metabolism and calcitonin secretion may be discussed (Becker *et al.*, 1973). Also in this study the glucagon infusion significantly lowered serum calcium levels in the fasting state.

Further investigations have to explain why exogenous glucagon *stimulates* the release of insulin (Samols *et al.*, 1965) and calcitonin (Bell *et al.*, 1970) and *inhibits* the release of gastrin (Becker *et al.*, 1973; Hansky *et al.*, 1973) and GIP (this study). The chemical structure of gastrin and GIP is not related. It will be also of interest to know if exogenous glucagon paradoxically stimulates GIP release from GIP-producing tumours, as has been well documented for gastrin release from gastrinomas (Becker *et al.*, 1973; Korman *et al.*, 1973; Creutzfeldt *et al.*, 1975). Finally, it has to be elucidated if physiological levels of glucagon influence GIP secretion and if endogenous glucagon participates in the regulation of GIP secretion as discussed recently (Ebert *et al.*, 1976a).

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